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Acaricidal investigation of commiphora swynnertonii (burtt) stem bark exudate

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NM-AIST

ACARICIDAL INVESTIGATION OF *Commiphora swynnertonii* (Burtt) STEM BARK EXUDATE

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A Dissertation Submitted in Partial Fulfilment of the Requirements of the Degree of Master's in Life Science of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

The present study was carried out to investigate acaricidal potencies of *Commiphora swynnertonii* (Burtt) stem bark exudate against ticks *Rhepicephalus appendiculatus* and evaluate its toxicity using mice and rats.

Commiphora swynnertonii exudate was prepared in soap solution and evaluated against the *R. appendiculatus* using adult immersion test method (AIT). The percent mortality, Index of laying eggs and percentage hatching were determined at concentrations of 100, 90, 80, 70, 60, 50, 25 and 12.5 mg/ml. The acute and sub-acute toxicity was determined by oral administration of the exudates to mice and rats at 500, 1000, 3000 and 250, 500, 1000 mg/Kg body weight respectively as a single (14 days) and daily dosing (28days). Physiological, behavioral changes, hematology, relative internal organs weights, biochemical and histopathology were assayed.

Results show that at concentration above 25mg/ml there was significant mortality *($p<0.05$)* of ticks treated with *C. swynnertonii* exudates. Inhibition of laid eggs was found to be significant at concentration greater than 90mg/ml *($p<0.05$).* In addition hatching of eggs was completely inhibited in all treated groups. On the other hand oral toxicity results showed that, administration of *C. swynnertonii* exudates in mice and rats did not result into any observable toxicity during the experimental period.

The current results indicate that *C. swynnertonii* exudate extract soap solution have acaricidal activity against *R. appendiculatus* and can be used as alternative measure to control ticks. Having lower toxicity levels reveal initial strategy for further findings and finally formulation acaricides from the plant exudate.

DECLARATION

I, **SYLVESTER GERALD TEMBA**, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree in any other Institution.

_____ _____

Name and Signature of the Candidate **Date**

The above declaration is confirmed

_____ _____

Name and Signature of the Supervisor 1 **Date**

_____ _____

Name and Signature of the Supervisor 2 **Date**

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CERTIFICATION

The undersigned certify that they have read the dissertation titled "**Acaricidal Investigation of *Commiphora Swynnertonii* (Burtt) Stem Bark Exudate**" and recommend for examination in partial fulfillment of the requirements for the degree of Master in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

_____ _____

Dr. Musa Chacha (Supervisor 1) **Date**

_____ _____

Dr. Paul Erasto Kazyoba (Supervisor 2) **Date**

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May the Almighty God bless all abundantly

DEDICATION

I would like to dedicate this work to my parents Gerald and Feliciana Temba and my wife Emiliana Sulle for their love and inspiration in my academic life.

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LIST OF ABREVIATIONS

AIT	Adult immersion test
ALP	Alkaline phosphate
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BOD	Biological Oxygen demand
CAA	Commiphora acaricidal assay
DMSO	Dimethyl sulphoxide
DPPH	Diphenyl-2-picryl hydrazyl
EDTA	Ethylene diamine tetra acetic acid
ECF	East coast fever
ERF	Estimated reproductive factor
Hb	Haemoglobin
IE	Index of laying eggs
IR	Inhibition of reproduction
LD	Lethal dose
NMAIST	Nelson Mandela African institution of science and Technology
OECD	Organization for Economic Cooperation and Development
RBC	Red blood cell
RDW	Red cell Distribution width
RH	Relative humidity
RUFORUM	Regional University Forum for capacity building in agriculture

SUA	Sokoine University of Agriculture
TPRI	Tropical Pesticides Research Institute
TBD	Tick borne diseases
TTBD	Tick and tick borne diseases
WBC	White Blood cell

CHAPTER ONE

INTRODUCTION

This chapter discusses the general introduction of the study. It covers background information of the tick and tick borne diseases, the ethnoveterinary use of *Commiphora swynnertonii* exudate for the control of ectoparasite in livestock. The toxicity of the *C. swynnertonii* exudate was assessed using animal models. It also covers the statement of the research problem, research objectives and the significance of the study as well as data analysis methods.

1.1 Background Information

Ticks are ectoparasites feeding in blood of the mammals, birds, reptiles and amphibians (Ravindran *et al*., 2011; Rafique, *et al.*, 2015). They transmit diseases which are distributed throughout the world, particularly in tropical and subtropical regions (Cutullé *et al*., 2009). Although species of ticks differ among ecological regions, their impact on animals is important whenever they occur. Ticks transmit a number of diseases to human and livestock being bacterial, viral and protozoan. Tick-borne diseases (TBD) that are of economic importance in sub-Saharan Africa are East Coast Fever (ECF), babesiosis, anaplasmosis, dermatophilosis, and cowdriosis (Kalala *et al.*, 2014b; Mkangara *et al.*, 2014). The effects associated with tick and tick-borne diseases (TTBD) includes economic losses due to the death of livestock, poor quality of the animal product such as hides, skin, meat, milk. Indirect economic loss occurs through the costs incurred for veterinary services and loss of labor animals (Dedkov *et al*., 2017). Losses due to TTBD tend to be lower in areas where indigenous animals, the tick vectors and the TBD have co-existed, resulting in endemic stability. Reducing the incidence of TBD leads to economic benefits such as increase in the quality of animal products, fertility and manure production (Kivaria, 2006). Losses due to TTBD in Tanzania reached US$ 364 million annually, mainly due to death of more than 1.3 million cattle; 68% caused by theileriosis, 13% by anaplasmosis, 13% by babesiosis and 6% by cowdriosis (Kivaria, 2006)

Synthetic acaricides are commonly used with promising results in the control of ticks (Ahmed, 2016). The main classes of commercial acaricides are organophosphates, carbamates, and pyrethroids (Ravindran *et al*., 2011; Kearney, 2013; Shyma *et al*., 2014; Abdisa, 2017). Apparently the spread use of the multiple and large use of synthetic acaricides led to resistant against ticks, environmental contamination, toxicity to non-target organisms and residues on animal products (Pretty and Waibel, 2005; Castro-Janer *et al*., 2010; Krishna *et al*., 2014). Thus there has been a need to development alternative acaricidal agents which are affordable, eco-

friendly, effective with less effect to non-targeted organisms and applicable to small holder farmers.

Validation of indigenous plants used by ethnic groups for the management of veterinary diseases is viewed as a plausible solution against resistance of synthetic acaricides, contamination of food and environment, unavailability, inaccessibility as well as reduced cost (Chandler *et al*., 2011; Maia and Moore, 2011). *Commiphora swynnertonii* offers best ethnoveterinary medicine and have been used by various ethnic groups in Tanzania to control livestock diseases. A study done by Nagagi *et al.*, (2016) reveal that *C. swynnertonii* exudates have trypanocidal activities against *Trypanosoma congolense*. *Commiphora swynnertonii* extracts has been reported to be used in control of ectoparasite in livestock such as ticks, lice, bed bugs and mites (Kalala, *et al*., 2014a; Mkangara, *et al*., 2014). However this study report for the first time on the acaricidal activity of *C. swynnertonii* extracts prepared using soap solution which was assumed in this study to be the most affordable for small holder farmers to control ticks.

Majority of plants contain dozens mixture of bioactive compounds of different complexities such as mucilages, tannins, phenolic compounds, alkaloids, saponins, flavonoids and terpenes of which modify and modulate the effects of active compounds (Tamilselvan *et al.*, 2014). Pharmacological and toxicity effects of the *C. swynnertonii* exudates need to be studied in order to validate its uses. Plants extracts should not only be efficacious but safe for both human and livestock use (Zhang, 2004; Bulus *et al*., 2011). Therefore medicinal plants have to be scientifically evaluated for its possible short and long terms toxicology and efficacy in order to validate its use.

1.2 Research Problem and Justification

Tick and tick-borne diseases (TTBD) is a common veterinary health problem among the small holder farmers in sub-Saharan Africa (Hezron *et al*., 2012). The effects of tick infestations and other economic consequences associated with TTBD has remained a challenge to small holder farmers (Byaruhanga *et al.*, 2015). TTBD results into economic loss by affecting the quality of animal products such as meat, milk, hides and skin (Riaz and Ullah, 2017). To overcome TTBD challenge farmers depend extensively on the use of synthetic acaricides to control ticks. Despite of promising results through the use of synthetic acaricidal agents, unaffordability of veterinary services and reduced susceptibility of ticks to some synthetic acaricides leads to serious loss of cattle among the poor small holder farmers. Thus there has been a need to develop an alternative acaricidal agents which are affordable, eco-friendly, effective and applicable to rural small holder farmers where the plants are endemic.

The current work was aiming at Investigation of acaricidal activity of soap solution extract from *Commiphora swynnertonii* stem bark exudate on *Rhipicephalus appendiculatatus* and its oral toxicity effects on mice and rats.

1.3 Research Objectives

1.3.1 General Objective

The main objective of this study was to evaluate the acaricidal efficacy of *C. swynnertonii* stem bark exudate soap solution extract against *R. appendiculatus* and evaluation of its toxicity levels.

1.3.2 Specific Objectives

i. To evaluate mortality rates of *R. appendiculatus* treated with *C. swynnertonii* exudate soap solution extract.
ii. To evaluate index of laying eggs of *R. appendiculatus* treated with *C. swynnertonii* exudate soap solution extract.
iii. To evaluate fecundity of eggs laid by *R. appendiculatus* treated tick with *C. swynnertonii* exudate soap solution extract.
iv. To evaluate acute and sub-acute oral toxicity of *C. swynnertonii* stem bark exudate against rats and mice.

1.4 Hypothesis

i. *Commiphora swynnertonii* exudate soap solution extract have no significant mortality activity against *R. appendiculatus*.
ii. *Commiphora swynnertonii* exudate soap solution extract have no significant effect on index of laying eggs of *R. appendiculatus*.
iii. *Commiphora swynnertonii* exudate soap solution extract have no significant effect on eggs fecundity of *R. appendiculatus*.
iv. *Commiphora swynnertonii* stem bark exudate have no significant effects on acute and sub-acute oral toxicity against rats and mice.

1.5 Significance of the Study

The result obtained from this research will be used as base for development of ecofriendly and cheaper acaricidal agents suitable for small holder farmers.

CHAPTER TWO

LITERATURE REVIEW

2.1 Tick-Borne Diseases

In Africa many societies depend on livestock keeping as source of food, income and providing power for farming and other domestic benefits (Gonzo *et al*., 2014). The livestock diseases affect the livelihood of the pastoralists as they depend on those animals for income generation (Laisser *et al*., 2016). Domestic and grazed livestock have always suffered from a wide range of diseases in Africa (Gilioli *et al*., 2009). As livestock are concentrated in larger numbers, the problems of major epidemics have become more severe. The transmission of livestock diseases in Africa, specifically in the tropics is contributed by favorable temperature, humidity and precipitation (Jabbar *et al.,* 2015). The diseases which are common in livestock keeping include East coast fever (ECF), trypanosomosis, babesiosis, anaplasmosis, dermatophilosis, cowdriosis, ectoparasite, diarrhea, fungal, bacterial and viruses (Uilenberg, 1995; Kalala *et al*., 2014b; Mkangara *et al*., 2014; Vudriko *et al*., 2016).

Tick and tick-borne diseases (TTBD) cause death of livestock, elevate cost of veterinary services, reduce quality and quantity of the animal product, loss of labor animals and lower reproduction capability (Kivaria, 2006; Ilham *et al.,* 2014; Mkangara *et al*., 2014). Reducing the incidence of TTBD leads to economic benefits such as increasing the quality of animal products, fertility, lowers cost for veterinary services and increased labor animal efficiency and manure production (Kivaria, 2006).

Farmer in the local areas through inherited knowledge since ancient use plants parts to control or treat the livestock diseases (Adedeji *et al*., 2013). The small holder farmers living in marginal areas which are endemic to pathogens, vectors and diseases are the one which are more affected (Jabbar *et al*., 2015; Region *et al*., 2017). The livestock suffers from the diseases as the conventional veterinary services are less available due to remoteness or an affordability by small holder farmers (Ajayi *et al*., 2012). The only solution for the small holder farmers who are not accessing the modern veterinary service is to depend on their ethnoveterinary information to manage the livestock diseases (Kubkomawa *et al*., 2013).

2.2 Use of Synthetic Acaricides to Control Ticks

For many years, tick infestations has been controlled through the use of synthetic acaricides usually suspended in water (Brito *et al*., 2011). The synthetic acaricides have been reported to

be problem in the world due to unlimited control in use, multiple application, wrong dilution, poor application methods, improper frequency of application as well as the use of substandard synthetic acaricides. The practices results in to the development of resistance to ticks, environmental contamination, residues in the animal products and affect untargeted organisms (Mugisha *et al*., 2005; Thullner *et al.*, 2007; Abbas *et al*, 2014; George *et al*., 2014; Shyma *et al*., 2014; Nene *et al*., 2016; Vudriko *et al*., 2016; Yessinou *et al*., 2016). Acaricides residues in in animal products is a growing concern for producers, traders, and consumers in many parts of the world (Pretty and Waibel, 2005; Castro-Janer *et al*., 2010; Krishna *et al*., 2014). Several strategies have been established to reduce the use of synthetic acaricides, one of them is the use of botanicals acaricides which have less adverse effects (Dadang and Prijono 2009). Botanical acaricides which contain plant extracts as active components, are safer as well as environmentally friendlier than synthetic acaricides.

