Advancement in Medicinal Plant Research
Vol. 3(2), pp. 46-54, April 2015
ISSN: 2354-2152
Full Length Research Paper

Chemical composition and acaricidal activity of *Salvia nilotica* essential oil against *Rhipicephalus appendiculatus*

Christine T. Nyabayo[1]*, Josphat C. Matasyoh[1] and Charles Mwendia[2]

[1]Department of Chemistry, Egerton University, P. O. Box 536, Egerton -20115, Kenya.
[2]Department of Biochemistry, Egerton University, P. O. Box 536, Egerton -20115, Kenya.

Accepted 21 April, 2015

ABSTRACT

Cattle tick, *Rhipicephalus appendiculatus,* is one of the major vectors of East Coast Fever causative pathogen. The estimated economic loss associated with East Coast Fever in East African countries is US$ 168 million annually. Development of resistance and side effects associated with synthetic acaricides has triggered intense research efforts towards natural products such as essential oils due to their efficacy and safety. The aim of this study was to determine the acute toxicity of essential oil of *Salvia nilotica* (Sage) against *R. appenduculatus*. The essential oil was extracted by hydrodistillation and its chemical composition determined by gas chromatography-mass spectrometry. The oil was dominated by monoterpenes (39.39%) and sesquiterpenes (21.78%). The major monoterpenes were β-phellandrene (11.52%) and δ-3-carene (6.62%). Only caryophyllene oxide (6.85%) was found to be the major component for sesquiterpenes. Bioassays were determined through contact toxicity and mortality data was collected after 3, 6, 12, 24 and 48 h. Probit regression analysis was used to estimate concentration dependent mortality for LC_{50} and LC_{90} values in mg/ml. The essential oil exhibited potent acaricidal activity with LC_{50} / LC_{90} values of 1.8/3.3, 1.7/3.1, 1.5/3.0, 1.37/2.85, 1.37/2.55 mg/ml against the larvae and 3.9/5.8, 2.7/4.7, 2.2/4.1, 2.1/3.8 mg/ml against the adults of *R. appendiculatus* at 3, 6, 12, 24 and 48 h, respectively. The results obtained indicated that the essential oil of *S. nilotica* exhibits potential as a promising acaricide.

Keywords: Acaricide, essential oil, *Rhipicephalus appendiculatus*, *Salvia nilotica*.

*Corresponding author. E-mail: nyabayochristine@yahoo.com.

INTRODUCTION

The biggest challenge to livestock industries among pastoralist communities especially in Africa is tick infestation and tick-borne diseases (Olwoch et al., 2008). These ticks are vectors to several problems afflicting both humans and livestock. Among the most economically important vector of the causative agent of East Coast Fever (ECF) is the brown ear tick, *Rhipicephalus appendiculatus.* In Machakos District in Kenya, the disease was perceived to be fatal and losses associated with it amounted to 1.1 million cattle deaths annually (Jongejan, 1998). More so, infestation by *R. appendiculatus* causes economic losses such as general stress and irritation, anemia, decrease in productivity, depression of immune system and damage to hides (Valente et al., 2014). Prevention and control of both vector and pathogen has faced a lot of setbacks which include; continuous widespread and increasing resistance to synthetic acaricides such as organophosphate (OP), pyrethroid and N, N-diethyl-3-methylbenzamide (DEET), hence becoming a major concern worldwide (Miller et al., 2005; Li et al., 2003). The use of N, N-diethyl-3-methylbenzamide (DEET) currently has demonstrated adverse toxic effects on some species of zooplankton and fish (Seo et al., 2005). Other challenges faced by the use of these synthetic acaricides include; environmental pollution (Bhattacarya

et al., 2003), residues in food, and toxicity to non-target organisms (Graf et al., 2004). Over 200,000 people die worldwide each year due to the direct result of pesticide poisoning as estimated by the World Health Organization (WHO) (Feng et al., 2007). Herbal acaricides are eco-friendly, cheaper and cause minimal mammalian toxicity (Habeeb, 2010). Despite the capability of these botanical pesticides to provide novel modes of action against Acarina, they can also reduce the risk of cross-resistance and offer new leads of target-specific acaricides (Isman, 2006). It is therefore necessary to come up with a range of compounds that possess various modes of action that will enable rotation of new chemicals that will curb the problems of acaricidal resistance (Graft et al., 2004).

