Available online at http://ajol.info/index.php/ijbcs

Int. J. Biol. Chem. Sci. 8(4): 1938-1947, August 2014

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper

http://indexmedicus.afro.who.int

Chemical composition and larvicidal activity of essential oil of *Lippia kituiensis* against larvae of *Rhipicephalus appendiculatus*

Caroline J. KOSGEI [1*], Josphat C. MATASYOH [2], Charles M. MWENDIA[1], Samuel T. KARIUKI [3] and AbdI Y. GULIYE [4]

[1]*Department of Biochemistry, Egerton University. P. O. Box 536, Egerton-20115, Kenya*
[2]*Department of Chemistry, Egerton University.P. O. Box 536, Egerton-20115, Kenya.*
[3]*Department of Biological Sciences, Egerton University.P. O. Box 536, Egerton-20115, Kenya.*
[4]*Department of Animal Science, Egerton University.P. O. Box 536, Egerton-20115, Kenya.*
[*]*Corresponding Author; E-mail:rocachep@yahoo.com, cell phone: +254-707-309-338*

ABSTRACT

This study evaluated the larvicidal activity of essential oil of *Lippia kituiensis* leaves against larvae of *Rhipicephalus appendiculatus*. The oil was obtained by hydro-distillation of fresh leaves and analysed using Gas Chromatography-Mass Spectrometry (GC-MS). Analysis showed that sesquiterpenes were dominant (56.57%), followed by monoterpenes (36.36%), diterpenes (2.59%) and others (5.19%). Major sesquiterpenes were germacrene D, β-bourbonene, gamma cadinene and 2-isopropyl-5-methyl-9-methylene-bicycle (4.4.0)dec-1-ene. Major monoterpenes were (1S, 4S)-(-)-camphor, *trans*-sabinene hydrate, gamma-terpinene, dl-limonene, alpha-terpinolene, l-Phellandrene, beta-Myrcene, sabinene, camphene, alpha-pinene, 4-terpineol, 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol, 14.29 borneol (=endo-borneol), camphore, and neo-allo-ocimene. At 6, 12, 24, and 48 hrs after larval treatment, the oil showed activity against *R. appendiculatus* larvae with LC_{50}in mg/ml of 3.26(3.14-3.38), 3.21(3.08-3.32), 3.15(3.03-3.26), 3.09(2.97-3.20) while LC_{90}in mg/ml were 4.15 (3.95-4.45), 4.03 (3.85-4.30), 3.94 (3.77-4.19), 3.86 (3.69-4.09) respectively. Results of one way ANOVA (Analysis of Variance) showed there was no significant difference in activity of the oil against the larvae, between 6, 12, 24 and 48 hrs in all the concentrations used P = 0.97, 95% confidence. The findings indicatedthat essential oil of *L. kituiensis* possessed larvicidal properties and can be used to control tick larvae.

Keywords: *Rhipicephalus appendiculatus;* essential oils; acaricidal activity, ticks.

INTRODUCTION

R. appendiculatus is a three-host ixodid tick and is a vector of major economic importance in Africa. It transmits *Theileria parva* which causes East coast fever, a threatening disease in the livestock industry in Eastern, South-eastern and Central Africa (Olwoch et al., 2008).The use of synthetic chemical acaricides to control ticks in cattle is expensive and ineffective, besides, causing environmental pollution (Annanet al., 2009).

Plant products have been used for curative purposes since ancient times (Pattanayak et al., 2010), and present-day research is geared towards finding effective natural products to replace synthetic chemicals. There are huge prospects in the use of extracts and essential oils of plants from tropical and subtropical regions of Africa,

DOI : http://dx.doi.org/10.4314/ijbcs.v8i4.53

Asia and South America, to come up with acaricides that can decrease the cost of tick control (Habeeb, 2010).

Essential oils from plants constitute a rich source of bioactive chemicals which have broad insecticidal activity (Abad et al., 2012). This is because they possess various bioefficacies that include repellent, antifeedant and respiration inhibitors (Akhtar and Isman, 2004). Among the plant essential oils that exhibited acaricidal properties is oil of *Chenopodium ambrosioides* which has potential to increase the mortality of adults and eggs of two-spotted spider mite, *Tetranychus urticae* (Chiassonet al., 2004). Likewise, essential oil from flowers of *Ageratum houstonianum* showed activity against *Rhipicephalus lunulatus* (Pamo et al., 2004) while essential oil of *Origanum onites* was acaricidal against *Rhipicephalus turanicus* (Coskunet al., 2008).