2.3 Use of Plants to Control Diseases in Livestock

Individuals from different cultures use ethnoveterinary information in the prevention of livestock disease (Damtew, 2012). Ethnoveterinary information has been incorporated by pastoralists and is therefore part and parcel of their livestock diseases management measures as the society accept its efficacy (Fajimi and Taiwo, 2005; Corrigan *et al.*, 2011). The emergence and spread of microbes resistant to conventional drugs, concerns over chemical residues in food product and the environment, as well as ever increasing cost of modern drugs are contributing factors which has led for search of botanicals as an alternative approach for management of livestock diseases (Rahman *et al*., 2008; Nalubega *et al.*, 2012; Usman *et al*., 2014).

Plants with medicinal values have been used in management of various ailment in livestock farming. In livestock keeping *C. swynnertonii, Taarindus indica, Euphobia tirucalli* and *Acasia nilotica* have been used in management of endoparasites and ectoparasite in cattle, donkey and other small ruminants (Luseba and Tshisikhawe, 2013). In poultry production, *Aloe vera, Solonum incanum, capsicum frutescens* are used to manage coccidiosis, Newcastle and worms (Masola *et al*., 2009; Adamu *et al*., 2012; Adedeji *et al*., 2013; Mwatawala and Mlinjanga, 2016).

2.4 Screening of Medicinal Plants

Plants are used as source of food for both human beings and animals due to its diversity, they are also used for health beneficial as they are offering the primary health care (Jothy *et al*.,

2011; Sandu *et al*., 2012). There is an increasing demand for users of medicinal plants as an alternative to clinical therapy and the demand for these remedies has currently increased (Sini *et al.*, 2010). The increase in number of users as opposed to the scarcity of scientific evidence on the safety of the medicinal plants, have been raised regarding toxicity and detrimental effects of these remedies (Saad *et al*., 2006; Alam *et al*., 2011; Udayabhanu *et al.*, 2014). The studies of medicinal plants using scientific approaches showed that various biological components of medicinal plants consists of different chemical properties which can be used to treat various ailment (Sini *et al.*, 2010). The popularity of plants remedies was contributed by its easy availability, therapeutic efficacy and relatively low cost (Castro *et al*., 2009; Jaradat *et al*., 2016). The wide spread public opinion is that being a natural product remedies are harmless and safe even if the expected efficacy is not met, as compared to synthetic drugs (Park *et al*., 2010; Rodrigues *et al*., 2010).

Most of the plants remedies are prepared in water or in ethanol and then ingested or applied externally hence its efficacy and potential toxicity employed is not known (Bussmann *et al*., 2011; George, 2011). Variety of study have reported that potentially bioactive compounds have been isolated from only few plants and tested, while a lot of plant not yet screened and tested for its potential toxicity (Tamilselvan *et al*., 2014). Among the challenges facing herbal remedies are due to confusion in nomenclature and inaccurate identification of the medicinal plants due to poor documentation (Said *et al.*, 2002; Lin *et al*., 2009). The distribution of active components in plants is not even, the concentration of the active components vary depending on which part of the plants harvested, maturity status of plants, sample preparation methods and stability of the active compounds (Saad *et al.*, 2006). However geographical location, soil contaminant composition, variation in soil acidity, variation of the weather and other growth factors can influence the composition of active components of plants remedies (Carson *et al*., 2006; Jayanthy *et al.,* 2013). The effect of plants remedies administered to livestock can be affected by various factors depending on the age of the animals and ability to metabolize the active ingredients which varies between one organism and another (Ben-Arye *et al*., 2016). However, other related parameters that contribute in variation of effects in livestock and human using herbal remedies includes variation in genetics, associated diseases, concurrent use with other drugs and the quality of the herbal (Jayanthy *et al*., 2013).

Most of the available remedies have no clear information on the content or medical information which have been validated by the recognized bodies (Mohammad, 2012), therefore its safety is still questionable (Ernst and Pittler, 2002; Alam *et al*., 2011). The internal or ectopic

applications of medicinal plants are associated with risk for both human and livestock (Nyigo *et al*., 2015).

Due to complexity of components of medicinal plants it is important to determine its safety level, efficacy and quality in order to validate its use (Kabubii *et al*., 2015). Plants have complex mixture of terpenes, alkaloids, saponin, mucilages, tannins, phenolic compounds, alkaloids and flavonoids. The mixture of this chemicals can increase the risk and adverse reactions to them or can increase the efficiency by synergistic effects (Hammer *et al*., 2006). Toxicity screening gives potential health information to help in selection of appropriate medicinal plants for both livestock and human being. The number of publications reporting on toxicity potentials of various medicinal plants are increasing enormously (Ernst, 2000).

2.5 Distribution and Uses of *Commiphora* species

Commiphora species are native to East Africa, found in Tanzania, Kenya, Uganda, Ethiopia and Somalia (Birkett *et al*., 2008). The species are small shrub tree with thorny branches which produces yellowish gum resin. To collect resin, incision is made on the bark of the tree with sharp object (Sarup *et al.*, 2015). The plants have been used since biblical times as medicine, healing injuries, and purification of rituals in women or as a perfume (Hanus *et al*., 2005).

Commiphora leptophloeos is a plant species usually known for its medicinal values and the use against *S. aureus*, *Mycobacterium smegmatis*, *Mycobacterium tuberculosis* have been documented (Pereira *et al.*, 2017). *Commiphora kerstingii* stem bark extract has antimicrobial activity against *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Pseudomonas fluorescens and Bacillus subtilis* (Musa, 2008). However Bakari (2012) revealed the potential of *Commiphora swynnertonii* in controlling gram positive and negative bacteria, fungi and virus.

Antioxidant activities are a possible mediators in protection against myocardial necrosis, inhibition of platelet aggregation, as well as increased fibrinolysis (Sarup *et al*., 2015). The *in vitro* antioxidant activity carried out using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) and nitric oxide assays reveal activities of *commiphora* species (Musa, 2008; Fraternale *et al*., 2011; Ibrahim *et al*., 2016; Mahboubi and Kazempour, 2016; Vani *et al*., 2016).

The anti-inflammatory activity of *Commiphora* was explained to be due to presence of flavonoids metabolites which acts by inhibiting production of prostaglandin (signaling molecule) and phosphosdiesterases involved in cell activation. This activity depend upon

biosynthesis of protein cytokines that mediate migration and diapedesis of circulating leucocytes to site of injury (Su *et al*., 2011; Compaoré *et al*, 2016). The extract from *Commiphora molmol* found to decreased body weight, normalized the high levels of blood lipids and decreased atherogenic index low-density lipoprotein/ high-density lipoprotein in obese hyperlipidemia rats (Francis *et al.*, 2004; Sivakumar *et al.*, 2008; Shalaby and Hammouda, 2014).

Commiphora swynnertonii has anti ectoparasitic and repellency activities against lice, mosquito, ticks, fleas, trypanosome and mites (Kalala *et al.*, 2014b; Mkangara *et al.*, 2015; Nagagi *et al*., 2016; Edwin *et al*., 2017). The effects is suggested due to the presence of sesquiterpenes hydrocarbons present in greater amount which is responsible for repellency activities (Birkett *et al*., 2008). Some of the *Commiphora species* has larvicidal, lowering oviposition and repellent activity against arthropods species such as mosquitoes (Deepa *et al*., 2015; Baranitharan *et al*., 2016).

2.6 Phytochemistry of *Commiphora* species

Plant derived substances have recently become of great interest owing to their importance and broad applications. Medicinal plants are the richest bio-resource of drugs in medicine, food supplements, and chemical entities for synthetic drugs (War *et al*., 2012). Plants tend to synthesize secondary metabolites which constitute phenols or their oxygen-substituted derivatives (Iriti and Faoro, 2009). In many cases, these substances serve as plant defense mechanisms against predation by higher animals, infestation by microorganisms and insects (Kunz and Kemp, 1994; Chandler *et al*., 2011; Jaiswal *et al*., 2013; Kant *et al*., 2015). *Commiphora* species resins have been found to contain terpenes, esters, cumunic aldehyde, eugenol alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, steroids, resin acids and phenolics and steroids (El Ashry *et al.*, 2003; Hanus *et al*., 2005; Anurekha and Gupta, 2006; Musa, 2008; Bissinger and Roe, 2010; Krishna *et al.*, 2014; Shyma *et al*., 2014). In addition, ethanolic extract of *Commiphora africana* was shown to contain phenolic compounds and tannins, whereas the hexane extract contain alkaloids, triterpenes and sterols (Adebayo *et al*., 2006; Aliyu *et al*., 2006). *Commiphora swynnertonii* consist of tannins, alkaloids, terpenes, flavonoids, steroids, and saponin (Musa, 2008; Bakari, 2013; Ibrahim *et al.*, 2016). The concentration of phytochemical compounds varies between species, season of the harvest, and geographical location. The phytochemical composition of some of *Commiphora* species are shown in Table 1.

Table 1: Phytochemical constituents of some of *Commiphora* species

***Commiphora* species**	**Compounds reported**
Commiphora flaviflora	Mansumbinone, mansumbionic acid, picropolygamain, lignin-1(methoxy 1,2,3,4-tetrahydropolygamain)
Commiphora africana	Dihydroflavonol glucoside – phellamurin
Commiphora angolensis	Condensed tannins found in powdered bark
Commiphora dalzielli	Dammarene, triterpenes, lupeol and βamyrin
Commiphora merkeri	Pentacyclic triterpene antiinflammatory activity, 2α, 3β, 2,3trihydroxylean-12-ene.

Source: Hanus *et al.* (2011)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials Collection

3.1.1 Description of the Study Area

Plant materials were collected from Mererani village, Simanjiro District in Manyara region while ticks were collected from Monduli Juu village, Monduli District in Arusha rfegion Tanzania. Simanjiro District is located 3^0 34S' to 36^0 59E' at an altitude of 980m above sea level, while Monduli District is located 03°17S' to 036°26E' at 1557m above sea level.

3.1.2 Climate

The Climatic condition of Simanjiro and Monduli Districts are characterized by semi-arid with annual rainfall 500mm per year. In Simanjiro District the area is covered by closed thick forest around the mountains with woody lands which are mainly acacia woodlands.

3.2 Plant Materials

The *C. swynnertonii* plants was identified by botanist from Tropical Pesticide Research Institute (TPRI) Arusha Tanzania with voucher specimen number CS 001 and deposited at Nelson Mandela African Institution of Science and Technology. The exudate were collected in bottle by incision of the stem bark with knife. Then it was transported in cool box to Nelson Mandela African Institution of Science and Technology laboratory and stored at 4℃ until use.

3.3 Preparation of Plants Extract

The extraction was done by using 10 mg/ml of a commercial powder soap OMO (purchased from Uniliver Kenya Ltd) prepared using distilled water (Dadang and Prijono, 2009). The following dilutions of exudate soap solutions were prepared: 100, 90, 80, 70, 60, 50, 25 and 12.5 mg/ml.

3.4 Ticks Collection

Ticks were collected from small holder farmers and stored in a small bottle closed with muslin cloth to prevent from escape and transported to TPRI for Identification. After Identification the ticks *R. appendiculatus* were fed on rabbit to engorge (Miller *et al.*, 2002; White *et al.*, 2004). Ticks were incubated in biological oxygen demand (BOD) incubator at 28 ± 2° C and relative humidity (RH) 80% to lay eggs, then the eggs were incubated until they hatch. The hatched larvae were fed and then molt to nymph, after feeding nymph molt to adult and continue fed

until engorged. The engorged female ticks were transported to NM-AIST laboratory where it was washed with distilled water and dried in by absorbent paper ready for the experiment as described by George *et al.* (2004) and Ravindran *et al.* (2014) with minor modification.

3.5 Adult Immersion Test (AIT)

A total of 216 adult engorged female ticks were randomly assigned into a control and eight treated groups, in four replicate each containing six ticks. The weight of each replicate of the female engorged ticks was recorded before commencing experiment. Each replicates was immersed to respective concentration (10 ml) of exudate extract at room temperature for 2 min in a 50 ml beaker with a gentle agitation according to Drummond *et al.* (1973) and Ravindran *et al.* (2011) with minor modifications and the untreated group was immersed in soap solution (10mg/ml). Then the ticks were placed in a petri dish over whatman filter paper number 1 and covered to prevent escape. The treated and untreated petri dishes were kept at room temperature for 24 hours. Then all petri dishes were incubated in biological oxygen demand (BOD) incubators at 28±2°C and relative humidity 85±2 %. The mortality of the ticks was confirmed by loss of mobility and pedal reflex after exposing to light. The mortality was observed and recorded for the period of 15 days then percentage mortality was determined (Drummond *et al.*, 1973).

3.6 Index of Laying Eggs

Following 15 days, the weights of laid eggs for both treated and untreated group were recorded. The Index of laying eggs (IE) is given by the following relation;

$$IE = \frac{Weight of\ the laid\ eggs(g)}{Weight of\ the\ females(g)}$$

The percentage inhibition of laying eggs is given by the relation;

$$Percentage Inhibition of\ laid\ eggs = \frac{IE\ untreated\ group - IE\ treated\ group}{IE\ untreated\ group} x100$$

3.7 Evaluation of Fecundity

The laid eggs for both treated and untreated group were incubated at 28 ± 2ºC for 30 days to estimate percentage hatching. The number of dead and live larvae, and unhatched eggs were determined and percentages hatched eggs were calculated. Estimated reproductive factor (ERF)

and inhibition of reproduction (IR) were calculated using the formula below (Drummond *et al*., 1973).

$$ERF = \frac{20{,}000\,XY}{Z}$$

Where: 20,000 average number of eggs per gram

X= weight (g) of the eggs produced

Y= estimated percentage hatchability of the eggs

Z= weight (g) of the females

$$\%IR = \frac{ERF\,untreated\,group - ERF\,treated\,group}{ERF\,untreated\,group} x100$$

IR = Inhibition reproduction

3.8 Toxicity Assay

3.8.1 Preparation of the Doses

Dimethyl Sulphoxide (DMSO) manufactured by Avantor Performance materials India Limited was used to prepare various concentrations. All concentrations were prepared in 10% DMSO in distilled water. For acute toxicity, the concentrations of 500, 1000 and 3000 mg/Kg body weight were used while 250, 500 and 1000 mg/Kg body weight were used for sub-acute toxicity. The test concentrations (doses) were selected based on Hodge and Sterner scales (Ahmed, 2015)

3.8.2 Experimental Animals

Acute and sub-acute oral toxicity test was performed as per Organization for Economic Co-operation and Development number 407 guidelines (OECD, 2001). All experiments were carried out in accordance with ethical guidelines of the Sokoine University of Agriculture (SUA) Morogoro, Tanzania. Swiss albino mice and rats of both sexes and non-pregnant were randomly collected from SUA Morogoro, Tanzania. The age of mice and rats ranged between 8-12 weeks while weights of mice were between 23.8 and 35.4g and that of rats were 80.33 to 221g. All animals were housed in the wire marsh cages, supplied with synthetic diet grower marsh (Manufactured by Harsho Trading Co Ltd Moshi, Tanzania) and water *ad libitum*. In addition, room temperature was maintained at 28 ± 5°C and the lighting was controlled to

supply 12 hours of light and 12 hours of darkness for each 24 hours period. Before dosing, animals were acclimatized for 7 days and their body weights were determined.