An upsurge interest in the use of essential oil as phytomedicines has resulted in a thorough investigation into plant resources. Essential oils from plants constitute a rich source of bioactive chemicals which have been reported to have broad insecticidal activity (Abad et al., 2012). Several researchers indicate that essential oils possess healing, antiseptic, anti-inflammatory, antipyretic, antispasmodic, insecticidal and bactericidal activities among others (Pamo et al., 2004). Acaricidal active essential oils from *Ageratum houstonianum* (Pamo et al., 2004), *Origanum onites* (Coskun et al., 2008) *Clea serrata* (Oliveira et al., 2005) and *O. minutiflorum* (Cetin et al., 2009) have been studied against some of the *Rhipicephalus* species. Some past studies have been done on the toxicity and repellency of essential oils from different plant species against *R. appendiculatus,* for example, the essential oil of *Tagetes minuta* and *Tithonia diversifolia* were found effective against the species (Wanzala et al., 2014). In another study, Lwande (1998) demonstrated that *Gynandropsis gynandra* essential oil repels *R. appendiculatus*. Also *Cassia didymobotrya, Euphorbia hirta, Kigelia africana* and *Cissus adenocucaulis,* were shown to be toxic to adults of *R. appendiculatus* (Opiro et al., 2013).

Approximately 900 species are found in the genus *Salvia* (Akin *et al*., 2010). It is an important genus that is widely cultivated and used in flavoring and folk medicine all over the world. Most of the *Salvia* species are used for several treatments such as chronic bronchitis, anti-perspirations, fever, rheumatism, mental and nervous problems, as insecticides (Baricevic and Bartol, 2000), antibacterial (Ulubelen et al., 2001), anti-oxidants, inflammation relief and as an anticholinesterase (Perry et al., 2003). In Eritrea, the aerial parts of *S. nilotica* (also known as sage in English) are used to treat sunburns, as after-vomiting medicine and as a painkiller (Gebrehiwot et al., 2009). In Kenya, *S. nilotica* grows as a perennial shrub in marshy areas of the Sururu forest in the Mau-Narok County. Previous studies have shown essential oil of *S. nilotica* possess potent antiradical activity (Vagionas et al., 2007) and pesticidal properties (Mwangi et al., 1995a). However, no experimental evidence on the effects of essential oil from *S. nilotica* against *R. appendiculatus* has been found in the literature.

MATERIALS AND METHODS

Sample collection

Fresh leaves of *S. nilotica* were collected from Sururu forest, Mau-Narok, Nakuru County in Kenya (0°66′ 55.2″S 36°1′ 47.3″E). The sample material was then identified by a taxonomist at the Department of Biological Science, Egerton University in Nakuru, Kenya, where a voucher specimen was deposited.

Essential oil extraction

Fresh leaves of the *S. nilotica* plant were subjected to hydro-distillation for 4 h in a modified Clevenger-type apparatus with a water-cooled oil receiver, to reduce formation of artifacts due to overheating during hydro-distillation. The essential oil was collected over water, separated and dried over anhydrous sodium sulphate (Na_2SO_4). It was stored in a sealed vial at 4°C.

Analysis of essential oil

The essential oil was diluted in methyl-t-butyl ether (MTBE) (1:100) and analyzed on Agilent GC-MSD apparatus which was outfitted with an Rtx-5SIL MS (‘Restek’)(30 mm × 0.25 mm,0.25 µm film thickness) fused silica capillary column. The GC column was maintained at 50°C for 2 min, and then programmed to 260°C at 5°C/min and held for 10 min at 260°C. The carrier gas was Helium (at 0.8 ml/min). Injection of the samples was done in split mode at a ratio of 1:10 to 1:100. The temperature was maintained at 250°C and the transfer line was kept at 280°C. The MS was operated in the electron impact ionization (EI) mode at 70 eV, in *m/z* ranging from 42 to 350. Compound identification was achieved by comparing the mass spectra and retention indices with ones from the literature (Adams, 2007).

Tick rearing

The larvae and adults of *R. appendiculatus* were reared according to the method adopted from Bailey (1960) with slight modifications.