Lippia is a genus of flowering plants in the verbena family (*Verbenaceae*) that are aromatic due to their essential oils (Arthur et al., 2011). The leaves of certain *Lippia* species such as *Lippia graveolens*, can be used as a culinary herb (Tucker et al., 2009). Other therapeutic functions of genus *Lippia* include remedy for gastrointestinal and respiratory disorders, and are also reported to have antimalarial, spasmolitic, sedative, hypotensive, and anti-inflammatory effects (Pascualet al., 2001; Jigam et al., 2009). Camphor isolated from *L. kituiensis* essential oil was found to have a strong repellent activity against maize weevil compared with NN-diethyltoluamide (DEET) (Mwangi, 1992).

Essential oil of *Lippia graveolens* and *Lippia javanica* are among oils of *Lippia* species whose acaricidal efficacy have been ascertained (Magano et al., 2011; Martinez-Velazquez et al., 2011). It was shown that *L. graveolens* can cause 90-100% mortality on 10 day *Rhipicephalus microplus* tick larvae (Martinez-Velazquez et al., 2011) while *L. javanica* was found to be repellant against adults cattle ticks of *Hyalomma marginatum* (Maganoet al., 2011). To the best of our knowledge, acaridal efficacy of *L. kituiensis* essential oil has not been done. The aim of this study was thus to determine the chemical components present in the oil and its larvicidal properties against *R. appendiculatus.*

MATERIALS AND METHODS

Sample collection

Leaves of *L. kituiensis* were collected from botanical-garden of Egerton University in Kenya which is at an altitude of 2127 m. A voucher specimen has been deposited at the Department of Biological Sciences, Egerton University.

Extraction of essential oil

Fresh leaves of *L. kituiensis* were subjected to hydro-distillation in a modified clevenger-type apparatus for at least 4 hrs according to British pharmacopoeia (Papachristos and Stamopoulos, 2004). The essential oil obtained was dried over anhydrous sodium sulphate then stored in sealed glass vials at 4 ^{o}C awaiting chemical composition analysis using GC-MS and larvicidal bioassay done.

GC-MS analysis of oil

Samples of essential oils were diluted in methyl-t-butyl ether (MTBE) (1:100) and analyzed on an Agilent GC-MSD apparatus equipped with an Rtx-5SIL MS (‘Restek’) (30 m x 0.25 mm, 0.25 µm film thickness) fused-silica capillary column. Helium (at 0.8 mL/min) was used as a carrier gas. Samples were injected in the split mode at a ratio of 1:10 – 1:100. The injector was kept at 250 ºC and the transfer line at 280 ºC. The column was maintained at 50 ºC for 2 min and then programmed to 260 ºC at 5 ºC /min and held for 10 min at 260 ºC. The MS was operated in the electron impact ionization (EI) mode at 70 eV, in m/z range 42-350. The identification of

compounds was performed by comparing their retention indices and mass spectra with those found in literature (Adams, 2007) and supplemented by Wiley 7N.l, HPCH 1607.L and FLAVORS.L GC-MS libraries. The relative proportions of the essential oil constituents are expressed as percentages obtained by peak area normalization, all relative response factors being taken as one.

Larval bioassay

The larvae used for the bioassay were reared according to Bailey (1960). The oil was first dissolved in 2% Dimethly Sulfoxide (DMSO) and a stock solution of 4.5 mg/ml made. The stock solution was obtained after conducting preliminary screening. Serial dilution of 4.5 mg/ml resulted in 14 concentrations ranging from 1.5 mg/ml to 4.5 mg/ml. The bioassay was done using contact toxicity according to Pamo et al. (2005) with modification. This involved attaching Whatman No. 1 filter paper to the bottom of the Petri dish (15 cm) using double sided cellophane tape and 20 larvae placed inside. Different concentrations of the oil was then sprinkled on the Petri dish containing the larvae using a pasture pipette making sure that filter paper was wet and the larvae came in contact with the sprinkled extract. The Petri dish was kept at 25 ^{o}C and 75% relative humidity and experiment replicated three times. From the start of the experiment, mortality data was obtained at 6, 12, 24, and 48 hrs under a microscope x 4.5. The larvae were considered dead if they do not move their appendages when prodded with a pin. A negative control was set consisting of 2% DMSO while positive control consisted of 0.2% v/v of amitraz®.