3.8.3 Experimental Design for Acute Toxicity

Sixteen mice were randomly assigned into a control and three treated groups each containing two males and two females. Animals were kept fasting overnight and their body weight was recorded prior to dose administration. Mice in control group were administered with 10% (DMSO) while treated groups received doses of 500, 1000 and 3000 mg/Kg body weight. The doses were given orally as a single dose through oral gavage (Jones, 2015). Food and water were suspended for additional three hours post dose administration. After dose administration mice were observed individually for any physiological or behavioral changes during the first 30 min, periodically for the first 24 hours (with special attention given during the first 4 hours), and daily thereafter for a period of 14 days. At the end of the experiment, the body weight of each mice was recorded.

3.8.4 Experimental Design for Sub-Acute Toxicity

A total of 24 rats were randomly assigned into a control and three treated groups, each containing three males and three females. The control group were given carrier substance a 10% DMSO in distilled water by oral route, daily for a period of 28 days while treated groups administered dose of 250, 500 and 1000 mg/Kg body weight. After dose administration mice were observed individually during the first 30 min, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter for a period of 28 days. The body weights of animals were recorded after 28 days of experimentation period prior sacrifice.

3.8.5 Blood Analysis

At the end of the 28^{th} sub-acute toxicity study, blood samples (approximately 1.3 ml) were collected in ethylene diamine tetra acetic acid (EDTA) vacutainer tube from orbital sinus to perform hematological tests in an ABX micros 60 automated hematology analyzer (manufactured by HORIBA ABX-USA). Similarly blood samples (approximately 2 ml) were collected in plain vacutainer tube and serum was obtained by centrifuging at 3000 rpm for 10 min to perform biochemical tests (by UV-2800 Spectrophotometer manufactured by Unico USA).

3.8.6 Hematology Assays

White blood cell (WBC), Red Blood cell (RBC), Red Cell (erythrocyte volume) Distribution Width (RDW) and hemoglobin (Hb) were evaluated for both control and treated groups of rats.

3.8.7 Biochemical assays

Total protein, albumin, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined in all groups.

3.8.8 Histopathology

All the rats were anaesthetized and sacrificed to examine gross pathology of visceral organs after 28 days of dose administration. The internal organs such as liver, lungs, kidneys, spleen and heart were collected and weighed (OHAUS Scout pro SPG 202F, USA). Additionally, relative organ weights of each animal in both groups was calculated. Histopathology examination of the internal organs were performed for high dose (1000 mg/kg body weight) and control group animals. Tissues were fixed with neutral formalin 10% and embedded in paraffin. Thereafter, they were manually sectioned with a microtome to obtain 4-5 μm-thick paraffin sections. Dewaxed sections were then stained with Hematoxylin and Eosin (H and E) and left to dry for about 30 minutes. The histopathological slides were examined under the microscope (Optika B 350, Italy) for different histopathological lesions.

3.9 Data Analysis

Several analytical packages were used in this study. For analyzing LC_{50} and LC_{99} for mortality of ticks Microsoft excel 2013 was used to obtain the regression equation. Ticks mortality, index of laid eggs, percentage inhibition of laid eggs hatching percentages and inhibition of hatching was carried out by Genstat software version 10 where by one way analysis of variance (ANOVA) was used to determine significance differences among doses. In toxicity study Genstat was used to group mean and standard error of the mean for comparison between control and treated animals and significance differences was assessed by ANOVA. The p values less than 0.05 was considered significant.

CHAPTER FOUR

RESULTS AND DISCUSSION

The *C. swynnertonii* stem bark exudates were tested against tick mortality in which it was found to be significant ($p<0.05$) at doses greater than 25mg/ml. The index of laying eggs was found to be significant ($p<0.05$) at dose greater than 90mg/ml whereas fecundity on the laid eggs was totally blocked in all treated groups. In acute and sub-acute toxicity study there was no significant ($p>0.05$) changes in physiological or behavioral, body weight, relative weight of the internal organs, hematology, biochemical and histopathology assays.

4.1 Acaricides Properties of *C. swynnertonii* Stem Bark Exudate

4.1.1 Mortality of Ticks

Commiphora swynnertonii stem bark exudate was evaluated for acaricidal activity against *R. appendiculatus* and the findings emanated from this study are summarized in Table 2. The tested concentrations were prepared by dissolving a known amount of *C. swynnertonii* stem bark exudate in a known volume of diluted soap solution. It was evident that a complete mortality of ticks was observed at 100 mg/ml for the period of fifteen days of the experiment. Mortalities above concentrations of 25 mg/ml was found to be significant ($p<0.05$) as compared to untreated group. The mortality of ticks using synthetic acaricides (*Alpha cypermethrin*) as manufacturer use instructions was reported to be 100 percent within two days of exposure (Musa, 2008). The highest tick mortality of the tested *C. swynnertonii* stem bark exudate occurred between day one and day nine post treatment. The lethal concentrations which can result into mortality for at least 50 and 99 percent ($LC_{50,}$ LC_{99}) of the ticks was established to be 42.9 and 92.8 mg/ml respectively (Fig. 1).

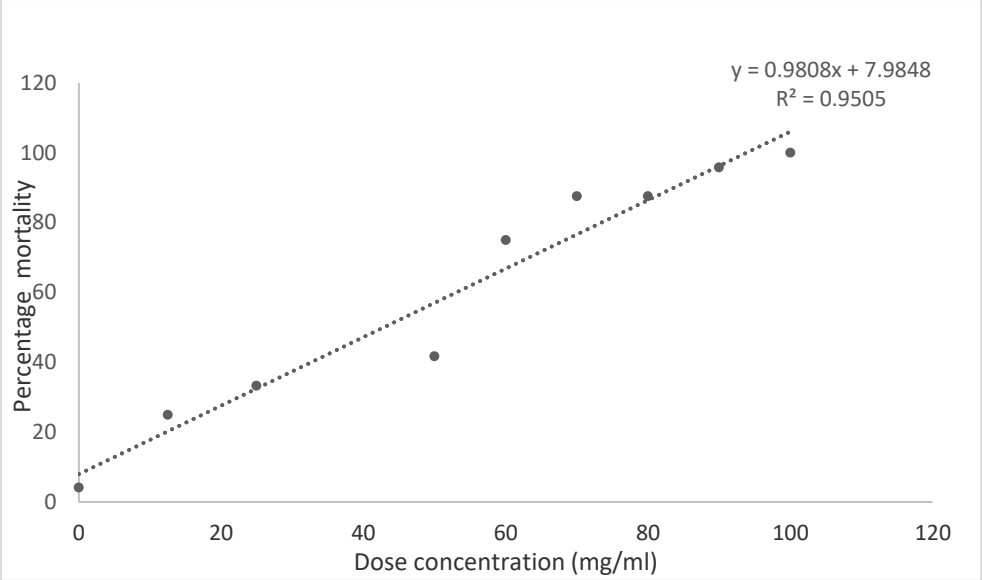

Figure 1: Effectiveness of *C. swynnertonii* stem bark exudates on tick mortality at different tested concentrations

Table 2: Effect of *C. swynnertonii* exudates against *R. appendiculatus*

Dose (mg/ml)	% Mortality	Laid eggs (g)	No. eggs incubated	Females ticks (g)	Index of laid eggs	% inhibition in laying eggs
Control	0.0^{d}	0.19^{b}	7128^{a}	1.2^{ab}	0.157^{cde}	0.0^{abc}
12.5	25^{cd}	0.31^{b}	13592^{a}	1.45^{a}	0.212^{d}	-48^{a}
25	33.3^{c}	0.1^{a}	4413^{bc}	0.67^{b}	0.187^{cd}	-32^{ab}
50	41.7^{c}	0.05^{cd}	2300^{c}	0.56^{b}	0.099^{abcd}	27.2^{bcde}
60	75^{b}	0.16^{bc}	6808^{b}	1.23^{ab}	0.128^{bcde}	15.8^{abcd}
70	87.5^{ab}	0.06^{cd}	2833^{bc}	1.07^{ab}	0.059^{abc}	62.6^{cde}
80	87.5^{ab}	0.08^{cd}	3573^{bc}	1.25^{ab}	0.064^{abc}	48.6^{cde}
90	95.8^{ab}	0.03^{d}	1250^{c}	1.63^{a}	0.025^{ab}	86^{de}
100	100^{a}	0.02^{d}	576^{c}	1.12^{ab}	0.08^{a}	91^{e}

Values are expressed as mean ± SEM, values with the same superscript within the column means do not show statistically significant differences ($p>0.05$) based on Duncan multiple comparison, % percentage.

4.1.2 Index of Ticks to Lay Eggs

Commiphora swynnertonii exudate was evaluated for its effect on ability of engorged female ticks to lay eggs. The weight of the engorged female ticks involved in the experiment was not different from each other (*P>0.05*). The engorged female ticks were treated with 12.5, 25, 50, 60, 70, 80, 90, and 100 mg/ml of *C. swynnertonii*. The weights of the laid eggs were found to decrease as tested concentration of *C. swynnertonii* stem bark exudates increased (Table 2). The weight of eggs laid by the treated engorged female ticks was found to be significantly different from those of untreated group (*p< 0.05*).

The index of laid eggs by female ticks was affected by the concentration of *C. Swynnertonii* exudates. The female ticks treated with high concentrations were found to have lower ability to lay eggs as compared to those treated with lower concentrations of the exudates. The index of laying eggs of treated engorged female ticks with *C. swynnertonii* stem bark exudates at concentration 100 and 90 mg/ml was found to have significance difference to those of untreated group ($p< 0.05$).

4.1.3 Fecundity of the Ticks Eggs

On visual examination of the incubated eggs, it was observed that all eggs laid by ticks treated with *C. swynnertonii* stem bark exudates did not hatch while those eggs laid by untreated ticks hatched (Table 3). *C. swynnertonii* stem bark exudate was found to interfere the development of eggs hence block hatching rates.

Table 3: Mean estimated reproductive factor and Inhibition of reproduction of *R. appendiculatus* engorged females subjected to different concentrations of *C. swynnertonii* stem bark exudates**.**

Dose (mg/ml)	**Untreated**	**12.5**	**25**	**50**	**60**	**70**	**80**	**90**	**100**	**P-value**
ERF ± SEM	314162.6 ± 51456.6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.001
IR ± SEM		100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	0.002

Values are expressed as mean ± SEM, ERF= Effective reproductive factor**,** IR= Inhibition of reproduction

4.2 Acute Oral Toxicity

No mortality and signs of toxicity were observed in both groups of mice in control and treated with *C. swynnertonii* stem bark exudates up to 3000 mg/Kg body weight during the 14 days observation period. The general behavior and physiological activities of the mice were found to be normal throughout the study period (Table 4). The Lethal dose required to cause mortality of 50 % of the tested animals (LD_{50}) was found to be above 3000 mg/Kg, as there was no mortality observed up to this dose. Additionally, there was a gradual increase in body weight of both control and treated mice (Table 5).

Table 4: Effects of oral administration of *C. swynnertonii* stem bark exudates on physiological and behavior change in mice

Parameter	**Doses (mg/Kg body weight)**			
	Control	**500**	**1000**	**3000**
Eye lid closure	-	-	-	-
Difficulty in breathing	-	-	-	-
Change in skin fur	-	-	-	-
Eyes mucus membrane	-	-	-	-
Sleep	-	-	-	-
General weakness	-	-	-	-
Loss of appetite	-	-	-	-
Diarrhea	-	-	-	-
Excitement	-	-	-	-
Mortality	nm	nm	nm	nm

Keys: – = no physiological/behavior changes, + = observed physiological/behavior changes, nm = no mortality

Table 5: Effects of oral administration of *C. swynnertonii* stem bark exudates on body weight (g) in mice

Dose (mg/Kg)	Sex	Mean weight at day 0	Mean weight at day 14	p- value
Control	M	31.26 ± 1.55	32.6 ± 2.17	0.587424
	F	27.4 ± 1.58	29.18 ± 1.32	0.228511
500	M	28 ± 1.82	29.64 ± 1.49	0.385250
	F	24.8 ± 1.72	26.2 ± 1.34	0.391518
1000	M	35.4 ± 1.35	37.57 ± 0.69	0.32142
	F	23.8 ± 1.76	25.44 ± 0.94	0.36085
3000	M	30.74 ± 1.5	32 ± 0	0.294342
	F	28 ± 2	29.26 ± 1.40	0.545162

Values are expressed as mean ± SEM, $p < 0.05$ values are considered significant

4.3 Sub-acute Toxicity

There was no mortality observed in both treated and control groups within 28 days of oral administration of *C. swynnertonii* exudates. The general behavior and physiological activities of the rats were found to be normal throughout the study period. Both controls and treated groups were observed to be relatively healthy throughout the study.