Larvicidal assay

Larvicidal bioassay was done using contact toxicity according to FAO, (2004). Preliminary screening was done to determine the stock solution to be used. The oil was first dissolved in 1% dimethyl sulfoxide (DMSO), and a stock solution of 5 mg/ml made. Larval mortality data was obtained at 3, 6, 12, 24, and 48 h post treatment. The stock solution of 5 mg/ml was serial diluted and resulted in 16 concentrations ranging from 0.5 to 5 mg/ml. Whatman No. 1 (15 cm) filter papers were soaked with each test concentrations and placed in petri dishes using a double sided cellophane tape. 20 larvae of *R. appendiculatus* were exposed to the soaked filter papers in petri dishes and covered. A triplicate set of experiment was done and petri dishes held at 75% relative humidity at 25°C.The larvae were considered dead if they did not exhibit their usual behavior when breathed upon or could not move their appendages when prodded with a pin. The negative and positive controls used were 1% DMSO+ distilled water and 0.2% v/v of Amitraz® respectively.

Adulticidal assay

The Adult Immersion Test (AIT) was conducted according to the method described by Drummond et al. (1973) with slight modifications. First the stock solution of 5 mg/ml was made and serially diluted resulting in 16 concentrations ranging between 0.5 and 5 mg/ml. Whatman No. 1 (15 cm) filter papers were soaked with each test concentrations and placed in petri dishes using a double sided cellophane tape. Each group of 20 adults of the *R. appendiculatus* was immersed for 5 min in 30 ml of the concentration to be tested in test tubes. They were then picked with a brush and placed in the prepared Petri dishes and observed for 3, 6, 12, 24 and 48 h post-treatment. All solutions were kept under constant agitation during the immersion period. 20 other adults were immersed in the positive control made of 0.2%v/v Amitraz® while other 20 were immersed in a negative control made of 1% DMSO+ distilled water. The experiment was carried out in triplicates and Petri dishes held at 75% relative humidity at 25°C as those of the larvae. The mortality of the adults was achieved when they did not exhibit their usual behavior when breathed upon or could not move their appendages when prodded with a pin.

Statistical analysis

The data collected was submitted to probit regression analysis using SPSS 20. Concentration dependent mortality for the lethal concentration at 50 and 90% calculate at the associated 95% confidence interval.

RESULTS

The light yellow essential oil of the fresh aerial parts of *S. nilotica* obtained by hydro distillation in 0.17% (v/w) was analyzed by the GC-MS and the results are demonstrated in Table 1. Compounds identification in the oil was done by comparison of the electron impact mass spectrum of the compounds in the oil with those in the Wiley7N.1, FLAVORS.L and HPCHI607 computer library database. From the data obtained it was evident that the oil was dominated by monoterpenes (39.39%) followed by sesquiterpenes (21.78%), diterpenes (1.70%) and others (10.20%). Major components identified were those with a percentage above two. Major monoterpenes were β- phellandrene (11.52%), α-3-carene (6.62%), α-terpineol (3.27%), γ-terpinene (2.70%), β-pinene (2.02%), allocemene (2.02%), pseudolimonene (2.00%), while major sesquiterpenes were caryophyllene oxide (6.85%), viridiflorol (2.25%). A summary of the constituents with their retention time is shown in Table 1.

The results presented in Tables 2 and 3 indicated that the % mortality was directly proportional to concentration of the essential oil at 3, 6, 12, 24 and 48 h post treatment for both the larvae and adults. The LC_{50} in mg/ml for the larvae and adults were: 1.8 (1.2 to 2.1), 1.7 (1.5 to 1.9), 1.5 (1.2 to 1.7), 1.4 (1.2 to 1.6), 1.4 (1.2 to 1.5) and 0.0 (0.0 to 0.0), 3.9 (3.6 to 4.2), 2.8 (2.5 to 2.9), 2.2 (2.0 to 2.4) and 2.1 (1.9 to 2.3) respectively. The LC_{90} in mg/ml for the larvae and adults were: 3.3 (2.9 to 4.0), 3.1 (2.7 to 3.7), 3.0 (2.6 to 3.7), 2.9 (2.4 to 3.5), 2.6 (2.3 to 2.9) and 0.0 (0.0 to 0.0), 5.8 (5.2 to 7.0), 4.7 (4.2 to 5.5), 4.1 (3.7 to 4.8), 3.8 (3.4 to 4.4), respectively.