Statistical analysis

The mortality data obtained was subjected to Probit regression analysis to calculate concentration dependent mortality for the LC_{50} and LC_{90} values and the associated 95% confidence interval. The significant difference in activity between 6, 12, 24 and 48 hrs was analyzed using one way ANOVA.

RESULTS

Table 1 shows the results of the GC-MS analysis of *L. kituiensis* oil. Sesquiterpenes were dominant (56.57%) followed by monoterpenes (36.36%), diterpenes (2.59%) and others (5.19%). Major sesquiterpenes were germacrene D, β-bourbonene, gamma-cadinene and 2-isopropyl-5-methyl-9-methylene- bicyclo-(4.4.0)dec-1-ene while major monoterpenes include (1S,4S)-(-)- camphor, trans-sabinene hydrate, gamma-Terpinene, dl-limonene, alpha-terpinolene, l-phellandrene, beta-myrcene, sabinene, camphene, alpha-pinene, (-)- 4-terpineol, 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol, 14.29 borneol (=endo-borneol), camphore, and neo-allo-ocimene. Major sesquiterpenes and monoterpenes are those with % area above 1 and are highlighted in Table 1.

Mean percentage larval mortalities at 6, 12, 24, and 48 hrs after treatment together with LC values are shown in Table 2. LC_{50} in mg/ml were: 3.26 (3.14-3.38), 3.21(3.08-3.32), 3.15(3.03-3.26), 3.09(2.97-3.20), while LC_{90} were 4.15 (3.95-4.45), 4.03(3.85-4.30), 3.94 (3.77-4.19), 3.86(3.69-4.09) at 6, 12, 24, and 48 hrs respectively. The highest concentration of 4.5 mg/ml caused 100% mortality at the first 6 hrs while amitraz (0.2% v/v) ® recorded the same % mortality at 48 hrs. The negative control used was 2% of DMSO which showed no activity against the larvae within 0 to 48 hrs. The results of one way ANOVA showed there was no significant difference in activity between 6, 12, 24 and 48 hrs in all the concentrations of the oil used against the larvae P =0.97 at 95% confidence.

Table 1: *L. kituiensis* essential oil components.

Name of Compound	Retention Time	Percent Concentration	Method of Detection
Monoterpenes			
delta3 carene	6.44	0.08	GC-MS
2-methyl-5-(1-methylethyl)-Bicyclo(3.1.0)hex-2-ene	6.60	0.24	GC-MS
alpha-pinene, (-)-	**6.77**	**2.04**	GC-MS
Camphene	**7.23**	**7.26**	GC-MS
Sabinene	**7.91**	**2.15**	GC-MS
beta-myrcene	**8.36**	**1.49**	GC-MS
l-Phellandrene	**8.72**	**1.11**	GC-MS
alpha-terpinene	9.08	0.55	GC-MS
dl-limonene	**9.52**	**6.52**	GC-MS
cis-ocimene	9.69	0.07	GC-MS
beta-trans-ocimene	10.01	0.24	GC-MS
gamma-terpinene	**10.31**	**1.22**	GC-MS
trans-sabinene hydrate	**10.71**	**4.45**	GC-MS
cis-sabinene hydrate	10.77	0.88	GC-MS
alpha.-terpinolene	**11.20**	**1.44**	GC-MS
linalool	12.11	0.20	GC-MS
neo-allo-ocimene	**12.45**	**2.39**	GC-MS
camphor, (1S,4S)-(-)-	**13.26**	**18.29**	GC-MS
Camphor	**13.35**	**3.49**	GC-MS
Pinocarvone	13.57	0.45	GC-MS
14.29 borneol (=endo-borneol)	**13.87**	**1.77**	GC-MS
4-methyl-1-(1-methylethyl)- 3-Cyclohexen-1-ol	**13.97**	**1.43**	GC-MS
4-terpineol	**14.10**	**3.03**	GC-MS
p-cymen-8-ol	14.18	0.39	GC-MS
l-verbenone	14.82	0.36	GC-MS
trans-(+)-carveol	15.01	0.17	GC-MS
dill ether	15.27	0.09	GC-MS
7-(1-methylethylidene)- Bicyclo[4.1.0]heptane	15.63	0.23	GC-MS
Total	**28**	**36.36%**	
Sesquiterpenes			
alpha-Cubebene	18.43	0.03	GC-MS
Ylangene	19.01	0.06	GC-MS
Copaene	19.16	0.48	GC-MS
beta-bourbonene	**19.43**	**1.36**	GC-MS
beta-Cubebene	19.50	0.26	GC-MS
Italicene	19.85	0.05	GC-MS
alpha-cedrene	20.08	0.06	GC-MS
Germacrene D	**20.35**	**3.20**	GC-MS
alpha-Guaiene	20.75	0.02	GC-MS
alpha-Elemene	21.01	0.06	GC-MS
gamma-Cadinene	**21.17**	**1.00**	GC-MS
allo-aromadendrene	21.35	0.88	GC-MS