4.3.1 Body Weight

There was gradual increase in body weight from day 0 to day 28 for both treated and control groups of rats. However, the increase in weights for both groups revealed no statistically significant difference ($p>0.05$) as shown in Table 6.

Table 6: Effects of oral administration of *C. swynnertonii* stem bark exudates on body weight (g) in rats

Dose mg/Kg	Sex	Mean weight at day 0	Mean weight at day 28	p- value
Control	M	221.67 ± 0.9	223.67 ± 11.84	0.753062
	F	102.33 ± 0.9	111.67 ± 0.88	0.102705
250	M	145.67 ± 9.5	180 ± 8.66	0.056006
	F	133 ± 2.3	153 ± 9.24	0.103604
500	M	170.67 ± 28	181.67 ± 19.34	0.762721
	F	133 ± 2.3	134 ± 5.20	0.153462
1000	M	168.67 ± 4.9	179 ± 3.46	0.160631
	F	80.33 ± 0.3	91.67 ± 3.76	0.139738

Values are expressed as mean ± SEM, $p< 0.05$ values are considered significant

4.3.2 Relative Organ Weight

Results of relative organ weights of rats treated with *C. swynnertonii* exudates are shown in Table 7. In this study, there were significant differences ($p<0.05$) in heart and lungs for female rats at doses of 250 and 1000 mg/kg body weight respectively.

4.3.3 Hematological Parameters

Results on hematological parameters are shown in Table 8. It was revealed that there were a significant difference ($p<0.05$) in Hb for treated male rats with *C. swynnertonii* exudates at dose of 1000 mg/kg body weight.

Table 7: Effects of oral administration of *C. swynnertonii* stem bark exudates on relative internal organs weight (g) in rats

	Dose (mg/Kg)	Kidney	Liver	Lungs	Spleen	Heart
Male	Control	0.00393 ± 0.000928^{ab}	0.028 ± 0.00681^{a}	0.00543 ± 0.0014^{a}	0.00253 ± 0.000809^{a}	0.0039 ± 0.000601^{a}
	250	0.0067 ± 0.00055^{ab}	0.0400 ± 0.002^{ab}	0.0053 ± 0.000907^{a}	0.00348 ± 0.00136^{a}	0.0056 ± 0.000289^{ab}
	500	0.00567 ± 0.000611^{ab}	0.033 ± 0.00404^{ab}	0.00503 ± 0.00118^{a}	0.00361 ± 0.000647^{a}	0.00473 ± 0.00147^{ab}
	1000	0.005 ± 0.000503^{ab}	0.039 ± 0.00404^{ab}	0.0056 ± 0.000113^{a}	0.00314 ± 0.00093^{a}	0.0056 ± 0.000115^{ab}
Females	Control	0.00653 ± 0.00264^{ab}	0.045 ± 0.00551^{b}	0.00893 ± 0.0000881^{ab}	0.00421 ± 0.0011^{a}	0.00806 ± 0.00083^{c}
	250	0.0038 ± 0.000702^{a}	0.042 ± 0.00458^{b}	0.01317± 0.000809^{bc}	0.00459 ± 0.00033^{a}	0.00493 ± 0.00119^{ab}
	500	0.00427 ± 0.00101^{ab}	0.0437 ± 0.00318^{b}	0.01362 ± 0.00284^{bc}	0.00384 ± 0.000416^{a}	0.00677 ± 0.000667^{bc}
	1000	0.00773 ± 0.00198^{b}	0.0437 ± 0.00176^{b}	0.01423 ± 0.0029^{c}	0.00296 ± 0.000463^{a}	0.00435 ± 0.000565^{ab}

Values with the same superscript within the column means do not show statistically significant differences based on Duncan Multiple comparison, mean ± SEM

Table 8: Effects of oral administration of *C. swynnertonii* stem bark exudates on hematological parameters in rats

Sex	Dose (mg/Kg)	WBC (M/mm^3)	RBC (M/mm^3)	RDW	Hb (g/dl)
Male	Control	0.7± 0.22^a	4.9 ± 1.67^a	7.617 ± 2.3^a	10.2 ± 3.72^a
	250	5 ± 1.98^a	5.9 ± 0.69^a	23.47 ± 6.9^a	14.1 ± 0.98^{ab}
	500	5.3 ± 1.37^a	4.7±1.55^a	30.13± 10.7^a	12.9 ± 2.22^{ab}
	1000	1.8 ± 0.28^a	6.6 ± 0.12^a	12.5± 0.4^a	15.7 ± 0.40^b
Female	Control	2.9 ± 0.07^a	5.8± 0.25^a	11.3 ± 0.21^a	13.1±0.58^{ab}
	250	2.7 ± 0.12^a	5.7 ± 0.2^a	11.3 ±0.34^a	11 ± 1.16^{ab}
	500	2.8 ± 0.09^a	5.6 ±0.23^a	11.3 ± 0.21^a	13.1± 0.55^{ab}
	1000	2.9 ± 0.08^a	5.8 ±0.29^a	11.2 ±0.17^a	13.7 ± 0.40^{ab}

Values with the same superscript within the column means they are not show statistically significantly different ($p>0.05$) based on Duncan multiple comparison, mean ± SEM, WBC=White blood cell, RBC=Red blood cells, RDW=Red cell distribution width, Hb=Hemoglobin

Table 9: Effects of oral administration of *C. swynnertonii* stem bark exudates on biochemical parameters in rats

	Dose (mg/Kg)	**Total protein (mg/dl)**	**Albumin (mg/dl)**	**Triglycerides (mg/dl)**	**ALT (mg/dl)**	**ALP (mg/dl)**	**AST (mg/dl)**
Male	Control	7.24 ± 0.11^{b}	3.24 ± 0.42^{ab}	94.02 ± 12.91^{a}	35.4 ± 3.4^{a}	59.55 ± 10.76^{a}	72.53 ± 2.21^{b}
	250	7.95 ± 0.26^{c}	3.53 ± 0.05^{b}	11.1 ± 6.14^{a}	35.23 ± 1.01^{a}	33.41 ± 4.0^{a}	54.36 ± 0.94^{a}
	500	7.89 ± 0.09^{c}	3.31 ± 0.42^{ab}	102.87 ± 15.34^{a}	28.56 ± 8.48^{a}	53.97 ± 23.36^{a}	55.3 ± 3.21^{a}
	1000	7.19 ± 0.05^{ab}	2.6 ± 0.02^{a}	94.61 ± 6.31^{a}	30.43 ± 4.31^{a}	73.57 ± 19.92^{a}	73.17 ± 5.89^{b}
Female	Control	6.82 ± 0.17^{a}	2.8 ± 0.15^{ab}	94.07 ± 6.72^{a}	31.12 ± 5.22^{a}	46.81 ± 6.7^{a}	48.07 ± 0.58^{a}
	250	7.13 ± 0.09^{ab}	2.93 ± 0.09^{ab}	93.13 ± 5.75^{a}	31.13 ± 4.36^{a}	44.5 ± 7.43^{a}	69.6 ± 4.35^{b}
	500	7.13 ± 0.09^{ab}	3.0 ± 0.02^{ab}	93.43 ± 6.81^{a}	28.4 ± 2.52^{a}	49.24 ± 8.71^{a}	72.13 ± 5.23^{b}
	1000	7.10 ± 0.06^{ab}	3.03 ± 0.07^{ab}	102.59 ± 2.34^{a}	30.4 ± 4.84^{a}	41.77 ± 7.80^{a}	68.7 ± 4.35^{b}

Values with the same superscript within the column means do not show statistically significant differences ($p>0.05$) based on Duncan multiple comparison, mean ± SEM, ALT=Alanine aminotransferase, ALP=Alkaline phosphatase, AST=Aspartate aminotransferase

4.3.4 Biochemical Parameters

Results on biochemical parameters in this study are shown in Table 9. It was revealed that there were a significant difference ($p<0.05$) in Total protein and AST for both control and treated groups of male rats treated with *C. swynnertonii* exudates at 250 and 500mg/Kg doses.

4.3.5 Histopathology Findings

Histopathological findings of organs namely liver, heart, kidney, lungs and spleen in a group of rats treated with high dose (1000 mg/Kg body weight) of *C. swynnertonii* exudates are shown in Fig. 2. It was revealed that, there were no lesions observed in both control and treated animals.

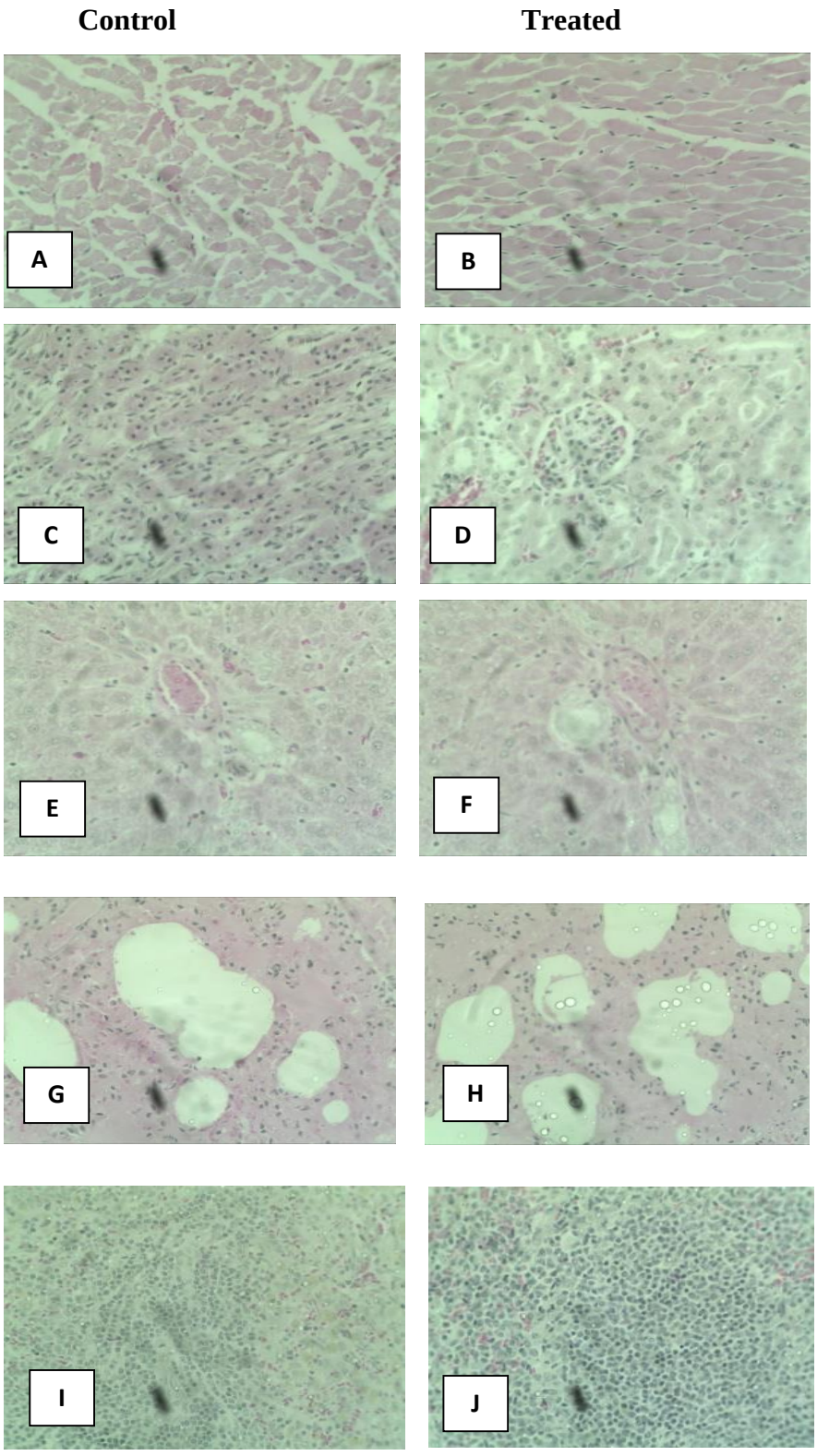

Figure 2: Microscopic examination of stained sections of A, B: Heart, C,D: Kidney, E,F: Liver, G,H: Lungs and I,J: Spleen of rats at magnification of 40X

4.4 Discussion

There is an increase interest on the use of botanicals especially by smallholder farmers to control ectoparasite as they are found to be relatively cheaper, eco-friendly, effective, readily available and safe (Shyma *et al*., 2014). Plants contain range of chemically active ingredients which can intervene all biological process of the organisms, interrupting its life cycle and its dispersal (Habeeb, 2010). In the current study *C. swynnertonii* stem bark exudate was evaluated for acaricidal activity against *R. appendiculatus*. The findings showed that *C. swynnertonii* exudate induces tick mortality, lowers ticks ability to lay eggs and inhibit the viability of laid eggs.

The mortality of the ticks when immersed in the *C. swynnertonii* stem bark exudates suggest that there was a contact effect of the exudates. At higher concentration the effect of the exudate was immediate while at lower concentration the effect was taking place slowly as the residual on the body stimulate effect. The variation in mortality based on concentration was attributed by the amount of the exudates in contact with body and penetrates to the inner cells. The amount of bioactive compounds of the exudate penetrates the inner cell is proportional to mortality caused thus exudates with higher concentration results into mortalities earlier than those with lower concentrations. However higher concentration is recommended to be used as lower concentration may result into resistance especially when the activity is not taking place shortly. The mode of action was similar to that of synthetic acaricides pyrethroids where it produces hyper excitation tremors and paralysis to the organism followed by mortality (Shyma *et al*., 2013). The nerve excitations occurs as a result of change in membrane permeability to sodium and potassium ions (Krishna *et al*., 2014). The use of detergent (OMO) is recommended as it has lipophilic properties which can remove the outside waxy cuticle layer of the ticks and allow the bioactive compounds to penetrate to the inner cells. Bioactive compounds revealed to interfere with basic metabolic, biochemical, physiological and behavioral functions of the ticks which results into mortality. The active components presents in the plant exudate is suggested to cause sterility in female ticks, due to its adverse effects in ovarian development. They also suggested to suppress and reduce the rate of ovary development hence reduced number of eggs laid. The laid eggs which was impaired by the active components of the exudate during ovarian development hindering the hatching ability (Khater, 2012; Shyma *et al*., 2014).