DISCUSSION

There is great inconsistency in essential oil composition of *S. nilotica* from different locations country wide. For example GC/MS of *S. nilotica* essential oil analyzed in Tanzania was dominated by camphor (9.70%), viridiflorol (7.91%), α-ylangene (5.19%), ρ-cymene (5.53%), α-amorphene (4.77%) and 3,7-guaidine (4.31%) (Vagionas et al., 2007), Ahfaha et al. (2008) in Eritrea, reported major constituents of *S. nilotica* to be germacrane D (28.48%), guaiol (13.99%) and trans-caryophyllene (12.96%) which differed from the oil analyzed in Kenya. There was difference in percentage constitution in *S. nilotica* analyzed in Kenya. The study demonstrated β-phellandrene (11.52%), caryophyllene oxide (6.85%), δ-3-carene (6.62%), α-terpineol (3.27%), γ-terpene (2.70%) and viridiflorol (2.25%) to dominate in the oil. The observed differences according to Perry *et al* (1999) may be due to edaphic factors, chemotypic variations and different collection times.

In most essential oils, monoterpenes have been shown to dominate and consequently are regarded as candidates for insecticidal activity. Most monoterpenoids are cytotoxic to animal tissues whereby they cause a drastic reduction in mitochondria and Golgi bodies populations while impairing respiration and reducing cell membrane permeability (Tripathi et al., 2009), while most synthetic acaricides target the nervous system by paralyzing and disordering the nervous impulses, prolonging sodium channel activation, causing a state of hyperrexcitation in ectoparasites (Dong, 2007; Roma et al., 2013). The essential oil from *S. nilotica* was found to be dominated by monoterpenes and hence the more reason for the strong toxic effects observed in the *R. appendiculatus'* larvae and adults. Based on the results obtained from the GC-MS analysis of the essential oils, it is possible that the acaricidal property of the *S. nilotica*'s essential oil was attributed to synergism action of the major and the minor terpenoids present in the oil, whose insecticidal properties have been proven in previous studies (Iacobellis et al., 2005). Little is known of the exact physiological action that led to the mortality of the ticks. But studies have shown that when the ticks are exposed to the essential oil they inhale the vapor and the essential oils acts in the vapor phase via respiratory system (Khater, 2011).

Previous scientific reports have shown that most of the identified components of the essential oil possess either acaricidal or insecticidal activity. For example linalool, one of the major constituents of *Lippia kituensis* (Kosgei et al., 2014), *L. javanica* (Viljoen et al., 2005) and *Melinis minutiflora* (Prates et al., 1995) was found to possess acaricidal activity. Moreover, the monoterpenoid linalool present relatively in good amount of the oil was

Table 1. *S. nilotica* essential oil components.

Compound name	Retention time (min)	% Conc	Detection method
Monoterpenes			
β-phellandrene	9.62	11.52	GC-MS
δ-3-carene	6.90	6.62	GC-MS
α-terpineol	14.47	3.27	GC-MS
γ-terpinene	7.03	2.49	GC-MS
Pseudolimonene	8.82	2.00	GC-MS
β-pinene	7.98	2.02	GC-MS
Allocimene	12.44	2.02	GC-MS
Linalool	11.68	1.57	GC-MS
4-terpeneol	13.90	1.23	GC-MS
Camphene	7.24	1.22	GC-MS
β-Myrcene	8.46	1.14	GC-MS
Verbenol	12.95	0.82	GC-MS
Verbenone-1	14.91	0.86	GC-MS
β-Ocimene	10.13	0.69	GC-MS
Trans- verbenol	12.73	0.58	GC-MS
Borneol	13.66	0.47	GC-MS
D- fenchyl alcohol	12.01	0.29	GC-MS
Cis-5-Ethenyltetrahydro-.alpha.alpha.,5-trimethyl, Furanmethanol	10.77	0.32	GC-MS
Piperitone	15.98	0.06	GC-MS
1-Methyl-2-methylene-4-isopropenylcyclohexane	17.41	0.06	GC-MS
1-(2,3-dimethyl-2-butenyl)-2,2,6-trimethyl-7-Oxabicyclo[4.1.0]heptane	40.71	0.07	GC-MS
7-(1-methylethylidene)-bicyclo[4.1.0]heptane	18.44	0.04	GC-MS
Cis- sabinene hydrate	10.61	0.03	GC-MS
Subtotal		39.39%	
Sesquiterpenes			
Caryophyllene oxide	24.62	6.85	GC-MS
Viridiflorol	25.98	2.25	GC-MS
Cryptone	14.27	1.65	GC-MS
α- Muuroline	21.61	1.32	GC-MS
Eremophilene	22.02	1.16	GC-MS
γ- Cadenene	25.67	0.87	GC-MS
Copaene	19.18	0.70	GC-MS
γ- cis calamenene	22.84	0.69	GC-MS
Junipene	28.47	0.67	GC-MS
β-caryophyllene	28.86	0.66	GC-MS
α-Gurjunene	26.09	0.56	GC-MS
Trans- caryophyllene	20.30	0.54	GC-MS
γ- Muurolene	21.72	0.44	GC-MS
Aromadendrene	22.18	0.42	GC-MS
4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinylCycloheptane	24.83	0.39	GC-MS
β- bisabolene	22.48	0.35	GC-MS
Nerolidiol	24.04	0.28	GC-MS
β- farnesene	21.20	0.25	GC-MS
Solavetivone	29.10	0.25	GC-MS
γ-Selinene	23.12	0.24	GC-MS
α- Farnesene	20.64	0.15	GC-MS
(Z,E)- 3,7,11-trimethyl-1,3,6,10-Dodecatetraene	20.64	0.14	GC-MS