gamma muurolene	21.70	0.54	GC-MS
ar-Curcumene	21.83	0.64	GC-MS
1H-Cyclopropa[a]naphthalene, decahydro-1,1,3a-trimethyl-7-methylene-, [1as-(1a.alpha.,3a.alpha.,7a.beta.,7b.alpha.)]-	21.98	0.04	GC-MS
2-isopropyl-5-methyl-9-methylene- Bicyclo[4.4.0]dec-1-ene	**22.26**	**1.05**	GC-MS
(E,Z)-alpha-farnesene	22.41	0.09	GC-MS
Zingiberene	22.51	0.10	GC-MS
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-	22.59	0.06	GC-MS
30.19 cubebol<10-epi->	22.66	0.42	GC-MS
delta-Cadinene	22.81	0.22	GC-MS
alpha-selinene	22.86	0.03	GC-MS
cadina-1,4-diene	23.00	0.04	GC-MS
Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,[1R-(1.alpha.,4a.alpha.,8a.alpha.)]-	23.14	0.07	GC-MS
Cadala-1(10),3,8-triene	23.28	0.03	GC-MS
2,4a,8,8-tetramethyl-1,1a,4,4a,5,6,7,8-octahydro-cyclopropa[d]naphthalene	23.47	0.09	GC-MS
1,5-epoxysalvial-4(14)-ene	23.85	0.07	GC-MS
beta-Gurjunene	23.98	0.12	GC-MS
1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	24.07	0.09	GC-MS
1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.)]-	24.13	0.27	GC-MS
(-)-Caryophyllene oxide	24.25	0.11	GC-MS
28.15 germacrene d	24.74	0.16	GC-MS
Caryophyllene oxide	24.84	0.05	GC-MS
33.60 cubenol<1,10-di-epi->	24.98	0.27	GC-MS
Epizonarene	25.21	0.16	GC-MS
Valencene	25.55	0.34	GC-MS
1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)- Naphthalene (gamma-muurolene)	25.67	0.14	GC-MS
t-Muurolol	25.85	0.24	GC-MS
Isoaromadendrene epoxide	27.05	0.02	GC-MS
cis-4,11,11-Trimethyl-8-methylenebicyclo(7.2.0)undeca-4-ene	27.22	0.02	GC-MS

40.52 Cadinene<14-hydroxy-delta->	29.00	0.08	GC-MS
1-Methylidene-2b-hydroxymethyl-3,3-dimethyl-4a-(3-methylbut-2-enyl)-cyclohexane	29.11	0.04	GC-MS
caryophylla-3,8(13)-dien-5.beta.-ol	29.86	0.02	GC-MS
Total	**43**	**56.57%**	
Diterpenes			
phytol isomer	34.91	0.04	GC-MS
n-Eicosane	44.29	0.01	GC-MS
Total	**2**	**2.59%**	
Others			
beta-fenchyl alcohol	14.36	0.81	GC-MS
2-methoxy-3-(2-propenyl)- Phenol	18.69	0.16	GC-MS
Methyleugenol	19.93	0.12	GC-MS
(1s,2s,5R)-(+)-4-Isopropenyl-7-methyl-1-oxaspiro(2,5)octane	27.54	0.02	GC-MS
Total number	**4**	**5.19%**	

Table 2: Larvicidal activity of essential oil of *L. kituiensis* against larvae of *R. appendiculatus* between 0-48 hrs.