The toxicological evaluation of *C. swynnertonii* exudate did not reveal any sign of toxicity or mortality present in the stem bark exudates. The LD_{50} of *C. swynnertonii* stem bark exudates was found to be greater than 3000 mg/Kg. According to OECD 407 (2001) classifications,

Hodge and Sterner toxicity scale (Ahmed, 2015) classify compound with oral or dermal LD_{50} evaluation between 500 to 5000 mg/Kg body weight to be considered as less toxic and experimentally safe.

The body weight was found to be increasing gradually from the beginning of the experiment to the end. The increase in body weight was suggested to be contributed by improvement of nutrition as they were supplied with synthetic grower marsh and water daily. The differences in the increase in weight of the animals treated with *C. swynnertonii* compared to the untreated group was statistically not significant ($p>0.05$). Therefore it can be concluded that the stem bark exudate of *C. swynnertonii* did not influence animal weight gain or loss.

Organ weight is one of the most sensitive drug toxicity indicators, and it changes often precede morphological changes. A number of factors have been reported that may influence animal organ weights including strain of animal, age, sex and environmental and experimental conditions (Piao *et al*., 2013). Liver, lungs, heart, kidney and spleen were accessed because of their relevance in metabolic, inflammatory and neurodegenerative processes. Overall this organs increases in weight over aging in rats and mice up to 36 weeks. However in this study the changes in relative organ weights of heart and lungs in some group of treated animals was contributed with the aging of the animals as the relative weight was within the range of their age.

Hematology assessment parameter is important in determination of any adverse effect of toxins taken orally (Adeneye *et al*., 2006). Analysis of blood parameters therefore assist in evaluation of any changes in some hematological parameters such as WBC, RBC, RDW and Hb for prediction of toxicity (Gautam and Goel, 2014). According to Ouedraogo *et al.,* (2013) the hematological indices in animal is used to decide toxicity threat as the change in blood system had higher projecting values for animal toxicity. The variation in Hb observed in group of rats treated with 1000mg/Kg was within the standard range (Giknis and Clifford, 2008). Since the current study did not reveal any significant differences in hematological parameters for both control and treated animals with *C. swynnertonii* stem bark exudate, then therefore it is suggested that the plant exudate is safe for dermal and oral application.

In a routine health evaluation, monitoring of enzyme serum maker as biochemical changes is essential. For instance, there are several biochemical activities which occur in the liver including metabolism, degradation and synthesis (Reddy *et al*., 2013). Thus, in any toxicological study, it is very important to assess the liver and kidney enzymes functions as

these organs play a major role in the metabolism and removal of foreign substances from the body. The enzymes ALT and AST tend to be elevated whenever the conditions associated with toxicity prevails. ALT is an indicator of liver functions and biomarkers for toxicity predictions (Mukinda and Syce, 2007: Kripa *et al.*, 2011). In this study, the analyses of the serum were carried out in order to evaluate if there is any damage of liver and kidney induced due to oral administration of *C. swynnertonii* stem bark exudates. The observations indicated that, there was a significant difference in AST between female animals treated with *C. swynnertonii* exudates and untreated group ($p<0.05$). There was significance difference in protein observed between 250, and 500mg/Kg treated male rats and the untreated group. The observed differences in enzymes and proteins between treated and untreated was found to be within the normal rat range (Aleman *et al*., 2015). This indicates that liver and kidneys were functioning well even after administration of *C. swynnertonii* stem bark exudates on mice and rats.

The histopathology study of structures of all organs found that all internal organs were normal and there was no observable change in morphology between treated and untreated group. Generally histopathology was in line with body weight and organ weight index, thus this study suggests that the *C. swynnertonii* exudates have got low toxicity levels.

CHAPTER FIVE

5.1 Conclusion

The obtained results indicate that *C. swynnertonii* exudate soap solution extract is effective against control *R. appendiculatus*. The presence of factors in the exudates with acaricidal property make the plant to be valuable component of developing strategies for integrated tick management in Agriculture. Therefore zero grazing small holder farmers, in the remote settings where synthetic acaricides is unaffordable or unavailable, they can use *C. swynnertonii* exudate soap solution extract to control ticks. However this study has shown that the *C. swynnertonii* exudate does not have severe toxicological effects as there was no observable changes in physiological, behavior, body weight, and relative organ weight as well as hematological, biochemical and histopathological parameters.

5.2 Recommendations

Further studies should be conducted to determine proper formulations, and determine the mechanism of *C. swynnertonii* exudates mode of actions on ticks. Plant of *C. swynnertonii* from different parts should be studied in order to compare activities as phytochemical composition may be affected by extrinsic and intrinsic factors. Additionally on station and on field trial acaricidal efficacy including other species of ticks should be conducted to compare with laboratory results obtained. The ectopic toxicity study of *C. swynnertonii* is required to be conducted in order to evaluate any associated effects on ectopic application.

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RESEARCH OUTPUT

Toxicological evaluation of *Commiphora swynnertonii* stem bark exudates on mice and rats[1]

Abstract

The aim of this study was to evaluate the safety of *Commiphora swynnertonii* stem bark exudates by determining its acute and sub-acute toxicity in mice and rats. The *acute* toxicity of exudates was administered to mice by oral administration of a single dose of tested concentrations and observed for 14 days. The doses were 500, 1000 and 3000 mg/Kg body weight. The sub-acute toxicity test of the exudate was conducted on rats through a daily oral administration of various doses for 28 days. The doses were 250, 500 and 1000 mg/Kg body weight. The physiological, behavioral and body weight change as well as relative weight of internal organs were subsequently recorded. Hematological, biochemical and histopathological parameters for rats used in the sub-acute toxicity assay were also measured at day 28. Results showed that, administration of up to 3000 mg/Kg body weight the *C. swynnertonii* exudates in mice did not result into any observable toxicity or mortality within the period of 14 day. Furthermore, the sub-acute toxicity test revealed that the exudates did not induce mortality neither behavioral nor physiological changes after 28 days. It was further observed that, the rat's body and internal organs weight as well as hematological, biochemical and histopathological parameters did not change as results of receive the doses for the whole period of the study. Therefore this study has revealed that *C. swynnertonii* stem bark exudates is safe for oral administration at low and moderate doses.

Keywords: *Commiphora swynnertonii*, Toxicity, hematological, biochemical, histopathological

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Introduction

In recent years the use of medicinal plants as sources of alternative drugs and dietary supplement have increased in the developing countries (Bakari, 2012; Nagagi *et al*., 2016). The large population in these countries depends on medicinal plants as an alternative to modern drugs (Gautam and Goel, 2014). Due to the increase of diseases burden, disorders, side effects coupled with the costs of modern drugs, people in the developing countries switch to herbal remedies which are believed to be more safer and readily available (Alam *et al*., 2011). As a result, in the developing countries herbal medicines contribute about 80% of the human and animal health care (Bulus *et al*., 2011; Badarunisha *et al*., 2014; Mkangara *et al*., 2014).

Commiphora swynnertonii (*Burseracea*) is a small woody plant which can grow up the height of 2.5 meter (Van Wyk and Wink, 2004; Rahman *et al*., 2008). The plants is found in the arid and semi-arid environment and grows with tiny leaves and spiny (Paraskeva *et al*., 2008). *C. swynnertonii* has been reported to be used for the treatment of various human and animal diseases (Deepa *et al*., 2015; Baranitharan *et al*., 2016). Among human diseases, this plant species is used in the treatment of sexually transmitted diseases, ulcers and wounds (cut wounds and burn wounds), recalcitrant ulcers, and abscesses, swelling of legs, chesty cough and scabies (Kalala *et al*., 2014a). Previous pharmacological research have indicated that this plant species has higher antibacterial and antifungal properties (Musa, 2008; Rahman *et al*., 2008). Furthermore, *C. swynnertonii* extracts have been reported to be used in the control of ectoparasite in livestock such as ticks, lice, bed bugs and mites (Kalala *et al*., 2014b; Mkangara *et al*., 2014). Despite of these numerous reports there is a limited scientific evidence on the safety of *C. swynnertonii* stem bark exudates, when administered to animals and humans. Therefore the aim of this study was to evaluate the *in vivo* safety of *C. swynnertonii* stem bark exudates using albino mice and rats.

Materials and Methods

Plant materials

The exudates were collected from the stem bark of *C. swynnertonii* in Simanjiro District Arusha between June and October 2016. The *C. swynnertonii* plants were identified by botanist from Tropical Pesticide Research Institute (TPRI) Arusha Tanzania and its voucher specimen (CS 001) was deposited at Nelson Mandela African Institution of Science and Technology.

Preparation of the doses

Dimethyl Sulphoxide (DMSO) manufactured by Avantor Performance materials India Limited was used to prepare various concentrations. All concentrations were prepared in 10% DMSO in distilled water. For acute toxicity, the concentrations of 500, 1000 and 3000 mg/Kg body weight were used while 250, 500 and 1000 mg/Kg body weight were used for sub-acute toxicity.

Animals

Acute and sub-acute oral toxicity test was performed as per Organization for Economic Co-operation and Development number 407 guidelines (OECD, 2001). All experiments were carried out in accordance with ethical guidelines of the Sokoine University of Agriculture (SUA) Morogoro, Tanzania. Swiss albino mice and rats of both sexes and non-pregnant were randomly collected from SUA Morogoro, Tanzania. The age of mice and rats ranged between 8-12 weeks while weights of mice were between 23.8 and 35.4g and that of rats were 80.33 to 221g. All animals were housed in the wire marsh cages, supplied with synthetic diet grower marsh (Manufactured by Harsho Trading Co Ltd Moshi, Tanzania) and water *ad libitum*. In addition, room temperature was maintained at 28 ± 5°C and the lighting was controlled to supply 12 hours of light and 12 hours of darkness for each 24 hours period. Before dosing, animals were acclimatized for 7 days and their body weight were determined.

Experimental design for acute toxicity

Sixteen mice were randomly assigned into a control and three treated groups each containing two males and two females. Animals were kept fasting overnight prior to dose administration. Mice in control group were administered with 10% (DMSO) while treated groups received doses of 500, 1000 and 3000 mg/kg body weight. The doses were given orally as a single dose through oral gavage. Food and water were suspended for additional three hours post dose administration. After dose administration mice were observed individually during the first 30 min, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter for a period of 14 days. After the end of 14 days experimentation period, body weight measurements of each animal were recorded.

Experimental design for sub-acute toxicity

A total of 24 rats were randomly assigned into a control and three treated groups, each containing three males and three females. The control group were given vehicle substance of 10% DMSO by oral route daily for a period of 28 days while treated groups administered dose of 250, 500 and 1000 mg/kg body weight. After dose administration mice were observed individually during the first 30 min, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter for a period of 28 days. The body weights of animals were recorded after 28 days of experimentation period.

Blood analysis

At the end of the 28th sub-acute toxicity study, blood samples (approximately 1.3 ml) were collected in ethylene diamine tetra acetic acid (EDTA) vacutainer tube from orbital sinus to perform hematological tests in an ABX micros 60 automated hematology analyzer (manufactured by HORIBA ABX-USA). Similarly blood samples (approximately 2 ml) were collected in plain vacutainer tube and serum was obtained by centrifuging at 3000 rpm for 10 min to perform biochemical tests in semi auto biochemistry analyzer (manufactured by UV-2800 Unico Spectrophotometer-USA).

Hematology assays

White blood cell (WBC), Red Blood cell (RBC), mean cell volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell (erythrocyte volume) Distribution Width (RDW), hemoglobin (Hb), Mean Platelet (thrombocyte) Volume (MPV), and Platelet distribution width (PDW) were evaluated for both control and treated groups of rats.

Biochemical assays

Glucose, cholesterol, total protein, albumin, triglycerides, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and bilirubin were determined in all groups.

Histopathology

All the rats were anaesthetized and sacrificed to examine gross pathology of visceral organs. The internal organs such as liver, lungs, kidneys, spleen and heart were collected and weighed (OHAUS Scout pro SPG 202F, USA). Additionally, relative organ weights of each animal in

both groups was calculated. Histopathology examination of the internal organs was performed for high dose (1000 mg/kg body weight) and control group animals. Tissues were fixed with neutral formalin 10% and embedded in paraffin. Thereafter, they were manually sectioned with a microtome to obtain 4-5 μm-thick paraffin sections. Dewaxed sections were then stained with Hematoxylin and Eosin (H and E) and left to dry for about 30 minutes. The histopathological slides were examined under the microscope (Optika B 350, Italy) for different histopathological lesions.

Statistical analysis

Data were analyzed using GENSTAT (version 10 of 2014) to obtain group means and standard error of the mean (SEM) for comparison between the control and treated groups. The significance differences were assessed by one way analysis of variance ANOVA and the significant differences within the group was considered at $p<0.05$

RESULTS

Acute oral toxicity

No mortality and signs of toxicity were observed in both control and mice group treated with *C. swynnertonii* stem bark exudates at 3000 mg/Kg body weight during the 14 days observation period (Table 1). Additionally, there was a gradual increase in body weight of both control and treated mice (Table 2).

Sub-acute toxicity

There was no mortality observed in both the treated and control groups within 28 days of oral administration of *C. swynnertonii* exudates. General behavior and physiological activities of the rats were found to be normal throughout the study period. Both controls and treated groups were observed to be relatively healthy throughout the study.