Table 1. Continues.

Shyobunol	37.12	0.11	GC-MS
Ylangene	19.01	0.10	GC-MS
3,7-Dimethyl-2,6-octadiene-1-ol	16.32	0.09	GC-MS
Isoterpinolene	18.08	0.08	GC-MS
Eremophilene	28.23	0.08	GC-MS
1-methyl-2-(3-methyl-2-buten-1-yl)-1-(4-methyl-3-penten-1-yl) oxetane	31.18	0.08	GC-MS
(4S,5R)-5-Hydroxycaryophyll-8(13)-ene-4,12-epoxide	26.97	0.08	GC-MS
Longifolenaldehyde	27.71	0.07	GC-MS
2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane	36.26	0.07	GC-MS
Curcumene	21.85	0.06	GC-MS
Valencene	29.60	0.06	GC-MS
1-Formyl-2,2,6-trimethyl-3-(3-methyl-but-2-enyl)-6-cyclohexene	41.70	0.04	GC-MS
α-Salinene	33.19	0.03	GC-MS
Subtotal		21.78%	
Diterpenes			
Phytol	35.02	0.73	GC-MS
Isophytol	31.88	0.30	GC-MS
7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	33.99	0.23	GC-MS
Eicosane	42.84	0.12	GC-MS
Abieta-8,11,13-trien-7-one	38.24	0.11	GC-MS
Peucelinendiol	34.73	0.06	GC-MS
(1R,3S)-Cembra-4,7,11,15-tetraen-3-ol	13.25	0.04	GC-MS
(3S,4S,4aS,8aS)-3,4-Epoxy-3,4,4a,5,6,7,8,8a-octahydro-4a,8,8-trimethylnaphthalen-2(1H)-one	41.70	0.04	GC-MS
(1R,3S)-Cembra-4,7,11,15-tetraen-3-ol	43.25	0.04	GC-MS
Eicosane	42.84	0.03	GC-MS
Subtotal		1.70%	
Others			
2-Pentadecanone, 6,10,14 trimethyl-	30.05	2.85	GC-MS
Carvyl acetate	18.14	0.91	GC-MS
γ-Dodecalactone	26.57	0.91	GC-MS
Benzyl benzoate	28.32	0.62	GC-MS
n-Hexadecanoic acid	32.40	0.54	GC-MS
Isobornyl formate	15.30	0.51	GC-MS
Benzyl salycilate	30.52	0.44	GC-MS
Octacosane	45.94	0.39	GC-MS
Pinocarvyl acetate	17.20	0.31	GC-MS
Methyl eugenol	19.91	0.26	GC-MS
α- Ionone	20.49	0.24	GC-MS
Cis- Jasmone	19.80	0.22	GC-MS
Oleic acid	35.90	0.20	GC-MS
1-Cyclohexene-2,6,6-trimethyl-1-butyraldehyde	29.44	0.20	GC-MS
Nonadecane	44.33	0.18	GC-MS
Hentriacontane	53.55	0.18	GC-MS
Phenanthrene	28.52	0.17	GC-MS
Methyl palmitate	31.45	0.14	GC-MS
Elemicin	23.82	0.12	GC-MS
10-Heneicosene	36.36	0.12	GC-MS

Table 1. Continues.