Concentration in mg/ml	**Mean (%) larval mortality±SD at the hrs shown below**			
	6	12	24	48
1.50	0±0.00	0±0.00	0±0.00	0.00±0.00
2.00	1.67±2.87	1.67±2.87	1.67±2.87	1.67±2.87
2.20	3.33±2.87	3.33±2.87	3.33±2.87	3.33±2.87
2.50	11.67±2.87	11.67±2.87	11.67±2.87	13.33±2.87
2.80	20±5.00	20±5.00	23.33±5.77	28.33±2.87
3.00	35±5.00	38.33±5.77	41.67±5.00	46.7±2.87
3.30	43.33±7.64	50±5.00	51.67±7.64	56.6±7.640
3.45	51.67±7.64	56.67±7.64	61.67±2.87	65±5.00
3.65	60±2.87	66.67±2.87	70±5.00	75±5.00
3.70	73.3±2.87	75±5.00	78.33±5.00	83.33±2.87
3.85	81.7±5.77	85±5.00	88.33±5.77	91.7±7.64
4.00	90±5.00	96.67±2.87	100±0.00	100±0.00
4.20	98.33±2.87	100±0.00	100±0.00	100±0.00
4.50	100±0.00	100±0.00	100±0.00	100±0.00
LC_{50}	3.26(3.14-3.38)	3.21(3.08-3.32)	3.15(3.03-3.26)	3.09(2.97-3.20)
LC_{90}	4.15(3.95-4.45)	4.03(3.85-4.30)	3.94(3.77-4.19)	3.86(3.69-4.09)
Amitraz ® (0.2% v/v)[P]	0±0.00	56.67±11.55	90.00±10	100±0.00
DMSO +distilled H_2O[Q]	0±0.00	0±0.00	0±0.00	0±0.00

[P] Positive control; [Q] Negative control

DISCUSSION

GC-MS analysis of essential oil of *L. kituiensis* showed sesquiterpenes being dominant. The domination of sesquiterpenes in the present study compared with previous acaricidal studies on *Drimysbrasiliensis* essential oil, which contained predominantly sesquiterpenes (66%), and it caused 100% mortality on the larvae of *Rhipicephalus microplus*, at concentrations of 2.5%, 1.25% and 0.625% (Ribeiroet al., 2008).GC-MS analysis of *L. kituiensis* oil in Tanzania showed variation with current study with camphor having 36.5% and 4-thujanol having 18.5% (Chogo and Crank, 1982). This could be because of essential oils of the same *Lippia* species growing in different geographical areas have been found to vary significantly in composition because of climatic conditions, large genetic diversity within the species and stable genetic traits of individual plants (Catalan and de Lampasona, 2002).

The oil showed larvicidal activity against *R. appendiculatus*. The activity of the oil against the larvae could be attributed to synergistic action of major components present in the oil whose acaricidal bioefficacy have been ascertained (Park et al., 2008). However, minor components present in the oil may also have contributed to the biological activity of the oil (Iacobellis et al., 2005).

Among the major compounds present in that oil acaricidal activity include alpha pinene, camphor, germacrene D, and 4 - terpeniol. Oil of *Laurus novocanariensis* leaf has been identified to be dominated by alpha pinene (10.4%) and its acaricidal activity of 100% against *Psoroptes cuniculi* mites at concentrations of 10% and 5% after 24 hrs was attributed to the presence of this compound (Macchioniet al.,2006). Other studies have shown alpha pinene rich plant *Eucalyptus globulus* (9.93%), and dl-limonene rich plant *Eucalyptus staigeriana* have been reported to cause 100% mortality on gravid female ticks at concentrations of 15% and 12.5%, respectively in five different concentrations (Chagas et al., 2002). Camphor (56.07%) has been identified as a chief essential oil component of *Ocimum kilim* and *scharicum*, responsible for wide range of therapeutic importance such as antimicrobial, antispasmodic, bactericide, carminative, hepato-protective, antiviral and larvicidal properties (Runyoro et al., 2010). Commercial camphor and terpinen-4-ol were found to be 7 to 48 times more active against both male and female *A. obtectus* adults' mite than the less active monoterpenes tested (Papachristos et al., 2004). Germacrene-D, has been reported to be an effective arthopod repellent (Bruce et al., 2005).