Body weight

There was gradual increase in body weight from day 0 to day 28 for both treated and control groups of rats. However, the increase in weights for both groups revealed no statistically significant difference ($p>0.05$) as shown in Table 3.

Relative organ weight

Results of relative organ weights of rats treated with *C. swynnertonii* exudates are shown in Table 4. In this study, there were significant differences ($p<0.05$) in heart and lungs for female rats at doses of 250 and 1000 mg/kg body weight respectively.

Hematological parameters

Results on hematological parameters are shown in Table 4. It was revealed that there were a significant difference ($p<0.05$) in MCV, MCH, MCHC, Hb, MPV and PDW for both control and treated groups of male rats treated with *C. swynnertonii* exudates at all doses of 250, 500 and 1000 mg/kg body weight.

Biochemical parameters

Results on biochemical parameters in this study are shown in Table 5. It was revealed that there were a significant difference ($p<0.05$) in Total protein, AST, creatinine and bilirubin for both control and treated groups of male rats treated with *C. swynnertonii* exudates at all doses.

Histopathology findings

Histopathological findings of organs namely liver, heart, kidney, lungs and spleen in a group of rats treated with high dose (1000 mg/kg body weight) of *C. swynnertonii* exudates are shown in Figure 1. It was revealed that, there were no lesions observed in both control and treated animals.

Discussion

Medicinal plants have received greater attention as an alternative to clinical therapy and the demand for these remedies has currently increased (Sini *et al*., 2010). The increase in number of users as opposed to the scarcity of scientific evidence on the safety of the medicinal plants, have been raised regarding toxicity and detrimental effects of these remedies (Alam *et al*., 2011). This study evaluated the acute and sub-acute toxicity of *C. swynnertonii* stem bark exudate using animal models.

The body weight was found to be increasing gradually from the beginning of the experiment to the end. The increase in weight is suggested to be contributed by improvement of nutrition as they were supplied synthetic grower marsh and water daily. The differences in the increase in weight of the animals treated with *C. swynnertonii* compared to the untreated group was statistically not significant ($p>0.05$). Further observation showed that, the exudate did not stimulate thyroid hormone which controls metabolism (Bakari, 2012; Mdegela *et al*., 2017).

Therefore it can be concluded that the stem bark exudate of *C. swynnertonii* did not influence animal weight gain or loss. However for the internal organs, there was no any changes in relative organ weight of the animals when treated and control groups were compared.

Hematology assessment parameter is important in determination of any adverse effect of toxins taken orally (Adeneye *et al*., 2006). Analysis of blood parameters therefore assist in evaluation of any changes in some hematological parameters such as WBC, RBC, MCH, MCHC, RDW, Hb, MPV, PDW and prediction for toxicity (Gautam and Goel, 2014). According to Ouedraogo *et al*., (2013) the hematological indices in animal is used to decide toxicity threat as the change in blood system had higher projecting values for human toxicity. Since the current study did not reveal any significant differences in hematological parameters for both control and treated animals with *C. swynnertonii* stem bark exudate, it can be therefore suggested that the plant exudate is safe for dermal and oral application.

In a routine health evaluation, monitoring of enzyme serum maker as biochemical changes is essential. For instance, there are several biochemical activities which occur in the liver including; metabolism, degradation and synthesis. These processes or activities play key roles in the detoxification of chemical compounds (Reddy *et al*., 2013). Thus, in any toxicological study, it is very important to assess the liver and kidney enzymes functions as these organs play a major role in the metabolism and removal of foreign substances from the body. The enzymes ALT and AST tend to be elevated whenever the conditions associated with toxicity prevails. ALT is an indicator of liver functions and biomarkers for toxicity predictions (Mukinda and Syce, 2007: Kripa *et al*., 2011). In this study, the analyses of the serum were carried out in order to evaluate if there is any damage of liver and kidney induced due to oral administration of *C. swynnertonii* stem bark exudates. The observations indicated that, there was a significant difference in AST between animals treated with *C. swynnertonii* exudates and untreated group (control group) ($p<0.05$). There was significance difference in protein, creatinine and bilirubin observed between controls and treated male rats however the observed difference was found to be within the range. (Aleman *et al*., 2015). This indicates that liver was functioning well even after administration of *C. swynnertonii* stem bark exudates. Furthermore, there was no significance difference in glucose and cholesterol level between control group and treated groups, hence *C. swynnertonii* exudates has no effects on lipids and carbohydrate metabolism. Since most foreign substances are eliminated from the body through kidney (renal excretion) hence it is important to examine the creatinine level as a determinant of kidney function (Diallo *et al*., 2010). The study revealed that, there was no difference in level of creatinine among the

treated and control groups. This therefore confirmed that the kidneys were not damaged by the *C. swynnertonii* exudates.

The structures of all organs was found to be normal, there was no observable change in morphology between treated and control group. Generally histopathology was in line with body weight and organ weight index. This study suggest that the *C. swynnertonii* exudates have got lower toxicity levels.

Conclusion

This study has shown that the *C. swynnertonii* exudate does not have severe toxicological effects as there was no observable changes in physiological behavior, body weight, and relative organ weight as well as hematological, biochemical and histopathological parameters. This study suggest that *C. swynnertonii* stem bark exudates is safe for oral or dermal applications at lower dosage, however further studies is recommended.

Table 1: Effects of oral administration of *C. swynnertonii* stem bark exudates on physiological and behavior change in mice

Parameter	**Doses (mg/kg body weight)**			
	Control	**500**	**1000**	**3000**
Eye lid closure	-	-	-	-
Difficulty in breathing	-	-	-	-
Change in skin	-	-	-	-
Eyes mucus membrane	-	-	-	-
Sleep	-	-	-	-
General weakness	-	-	-	-
Loss of appetite	-	-	-	-
Diarrhea	-	-	-	-
Excitement	-	-	-	-
Mortality	nm	nm	nm	nm

Keys: – = no physiological/behavior changes, + = observed physiological/behavior changes, nm = no mortality

Table 2: Effects of oral administration of *C. swynnertonii* stem bark exudates on body weight (g) in mice

Dose (mg/kg)	**Sex**	**Mean weight at day 0**	**Mean weight at day 14**	**p- value**
Control	M	31.26 ± 1.55	32.6 ± 2.17	0.587424
	F	27.4 ± 1.58	29.18 ± 1.32	0.228511
500	M	28 ± 1.82	29.64 ± 1.49	0.385250
	F	24.8 ± 1.72	26.2 ± 1.34	0.391518
1000	M	35.4 ± 1.35	37.57 ± 0.69	0.32142
	F	23.8 ± 1.76	25.44 ± 0.94	0.36085
3000	M	30.74 ± 1.5	32 ± 0	0.294342
	F	28 ± 2	29.26 ± 1.40	0.545162

Values are expressed as mean ± SEM, $p < 0.05$ values considered significant

Table 3: Effects of oral administration of *C. swynnertonii* stem bark exudates on body weight (g) in rats

Dose mg/kg	**Sex**	**Mean weight (g) at day 0**	**Mean weight (g) at day 28**	**p- value**
Control	M	221.67 ± 0.88	223.67 ± 11.84	0.753062
	F	102.33 ± 0.88	111.67 ± 0.88	0.102705
250	M	145.67 ± 9.53	180 ± 8.66	0.056006
	F	133 ± 2.3	153 ± 9.24	0.103604
500	M	170.67 ± 28	181.67 ± 19.34	0.762721
	F	133 ± 2.3	134 ± 5.20	0.153462
1000	M	168.67 ± 4.91	179 ± 3.46	0.160631
	F	80.33 ± 0.33	91.67 ± 3.76	0.139738

Values are expressed as mean ± SEM, $p<0.05$ values considered significant

Table 4: Effects of oral administration of *C. swynnertonii* stem bark exudates on internal organs in rats

	Dose (mg/kg)	**Kidney (g)**	**Liver (g)**	**Lungs (g)**	**Spleen (g)**	**Heart (g)**
Male	Control	$0.00393 \pm 0.000928^{ab}$	0.028 ± 0.00681^{a}	0.00543 ± 0.0014^{a}	0.00253 ± 0.000809^{a}	0.0039 ± 0.000601^{a}
	250	0.0067 ± 0.00055^{ab}	0.0400 ± 0.002^{ab}	0.0053 ± 0.000907^{a}	0.00348 ± 0.00136^{a}	0.0056 ± 0.000289^{ab}
	500	$0.00567 \pm 0.000611^{ab}$	0.033 ± 0.00404^{ab}	0.00503 ± 0.00118^{a}	0.00361 ± 0.000647^{a}	0.00473 ± 0.00147^{ab}
	1000	0.005 ± 0.000503^{ab}	0.039 ± 0.00404^{ab}	0.0056 ± 0.000113^{a}	0.00314 ± 0.00093^{a}	0.0056 ± 0.000115^{ab}
Females	Control	0.00653 ± 0.00264^{ab}	0.045 ± 0.00551^{b}	$0.00893 \pm 0.0000881^{ab}$	0.00421 ± 0.0011^{a}	0.00806 ± 0.00083^{c}
	250	0.0038 ± 0.000702^{a}	0.042 ± 0.00458^{b}	$0.01317 \pm 0.000809^{bc}$	0.00459 ± 0.00033^{a}	0.00493 ± 0.00119^{ab}
	500	0.00427 ± 0.00101^{ab}	0.0437 ± 0.00318^{b}	0.01362 ± 0.00284^{bc}	0.00384 ± 0.000416^{a}	$0.00677 \pm 0.000667^{bc}$
	1000	0.00773 ± 0.00198^{b}	0.0437 ± 0.00176^{b}	0.01423 ± 0.0029^{c}	0.00296 ± 0.000463^{a}	$0.00435 \pm 0.000565^{ab}$

Values with the same superscript within the column means do not show statistically significant differences based on Duncan multiple comparison, mean ± SEM

Table 5: Effects of oral administration of *C. swynnertonii* stem bark exudates on hematological parameters in rats

Sex	Dose (mg/kg)	WBC (M/mm^3)	RBC M/mm^3)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW	Hb (g/dl)	MPV (fl)	PDW
Male	Control	0.727 ± 0.2^{a}	4.87 ± 1.67^{a}	36.067 ± 14.2^{a}	27 ± 3.12^{a}	21.2 ± 7.33^{a}	7.6 ± 2.3^{a}	10.2 ± 3.72^{a}	7.4 ± 0^{a}	7.1 ± 0.12^{a}
	250	5 ± 1.98^{a}	5.87 ± 0.69^{a}	95.367 ± 20.5^{bc}	37.8 ± 8.89^{a}	59.5 ± 12.70^{bc}	23.5 ± 6.9^{a}	14.1 ± 0.98^{ab}	11.9 ± 2.5^{b}	14.7 ± 3.90^{b}
	500	5.26 ± 1.37^{a}	4.727 ± 1.55^{a}	103.767 ± 26.2^{c}	73.13 ± 26.14^{b}	78.5 ± 23.04^{c}	30.1 ± 10.71^{a}	12.9 ± 2.22^{ab}	12.5 ± 2.6^{b}	15.2 ± 4.27^{a}
	1000	1.77 ± 0.28^{a}	6.62 ± 0.12^{a}	60.4 ± 0.64^{ab}	23.667 ± 0.20^{a}	39.3 ± 0.09^{ab}	12.5 ± 0.43^{a}	15.7 ± 0.40^{b}	7.5 ± 0.03^{a}	7 ± 0^{a}
Female	Control	2.823 ± 0.07^{a}	5.753 ± 0.25^{a}	59.1 ± 0.06^{ab}	23 ± 0.058^{a}	39.3 ± 0.38^{ab}	11.3 ± 0.208^{a}	13.1 ± 0.58^{ab}	7.8 ± 0.03^{a}	7.7 ± 0.33^{a}
	250	2.697 ± 0.12^{a}	5.733 ± 0.2^{a}	58.7 ± 0.3^{ab}	22.43 ± 0.38^{a}	39.4 ± 0.45^{ab}	11.3 ± 0.34^{a}	11 ± 1.16^{ab}	9 ± 0.577^{a}	7.8 ± 0.62^{a}
	500	2.757 ± 0.09^{a}	5.567 ± 0.23^{a}	59.033 ± 0.09^{ab}	23.03 ± 0.52^{a}	39.4 ± 0.32^{ab}	11.3 ± 0.21^{a}	13.1 ± 0.55^{ab}	7.6 ± 0.15^{a}	7.5 ± 0.260^{a}
	1000	2.857 ± 0.08^{a}	5.773 ± 0.29^{a}	59.167 ± 0.15^{ab}	23.3 ± 0.3^{a}	39.3 ± 0.49^{ab}	11.2 ± 0.17^{a}	13.7 ± 0.40^{ab}	7.7 ± 0.08^{a}	7.3 ± 0.37^{a}

Values with the same superscript within the column means they are not show statistically significantly different ($p>0.05$) based on Duncan multiple comparison, mean ± SEM, WBC=White blood cell, RBC=Red blood cells, MCV=Mean corpuscular volume, MCH=Mean concentration hemoglobin, MCHC=Mean corpuscular hemoglobin concentration, RDW=Red cell distribution width, Hb=Hemoglobin, MPV=Mean packed cell volume, PDW=Platelet distribution width

Table 6: Effects of oral administration of *C. swynnertonii* stem bark exudates on biochemical parameters in rats