Calamenen-10-one	27.05	0.10	GC-MS
Cyclotetracosane	39.68	0.09	GC-MS
Di-(2-ethylhexyl)phthalate	42.13	0.09	GC-MS
1,7-dimethyl-2-oxo-7-(4'-formyl-butyl)-norbornane	30.75	0.07	GC-MS
n-Tricosane	38.13	0.07	GC-MS
Tetracosane	39.76	0.06	GC-MS
Nonadecane	41.33	0.06	GC-MS
beta.-Cyclo-homogeraniol	36.20	0.04	GC-MS
Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-	18.62	0.04	GC-MS
(3S,4S,4aS,8aS)-3,4-Epoxy-3,4,4a,5,6,7,8,8a-octahydro-4a,8,8-trimethylnaphthalen-2(1H)-one	40.39	0.04	GC-MS
(3.beta.,5.alpha.)- 2-methylene,Cholestan-3-ol	45.06	0.02	GC-MS
Naphthalene, 1,2-dihydro-1,1,6-trimethyl-	18.55	0.01	GC-MS
Subtotal		10.20%	
Total		73.07%	

Table 2. Larvicidal activity of essential oil of *S. nilotica* against larvae of *R. appendiculatus* between 0 and 48 h.

Conc (mg/ml)	Mean % mortality ± SD				
	3 h	6 h	12 h	24 h	48 h
5	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
4	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
4.5	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
3.7	96.67 ± 0.58	98.33 ± 0.58	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
3.5	90.00 ± 1.00	93.33 ± 0.58	98.33 ± 0.58	100.00 ± 0.00	100.00 ± 0.00
3.3	78.00 ± 0.58	88.33 ± 1.53	93.33 ± 0.58	96.67 ± 0.58	98.33 ± 0.58
3	75.00 ± 1.00	86.67 ± 0.58	88.33 ± 1.53	95.00 ± 0.00	96.67 ± 0.58
2.7	68.33 ± 2.08	83.33 ± 1.53	86.67 ± 2.08	91.67 ± 1.15	95.00 ± 1.00
2.5	48.33 ± 1.15	78.33 ± 1.53	83.33 ± 1.53	85.00 ± 1.00	90.00 ± 1.00
2.3	41.67 ± 1.53	66.67 ± 1.53	76.67 ± 1.53	83.33 ± 1.53	85.00 ± 1.00
2	30.00 ± 1.00	56.67 ± 1.53	70.00 ± 1.00	75.00 ± 2.00	73.33 ± 0.58
1.7	21.67 ± 0.58	50.00 ± 1.00	60.00 ± 1.00	61.67 ± 1.53	66.67 ± 2.31
1.5	16.67 ± 0.58	26.67 ± 1.53	35.00 ± 1.73	38.33 ± 2.08	43.33 ± 1.15
1.3	8.33 ± 0.58	11.67 ± 0.58	25.00 ± 1.00	26.67 ± 0.57	30.00 ± 1.00
1	1.67 ± 0.58	5.00 ± 1.00	18.33 ± 0.58	23.33 ± 0.57	26.67 ± 0.58
0.5	0.00 ± 0.00	1.67 ± 0.58	6.67 ± 1.53	10.00 ± 2.00	11.67 ± 1.53
Amitraz® (0.2v/v)[x]	0.00 ± 0.00	0.00 ± 0.00	11.33 ± 1.53	90.00 ± 3.46	100.00 ± 0.00
2% DMSO + H_2O[y]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LC_{50}	1.8 (1.6 - 2.1)	1.7 (1.5 - 1.9)	1.5 (1.2 - 1.7)	1.4 (1.2 - 1.6)	1.4 (1.2-1.5)
LC_{90}	3.3 (2.9 - 3.9)	3.1 (2.7 - 3.7)	3.0 (2.4 - 3.7)	2.9 (2.4 - 3.5)	2.6 (2.3-2.9)

[x]Positive control, [y]Negative control.