	Dose (mg/kg)	**Total protein (mg/dl)**	**Albumin (mg/dl)**	**Triglycerides (mg/dl)**	**ALT (mg/dl)**	**ALP (mg/dl)**	**AST (mg/dl)**
Male	Control	7.24 ± 0.11^{b}	3.24±0.42^{ab}	94.02 ± 12.91^{a}	35.4 ± 3.4^{a}	59.55±10.76^{a}	72.53±2.21^{b}
	250	7.95±0.26^{c}	3.53± 0.05^{b}	11.1± 6.14^{a}	35.23±1.01^{a}	33.41±4.0^{a}	54.36±0.94^{a}
	500	7.89 ± 0.09^{c}	3.31± 0.42^{ab}	102.87±15.34^{a}	28.56±8.48^{a}	53.97±23.36^{a}	55.3±3.21^{a}
	1000	7.19±0.05^{ab}	2.6±0.02^{a}	94.61±6.31^{a}	30.43±4.31^{a}	73.57±19.92^{a}	73.17±5.89^{b}
Female	Control	6.82±0.17^{a}	2.8±0.15^{ab}	94.07±6.72^{a}	31.12±5.22^{a}	46.81±6.7^{a}	48.07±0.58^{a}
	250	7.13±0.09^{ab}	2.93±0.09^{ab}	93.13±5.75^{a}	31.13±4.36^{a}	44.5±7.43^{a}	69.6±4.35^{b}
	500	7.13±0.09^{ab}	3.0±0.02^{ab}	93.43±6.81^{a}	28.4±2.52^{a}	49.24±8.71^{a}	72.13±5.23^{b}
	1000	7.10±0.06^{ab}	3.03±0.07^{ab}	102.59±2.34^{a}	30.4±4.84^{a}	41.77±7.80^{a}	68.7±4.35^{b}

Values with the same superscript within the column means do not show statistically significant differences ($p>0.05$) based on Duncal multiple comparison, mean ± SEM, ALT=Alanine aminotransferase, ALP=Alkaline phosphatase, AST=Aspartate aminotransferase

Acaricidal Effect of Commiphora Swynnertonii Stem Bark Exudate against *Rhipicephalus Appendiculatus*[2]

Abstract

The objective of this study is to evaluate acaricidal activity of *Commiphora swynnertonii* (*Burseracea*) stem bark exudates against *Rhipicephalus appendiculatus*. *C. swynnertonii* is a small woody plant of 2.5 meter height. It is found in arid and semi-arid environments, the plants have fruits which are consumed by birds and animals. *R. appendiculatus* is the brown ear tick responsible for the transmission of east coast fever in cattle. It found in the savannah and temperate climatic condition. *C. swynnertonii* exudate was prepared using soap solution and evaluated against the *Rhipicephalus appendiculatus* using adult immersion test method (AIT). The percent adult mortality within 15 days and percentage hatching of laid ova were determined at concentrations of 12.5, 25, 50, 60, 70, 80, 90, and 100 mg/ml. The results showed that, mortality was significantly high at concentration greater than 25 mg/ml *($p<0.05$)*, while inhibition of laid eggs was found to be significant at 90 and 100 mg/ml *($p<0.05$)*. The *C. swynnertonii* exudates found to inhibit completely the hatching of tick's eggs. The current results indicate that *C. swynnertonii* exudates have high acaricidal activity against *R. appendiculatus* and can be employed as natural acaricides especially by small holder farmers to control ticks.

Key words: Mortality, ticks, inhibition, hatching, laid eggs.

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Introduction

In Africa many societies depend on livestock keeping as source of food, income and providing power for farming and other domestic benefits (Gonzo *et al.*, 2014). Tick infestation and consequently, tick borne diseases has been a key bottleneck in livestock farming in Africa. Ticks are responsible in the transmission of East Coast Fever (ECF), babesiosis, anaplasmosis, dermatophilosis, and cowdriosis in the Sub Saharan Africa (Kalala *et al.*, 2014; Mkangara *et al.*, 2014). *R. appendiculatus* is hard tick mainly found in the ears of the cattle, buffalo and antelope. It transmit a number of pathogens including *Theileria parva* (East coast fever), Nairobi sheep diseases virus and brown tick toxicosis (Yessinou 2016). Tick and Tick borne diseases (TTBD) cause death of livestock, elevate cost of veterinary services, reduce quality and quantity of the animal product, loss of labor animals and lower reproduction capability (Ilham *et al*., 2014; Kivaria, 2006; Mkangara *et al*., 2014). Reducing the incidence of TTBD leads to economic benefits such as increase quality of animal products, fertility, lowers cost for veterinary services and increased manure production (Kivaria, 2006).

For many years, tick infestations has been controlled through the use of synthetic acaricides (Brito *et al.*, 2011) usually suspended in water. Tick resistance to some common synthetic acaricides, the presence of acaricides residues on animal products and environmental contamination has been reported in the world (Castro-Janer *et al.*, 2010; Krishna et al., 2014; Lovis *et al.*, 2011; Pretty and Waibel, 2005). Resistance to synthetic acaricides has been linked to the multiple use and improper frequency application of synthetic acaricides to livestock (Eshetu *et al.*, 2013). Development of acaricidal agents targeting eggs, larvae, nymph and adults is currently viewed as a sustainable means of controlling ticks (Habeeb, 2010; White *et al.*, 2004). It is in this view that *C. swynnertonii* which is exploited by many African communities for ethnoveterinary use (Miller *et al*., 2002; Musa, 2008) was evaluated for acaricidal activity. *Commiphora swynnertonii* (family *Burseracea*) is a small woody plant which can grow up the height of 2.5 meter (Mukhlesur, *et al*., 2008; Van Wyk and Wink, 2004). The plants is found in the arid and semi-arid environment and grows with tiny leaves and spiny with fruits edible to birds and animals (Paraskeva, *et al*., 2008). *C. swynnertonii* claimed to be used as veterinary medicine (Deepa, *et al.*, 2015). This paper therefore reports acaricidal activities of *Commiphora swynnertonii* exudates against the *Rhipicephalus appendiculatus*.

Methods

Plant Materials

The plant exudates were collected from the stem bark of *C. swynnertonii* at Mererani in Simanjiro District, Arusha region Tanzania. The *C. swynnertonii* plants were identified by botanist from Tropical Pesticide Research Institute (TPRI) Arusha Tanzania. The voucher specimen CS 001 was deposited in the herbarium at Nelson Mandela African Institution of Science and Technology (NM-AIST) Arusha, Tanzania.

Preparation of Acaricides

The extraction was done by using 10 mg/ml of a commercial powder soap OMO (purchased from Uniliver Kenya Ltd) prepared using distilled water. The following dilutions of exudate soap solutions were prepared 100, 90, 80, 70, 60, 50, 25 and 12.5 mg/ml. The dilutions prepared were used to test the ticks' percentage mortality, index of laying eggs, and inhibition of hatching by adult immersion test method (AIT).

Collection of Ticks

The ticks were collected from small holder farmers at Monduli District Arusha region. Ticks collected were stored in a small bottle closed with muslin cloth to prevent from escape and transported to TPRI for identification. After Identification the female *R. appendiculatus* were fed on rabbit to engorge (Drummond et al., 1973; Reghu Ravindran et al., 2011). Ticks were incubated at biological oxygen demand (BOD) incubator at 28 ± 2°C and relative humidity (RH) 80% to lay eggs. The eggs were incubated and hatch, the larva were fed to nymph and then to adult. The adult female were fed on rabbit again to engorged and drop out. The engorged female ticks were transported to Nelson Mandela African Institution of Science and Technology laboratory, washed by distilled water and dried in by absorbent paper ready for the experiment (Abbas *et al*., 2014). A total of 216 adult engorged female ticks were used for this experiment.

Adult Immersion Test (AIT)

The AIT was conducted according to FAO (1984) guidelines with minor modifications (Miller et al., 2002; R. Ravindran et al., 2014). Four replicates of six ticks were used to test each extract concentration. The ticks were selected randomly and weight of each replicate of the female engorged ticks was recorded. Each replicates were immersed to the respective concentration (10

ml) at room temperature for 10 min in a 50 ml beaker with a gentle agitation according to (Hanus *et al*., 2005; R. Ravindran et al., 2014) with minor modifications. Ticks were recovered from the solutions and placed in plastic petri dishes. The control group was immersed in soap solution (10 mg/ml). Ticks were placed in a petri dish over whatman filter paper no 1 (purchased from Whatman international Ltd Maidstone England). The treated petri and untreated ticks were kept at room temperature for 24 hours, then the ticks were incubated in biological oxygen demand (BOD) incubators at 28±2°C and relative humidity 85±2 %. The ticks were observed for mortality and oviposition for the period of 15 days. After 15 days the percentage mortality was determined (Hanus *et al*., 2005). The weights of the eggs laid were recorded and were incubated for additional 30 days to estimate percentage hatching.

$$\text{The index of laying eggs (IE)} = \frac{\text{weight of the laid eggs (g)}}{\text{Weight of the females (g)}}$$

$$\text{Percentage inhibition of egg laying} = \frac{(\text{IE control group} - \text{IE treated group}) \times 100}{\text{IE control group}}$$

After the period of thirty days the number of dead and live larvae, and unhatched eggs were determined and percentages hatched eggs were calculated.

Estimated reproductive factor (ERF) and inhibition of reproduction (IR) were calculated using the formula below as described by Drummond *et al* (1973).

$$\text{ERF} = \frac{20{,}000\ XY}{Z}$$

Where: 20,000 average number of eggs per gram

X= weight (g) of the eggs produced

Y= estimated percentage hatchability of the eggs

Z= weight (g) of the females

$$\text{Percentage inhibition reproduction (IR)} = \frac{(\text{ERF control group} - \text{ERF treated group}) \times 100}{\text{ERF control group}}$$

Statistical Analysis

Microsoft excel 2013 computer software was used to obtain regression equation where LC_{50} and LC_{99} were calculated. One way analysis of variance (ANOVA) was carried out by Genstat software version 10 to determine significance differences among the doses in mortality, index of laying eggs, percentage inhibition of laid eggs, hatching percentages and inhibition of hatching. The p values less than 0.05 was considered significant.

Results

Mortality of Ticks

Commiphora swynnertonii stem bark exudate was evaluated for acaricidal activity against *R. appendiculatus*. The findings emanated from this study are summarized in Table 1. The tested concentrations were prepared by dissolving a known amount of *C. swynnertonii* stem bark exudate in a known volume of diluted soap solution. It was evident that a complete mortality of ticks was observed at 100 mg/ml for the period of fifteen days of the experiment. Mortalities at higher concentrations (50 to 100 mg/ml) was significantly higher ($p<0.001$) than in lower concentrations. The mortality of ticks using synthetic acaricides (Alpha Cypermethrin) was reported to be 100 percent within two days of exposure (Musa, 2008). The highest tick mortality of the tested *C. swynnertonii* stem bark exudate occurred between day one and day nine post treatment. The lethal concentrations which can result mortality for at least 50 and 99 percent ($LC_{50,}$ LC_{99}) of the ticks was established to be 42.9 and 92.8 mg/ml respectively (Fig. 1).

Effects of *C. swynnertonii* Exudates on Ticks Ability to Lay Eggs

Commiphora swynnertonii exudate was evaluated for its effect on ability of engorged female ticks to lay eggs. The body weights of engorged female ticks were not significance different from each other. The engorged female ticks were treated with 25, 50, 60, 70, 80, 90, and 100 mg/ml of *C. swynnertonii*. The weights of the laid eggs were found to decrease as tested concentration of *C. swynnertonii* stem bark exudates increased (Table 1). The weight of eggs laid by the treated engorged female ticks was found to be significantly different from those of untreated group ($p< 0.05$).

The index of laid eggs by female ticks was affected by the concentration of *C. swynnertonii* exudates. The female ticks tested with high concentrations found to have lower ability to lay eggs as compared to those tested with lower concentrations of the exudates. The index of laying eggs of treated engorged female ticks with *C. swynnertonii* stem bark exudates at concentration 100 and 90 mg/ml was found to have significance difference to those untreated group ($p< 0.05$).

Hatching of the Ticks Eggs

Eggs laid by both treated and un-treated engorged female ticks were incubated at 28 ± 2 ^{o}C for 30 days. On visual examination of the incubated eggs, it was observed that all eggs laid by ticks treated with *C. swynnertonii* stem bark exudates did not hatch while those eggs laid by untreated ticks were hatched (Table 2). *C. swynnertonii* stem bark exudate was found to interfere the development of eggs. The *C. swynnertonii* stem bark exudate lowers reproduction rates of the tick.

Discussion

There is an increase interest on the use of botanicals to control ectoparasite as they are found to be relatively cheaper, eco-friendly, effective, readily available and safe especially for smallholder farmers (Shyma et al., 2014). Plants contain range of chemically active ingredients which can intervene all biological process of the ticks interrupting its life cycle and its dispersal (Habeeb, 2010). In the current study, *C. swynnertonii* exudate was evaluated for acaricidal activity against *R. appendiculatus*. The findings showed that *C. swynnertonii* exudates induces tick mortality, lowers ticks ability to lay eggs and affects the viability of laid eggs from the treated engorged female ticks.

Previous phytochemical studies on *Commiphora* species reported on the presence of several classes of secondary metabolites namely alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, terpenes, steroids, resin acids and phenolics which found to cause mortality in arthropods (Hanus *et al*., 2005; Krishna *et al*., 2014; Musa, 2008; Shyma *et al*., 2014). Moreover it was reported that *Commiphora* species possessing antibacterial and antifungal activities (Bakari *et al*., 2012). The acaricidal effect of *C. swynnertonii* exudate was due to the presence of tannins, saponin, terpenes and flavonoids (Bakari, 2013; Bissinger and Roe, 2010). The observed mortality of ticks may be accounted by several possible mechanisms working separately or working in synergic (Chandler *et al*., 2011; Kunz and Kemp, 1994). In some cases insecticides can have both insecticidal and acaricidal properties (George *et al*., 2014). It is believed that the mechanism of

action of terpenoids and flavonoids might be similar to that of the organophosphate compounds. Therefore the acaricidal activities observed in this experiment might be contributed by one or more bioactive compounds present in *C. swynnertonii* exudate.