demonstrated by Re et al. (2000) to be neurotoxin to insects. It affects ion transport and the release of acetylcholine esterase to the nervous system. It was reported that linalool posted high acaricidal activity by vapor action against mobile stages of *Tyrophagus putrescentiae* and toxic to the eggs and larvae of insects (Tripathi et al., 2009). Another monoterpene present in appreciable amount in the oil was β-myrcene. Basing on previous literature β-myrcene isolated from *Clausena excavate* essential oil exhibited larvicidal activity against *Aedes aegyptii* and *A. albopictus* larvae (Cheng et al., 2008) and repellent activity against *R. appendiculatus* and *Sitophilus zeamais* (Ndungu, 1995b). Caryophyllene oxide also present in appreciable amount in *S. nilotica* oil had larvicidal activities against mosquito parasite *Anthropophagus* (Oztürk et al., 2009; Zhu and Tian, 2013),

Table 3. Adulticidal activity of essential oil of *S. nilotica* against adults of *R. appendiculatus* between 0 and 48 h.

Conc. (mg/ml)	Mean% Mortality ± SD				
	3 h	6 h	12 h	24 h	48 h
5	45.00 ± 1.00	81.70 ± 12.60	98.30 ± 2.90	100.00 ± 0.00	100.00 ± 0.00
4.5	21.70 ± 1.20	75.00 ± 5.00	95.00 ± 5.00	100.00 ± 0.00	100.00 ± 0.00
4	13.30 ± 1.20	56.70 ± 5.80	83.30 ± 10.40	95.00 ± 5.00	100.00 ± 2.90
3.7	6.70 ± 0.60	43.30 ± 7.60	70.00 ± 10.00	88.30 ± 2.90	93.30 ± 8.70
3.5	3.30 ± 1.20	30.00 ± 10.00	68.30 ± 7.60	76.70 ± 2.90	85.00 ± 5.80
3.3	0.00 ± 0.00	23.30 ± 7.60	63.30 ± 7.60	75.00 ± 5.00	83.30 ± 7.60
3	0.00 ± 0.00	16.70 ± 5.80	58.30 ± 7.60	70.00 ± 10.00	76.70 ± 2.90
2.7	0.00 ± 0.00	15.00 ± 5.00	50.00 ± 5.00	63.30 ± 7.60	66.70 ± 5.80
2.5	0.00 ± 0.00	11.70 ± 7.60	41.70 ± 2.90	53.30 ± 5.80	53.30 ± 2.90
2.3	0.00 ± 0.00	5.00 ± 5.00	36.70 ± 2.90	45.00 ± 5.00	46.70 ± 2.90
2	0.00 ± 0.00	3.30 ± 5.80	26.70 ± 5.80	35.00 ± 5.00	38.30 ± 2.90
1.7	0.00 ± 0.00	1.67 ± 2.90	20.00 ± 5.00	31.70 ± 2.90	33.30 ± 2.90
1.5	0.00 ± 0.00	0.00 ± 0.00	10.00 ± 0.00	26.70 ± 5.80	26.70 ± 5.80
1.3	0.00 ± 0.00	0.00 ± 0.00	5.00 ± 5.00	18.30 ± 5.80	20.00 ± 5.00
1	0.00 ± 0.00	0.00 ± 0.00	1.70 ± 2.90	10.00 ± 10.00	10.00 ± 10.00
0.5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.70 ± 2.90
Amitraz® (0.2%v/v)[x]	0.00 ± 0.00	0.00 ± 0.00	68.30 ± 16.07	96.67 ± 5.77	100.00 ± 0.00
2% DMSO + H_2O[y]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
LC_{50}	-	3.9 (3.6 - 4.2)	2.7 (2.5 - 2.9)	2.2 (2.1 - 2.4)	2.1 (1.9 - 2.3)
LC_{90}	-	5.8 (5.2 - 7.0)	4.7 (4.15 - 5.5)	4.1 (3.7 - 4.8)	3.8 (3.4 - 4.4)

[x]Positive control, [y]Negative control.

repellency activity against *A. gambiae* (Omolo et al., 2004) and acaricidal activity (Birkett et al., 2011). A monoterpene α-terpineol present in good amounts in the *S. nilotica* oil was reported to have insecticidal and acaricidal activity against *Pediculus humanus capitis* (Yang et al., 2009), rice weevil *Sitophilus oryzae* (Lee et al., 2008) and two house dust mite *Dermatophagoides farinae* and *D. pteronyssinus* (Kim et al., 2008).

According to Shelley et al. (2004), terpinen-4-ol and α–terpineol demonstrated real acaricidal activity against two-spotted mites with LC_{50} value of 96 ppm for the former. Commercial 4-terpineol was also found to be very toxic to both sexes of *Acanthoscelides obtectus* adult mites (Papachristos et al., 2004). Thorsell et al. (2006) reported the repellence of 4-terpineol and pinene against the nymphs of sheep tick *Ixodes ricinus*. α- pinene again exhibited strong acute toxicity against *Pear psylla* with LD_{50} and LD_{75} values of 1.34 and 11.76 µg/adult. The component α-pinene again a dominant in *Laurus novocanariensis* essential oil demonstrated potent acaricidal activity against *Psoroptes cuniculi* mites with 100% mortality at concentrations of 10% and 5% within 24 h (Macchioni et al., 2006). Viridiflorol in *Eucalyptus robust* also exhibited insecticidal activity (Liu et al., 2014). It was reported by Warner and Illman (1994) that the presence of the three monoterpene β-phellandrene, β-myrcene and δ-3-carene were important in host defence against spruce, and were also reported toxic or repellent to most *Scolytidis* species (bark beetles) (Bordasch et al., 1977). Also according to Kosgei et al. (2014) the active acaricidal activity demonstrated by the *L. kituiensis* oil was attributed to a synergism of various components presents which included δ-3-carene, phellandrene, β-myrcene and linalool among many that are mentioned in this study. Hence it was concluded that the presence of the mentioned components in *S. nilotica* essential oil are responsible in one way or another in the acute toxicity against *R. appendiculatus*.

Essential oils are volatile and hence prone to aerial oxidation, and thus it tends to lose its activity with time (Birkett et al., 2008). This is evident with an observed slight difference in lethal concentration values between 24 and 48 h .100% mortality of the *S. nilotica* oil was observed for 4.5, 3.7 and 3.5 mg/ml at 3, 12 and 24 h respectively for the larvae while for the adults 100% mortality was observed at 4.5 and 4.5 mg/ml at 24 and 48 h respectively. The oil was very active given that the positive control Amitraz® recorded 100% mortality at 48 h while the negative control recorded 0% mortality for all the hours observed. The difference in activity between the essential oil with the positive control could be attributed to the difference in concentrations of components involved synergistically (Ribeiro et al., 2011). Convulsions, spasms and tremors followed by paralysis were observed for both the larvae and the adults in the high concentrations of 3.7 to 5 mg/ml and positive control

immediately they were exposed. This is because the vapors of the essential oil interfere with the central nervous system by blocking the penetration of chloride ions in the cell which leads to tremors, spasms and paralysis and finally leading to death in the ticks (Roma et al., 2013; Mencke, 2006).

Paralytic signs were continuously observed, even though the ticks in lower concentration at 0.5 to 1.5 mg/ml moved their appendages when prodded by a pin. Acute toxicity of the oil was more effective against the larvae than the adults. This could be due to the morphological difference between the two. The larval membrane is more permeable than the adults hence the essential oil fat soluble permeated the membranes of the skin then captured by the micro-circulation and drained into the circulation system (Moss et al., 2003). The high acaricidal activity of the *S. nilotica* oil can, therefore, be attributed to the presence of β-phellandrene, δ-3-carene, γ-terpinene, α-terpineol, β-myrcene caryophyllene oxide, caryophyllene and pinene which has been reported in previous documents to possesses broad-spectrum insecticidal and acaricidal activity.

CONCLUSION

The acaricidal properties demonstrated by the *S. nilotica* essential oil and its components against *R. appendiculatus* in this study provide some scientific foundation for the integration of botanicals in this tick control. Follow-up studies more possible effects of the oil should be studied and exploited in partially refined products used to protect livestock against infestations by *R. appendiculatus* and other tick species.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. S. T. Kariuki from the Egerton University, Biological Sciences for identification of plant species; Egerton University for offering technical and administrative support and Technical Institute of Berlin, Germany for conducting the GC-MS analysis of the oil. The authors are also thankful to Richard Ochieng' for assisting in the rearing of *R. appendiculatus* larvae and adults.

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Citation: Nyabayo CT, Matasyoh JC, Mwendia C, 2015. Chemical composition and acaricidal activity of *Salvia nilotica* essential oil against *Rhipicephalus appendiculatus*. Adv Med Plant Res, 3(2): 46-54.