The mortality of the ticks when immersed in the *C. swynnertonii* stem bark exudates suggests that there is contact effect of the exudates. At higher concentration the effect of the exudates was immediate while at lower concentration the effect was taking place slowly as the residual on the body stimulate effect. The variation in mortality based on concentration was attributed by the amount of the exudates in contact with body and penetrates to the inner cells. The amount of bioactive compounds of the exudates penetrates the inner cell is proportional to mortality caused thus exudates with higher concentration results into mortalities earlier than those with lower concentrations. Higher concentration is recommended to be used as lower concentration may result into resistance especially when the activity is not taking place shortly. The mode of action was similar to that of pyrethroids in insect where by it produces hyper excitation tremors and paralysis followed by mortality (Shyma *et al*., 2013). The nerve excitations occurs as a result of change in membrane permeability to sodium and potassium ions (Krishna *et al*., 2014). The use of OMO (detergent) is recommended as it has lipophilic properties which can remove the outside waxy cuticle layer of the ticks and allow the bioactive compounds to penetrate to the inner cells. Bioactive compounds revealed to interfere with basic metabolic, biochemical, physiological and behavioral functions of the ticks. The penetration of the bioactive compounds through the outer layer of the ticks causes the mortalities, suppress and reduction in oviposition as well as development of the eggs hence blocking of the eggs hatching. The bioactive present in the exudates suggest interfering the ovaries as well as the egg formations (Shyma *et al.*, 2014).

This study supports the use *C. swynnertonii* as a potentially acaricidal agent for controlling *R. appendiculatus* and confirm the presence of some factors in the exudates with acaricidal property. Exudates having acaricidal, anti-oviposition, and inhibition of fecundity properties may make the plant valuable component of developing valuable strategies for integrated tick management.

Conclusion

The obtained results indicate that *C. swynnertonii* exudates may prove to be effective against control *R. appendiculatus*. Therefore small holder farmer especially those practice zero grazing, in the remote settings where synthetic acaricides is unaffordable or unavailable, they can use *C. swynnertonii* exudate to control ticks. Further studies should be conducted to determine the mechanism of *C. swynnertonii* exudates mode of actions. However plants from different parts should be studied to confirm if they have the same activity. Field trial should be conducted to compare the results with laboratory results.

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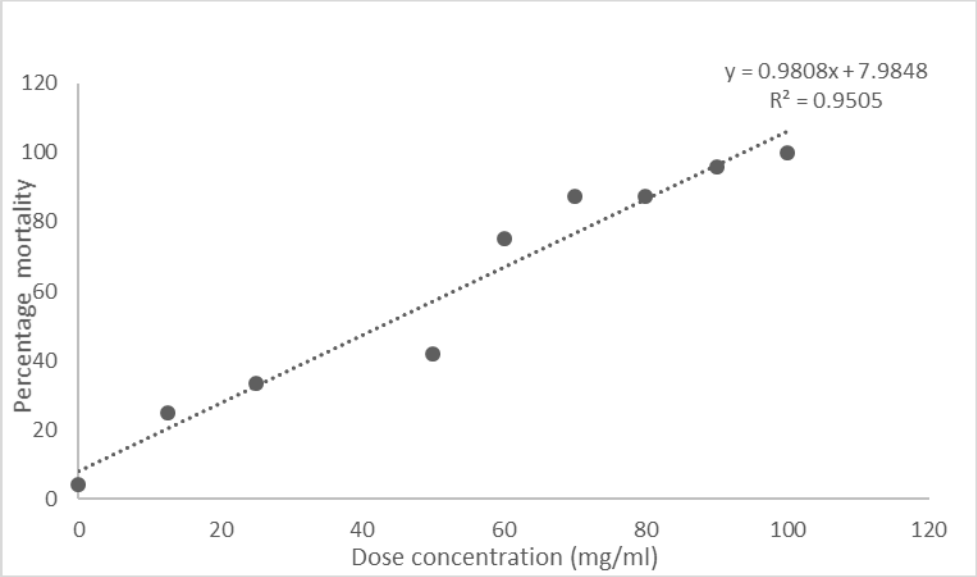

Figure 1: Effectiveness of *C. swynnertonii* stem bark exudates on tick mortality at different tested concentrations.

Table1: Effect of *C. swynnertonii* exudates against *R. appendiculatus*

Values are expressed as mean ± SEM, *< 0.05, values with the same superscript within the column means do not show statistically significant differences (p>0.05) based on Duncan multiple comparison, %= percentage, # =Number

Dose (mg/ml)	% Mortality	Laid eggs (g)	# eggs incubated	Females ticks (g)	Index of laid eggs	% inhibition in laying eggs
Control	0.0^{d}	0.19^{b}	7128^{a}	1.2^{ab}	0.157^{cde}	0.0^{abc}
12.5	25^{cd}	0.31^{b}	13592^{a}	1.45^{a}	0.212^{d}	-48^{a}
25	33.3^{c}	0.1^{a}	$4413b^{c}$	0.67^{b}	0.187^{cd}	-32^{ab}
50	41.7^{c}	0.05^{cd}	2300^{c}	0.56^{b}	0.099^{abcd}	27.2^{bcde}
60	75^{b}	0.16^{bc}	6808^{b}	1.23^{ab}	0.128^{bcde}	15.8^{abcd}
70	87.5^{ab}	0.06^{cd}	2833^{bc}	1.07^{ab}	0.059^{abc}	62.6^{cde}
80	87.5^{ab}	0.08^{cd}	3573^{bc}	1.25^{ab}	0.064^{abc}	48.6^{cde}
90	95.8^{ab}	0.03^{d}	1250^{c}	1.63^{a}	0.025^{ab}	86^{de}
100	100^{a}	0.02^{d}	576^{c}	1.12^{ab}	0.08^{a}	91^{e}

Table 2: Mean estimated reproductive factor and Inhibition of reproduction of R. appendiculatus engorged females subjected to different concentrations of C. swynnertonii stem bark exudates.

Dose (mg/ml)	**Control**	**12.5**	**25**	**50**	**60**	**70**	**80**	**90**	**100**	**P-value**
ERF ± SEM	314162.6±51456.6	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.001
IR ± SEM		100±0	100±0	100±0	100±0	100±0	100±0	100±0	100±0	0.002

Values are expressed as mean ± SEM, ERF= Effective reproductive factor**,** IR= Inhibition of reproduction

Utilization of African Ethnoveterinary Information for Management of Livestock Diseases[3]

Abstract

Smallholder farmers in Africa face various challenges in livestock keeping, livestock diseases being one of them. Due to poor infrastructure especially road and communication networks, smallholder farmers in rural areas do not easily access conventional veterinary drugs (CVD) and other veterinary services. Even when CVDs are accessible, they are often unaffordable to most of the smallholder farmers. Plants often provide an alternative to struggling smallholder farmers. The plants in use are traditionally known to have useful roles in the management of vectors transmitting veterinary disease pathogens. This accumulated knowledge on ethnoveterinary among smallholder farmer communities in Africa is largely untapped with respect to the management of livestock diseases. This paper reviews plants of ethnoveterinary significance in Africa.

Key words: East coast fever, ethno veterinary, livestock

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Background

Domestic and grazed livestock have always suffered from a wide range of diseases in Africa (Gilioli *et al*., 2009). As livestock are concentrated in larger numbers, the problems of major epidemics have become more severe. The transmission of livestock diseases is favored by environmental conditions in the continent (Jabbar *et al*., 2015). The diseases and complications of livestock include East coast fever, trypanosomosis, babesiosis, anaplasmosis, dermatophilosis, and cowdriosis, retaining placenta, wounds, ectoparasite, diarrhea, fungal and viruses. Diseases affect the livelihood of the small holder farmers as they depend on livestock for income food and social status (Laisser *et al*., 2016). Livestock disease control at community level particularly among smallholder farmers and pastoral groupings rely on ethnoveterinary information (Grade *et al*., 2009). Smallholder farmers living in marginal areas which are endemic to pathogens, vectors and diseases are the ones that are more affected. The health of their animals suffers from the diseases as the conventional veterinary services are not available, less effective and unaffordable. Ethnoveterinary information provides a reliable alternative to smallholder farmers in the management of livestock diseases (Kubkomawa *et al*., 2013).

However, ethnoveterinary information is barely tapped yet it is critical in the remote and rural areas for sustainability of animal health management (Khan, 2009; Marandure, 2016). Ethnoveterinary knowledge in many African societies is undocumented as it is contained and transferred from generation to generation through rituals and rites of passage as well as through observation. It is barely documented; this is threatening it is availability in posterity. Therefore this review is aimed to document of ethnoveterinary information that is being used by smallholder farming communities in Africa for livestock disease management.

Plants of Ethnoveterinary Importance

Ethnoveterinary medicine is the total knowledge, skills and practices based on the theories, beliefs and experiences of a people used in livestock disease management (Deeba *et al*., 2009). Different African cultures use ethnoveterinary information in livestock disease management (Damtew, 2012) and several smallholder farmers have incorporated ethnoveterinary information in their production systems (Fajimi and Taiwo, 2005).

Several plants used in the management of endoparasites in cattle, donkey and all small ruminants in Tanzania include: *Tephosia vogelii, Senna didymobotra, Azadirachta indica, Chassalia subckreata, Carica papaya adansonia digitata, Arachis hypogea, Clausena anisata, milletia grandis* and *cyathea dregei*. Meanwhile, plants such as: *Commiphora swynnertonii,*

Taarindus indica, Euphobia tirucalli and *Acasia nilotica* are commonly used for the management of ectoparasites. In poultry production, *Aloe vera, Solanum incanum, Capsicum frutescens* are used to manage coccidiosis, Newcastle and worms (Masola *et al*., 2009; Adamu *et al*., 2012; Adedeji *et al*., 2013; Mwatawala and Mlinjanga, 2016). Further, in Kenya, several researchers (Adedeji *et al*., 2013; Njoroge and Bussmann, 2006) have reported the use of *Mucuna spp, Aloe secundiflora, Agave americana, Synadenium compactum, Solanecio mannii* and *Senna didymobotrya*. Meanwhile the use of *Erythrina abyssinica* and *Capsicum annum* for the management of worms, Newcastle and bacterial infections in poultry (Lagu and Frederick, 2012) and *Balanites aegypticus, Carissa spinarum, Warburgia salutaris* and *Harrisinia abyssinica* in other livestock species in Uganda has been observed (Gradé *et al*., 2009).

Like in other dryland ecosystems of eastern Africa, extensive livestock grazing in the rangelands of Somalia and Ethiopia is the norm rather than the exception. In these areas pests such as lice, and ticks are common phenomenon, as a result, herders use a diversity of plants including among others; *Rumez patientia, Euphorbia somaliensis, Urtica dioica Eucalyptus spp, Capsicum frutescens* and *Thymus capitatus* (Mcgaw and Eloff, 2014; Wanzala *et al*., 2005; Adedeji *et al*., 2013). Further, in Southern Africa (in parts of Botswana and South Africa); *Terminalia serecea* roots, *Burkea africanum, Gunnera perpensa, Cissus quadrangularis* and *Jatropha zeyher* are used for the treatment of endometritis, and wounds in livestock (McGaw and Eloff, 2008; Mcgaw and Eloff, 2014; Moreki *et al*., 2012). *Adansonia digata, Zingiber officinale, Parkia filicoides* and *Nicotiana tabacum* are used by Fulani community in Nigeria for the treatment of microbial infections, worms, and skin disorders in livestock (Kubkomawa *et al*., 2013). The ethnoveterinary knowledge and information is not only limited to plants, but salt water and soap solutions are also used by some African communities to remove retained placenta in cattle (Mcgaw and Eloff, 2014).

Plant Selection for Ethnoveterinary Use

There are variations in cultural knowledge in the selection of plants for the management of livestock diseases. The number of uses a plant can provide is one of the criteria societies often rely in plant selection. For instance, Maasai use *Commiphora swynnertonii* exudates for the management of ticks, fleas and tsetse flies (Minja, 1999) while the same is used for the management of worm infestation among the Dorobo community (Willbrord *et al*., 2014). *Acacia drepanolobium* which is known as eluai (Maasai) is used by many communities in Tanzania for removal of retained placenta (Kiringe, 2006). In Tanzania ethnoveterinary information of such plants are fairly documented. The Karamojong of Uganda have similarly

used a range of plants to treat multiple livestock diseases, pests and conditions; for example, *Albizia coriaria* is used to treat rinderpest, pneumonia, contagious bovine/caprine Pleuropneumonia, and increase fertility (Grade, 2008; Egeru *et al*., 2015). Most of the knowledge and information has been preserved generations to generations through a unique adoption procedure. In the recent past, legal practitioners specialized in intellectual property rights, have been trained and have are interested in assisting local communities in developing patents of their local knowledge.

Application of Ethnoveterinary Knowledge

Ethnoveterinary medicines have been used for more than hundred years ago through trial and error leading to accumulated knowledge of a local people (Grade, 2008). However, few plants have been found to be sufficient to manage various ailments of animals. The traditional knowledge acquired by local people has been transferred through historical times by oral communication through songs, dance and practice. These patterns are beginning to weaken as cultural practices are similarly weakening. As such the quality and survival of ethnoveterinary knowledge is under immense challenge. Ethnoveterinary knowledge is applied in a range of fields including: plant extraction, concoction mixtures, administration, monitoring and observation of livestock performance and response to drug administration (Grade 2008; Grade *et al*., 2009).

Conclusion

This review has revealed that there is a wide spread use of plants for ethnoveterinary medicine in the management of livestock disease, pests and allied conditions. As such, ethnoveterinary knowledge is pivotal to the continued effective use and administration of plant materials. It is therefore vital that ethnoveterinary knowledge is documented to allow for a dependable source of information in the rapidly evolving societies and social systems.