From the results of the bioassay, there was no significant difference in activity between 6, 12, 24 and 48 hrs with $P = 0.97$ at 95% significant difference. This explained the fact that essential oil components are volatile compounds that are prone to rapid aerial oxidation and chemical re-arrangement thus lost activity quickly. Therefore, further increase in time could not increase larval mortality (Birkettet al., 2008). The oil of *L. kituiensis* caused 100% mortality at a concentration of 4.5mg/ml within the first 6 hrs while the positive control amitraz (0.2%v/v) reported the same % mortality at 48 hrs. The difference in activity between the oil extract and the positive control could be due to difference in concentration of the active ingredients present in both. This is because we could not isolate the active ingredient in the oil and quantify its concentration. Further studies should thus be done to isolate individual oil components, determine effects of each component on the tick larvae and quantify concentration of the active compound.

Due to various components present in the essential oils, the exact mode of action of these oils is not well proven. Because of their volatility, they could have been inhaled easily through the respiratory tract and lungs of the larvae which were then distributed in the bloodstream to various target sites (Adorjan and Buchbauer, 2010). Since essential oil are fat soluble, they might have permeated the

membranes of the skin before being captured by the micro-circulation and drained into the systemic circulation. Thus reaching all targets organs too long (Mosset al., 2003). Based on the poisoning symptoms observed on the larvae, there were convulsion and tremors followed by paralysis (knockdown) immediately the oil extract were exposed to larvae, followed by 100% mortality occurring within the first 30 mins at the highest concentration (4.5 mg/ml). The same behavior was observed in the positive control although convulsion and tremors persisted for a longer duration of more than 6 hrs before being knock down and 56.67 % of the larvae dying at 12 hrs.

The paralytic effect of the oil could be due to synergestic action of alpha pinene and camphor on the nervous system, since both have been reported to have anti-acetylcholinesterase activity (Picolloet al., 2008). Paralytic effects of amitraz® were due to blockage of octopamine receptors, which leads to paralysis and death of the larvae (Chenaet al., 2007).

Conclusion

Results of this study indicate that essential oils obtained from *L. kituiensis* plant have acaricidal properties and could be used to control larvae of *R. appendiculaus* and related ticks.

ACKNOWLEDGEMENTS

The authors are grateful to Egerton University for availing their research laboratories for conducting the research. They are also grateful to Technical Institute of Berlin, Germany for conducting the GC-MS analysis of the oil. The authors are also thankful to Richard Ochieng for helping in the rearing of *R. appendiculatus* larvae.

REFERENCES

Abad JM, Bedoya LM, Apaza L, Bermejo P. 2012. The *Artemisia* L. Genus: *A Review of Bioactive Essential Oils. J. Molecules*, **17**: 2542-2566.

Adams RP. 2007. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry* (4th edn). Allured publishing corporation: Carol Stream. Illinois. USA

Adorjan B, Buchbauer G. 2010. Biological properties of essential oils: an updated review. *Flavour Frag J*., **25**: 407-426.

Akhtar Y, Isman MB. 2004. Comparative growth inhibitory and antifeedant effects of plant extracts and pure allelochemicals on four phytophagous insect species. *J Appl Entomol.*, **128**: 32–38.

Annan K, Dickson R, Mensah A, Fleischer TC. 2009. Acaricidal Effect of *Plumbago zeylanica* L. Against *Amblyoma variegatum. Phcog J.*, **1**: 190-194.

Arthur H, Joubert E, De Beer D, Malherbe CJ, Witthuhn RC. 2011. Phenylethanoid glycosides as major antioxidants in *Lippia multiflora* herbal infusion and their stability during steam pasteurization of plant material. *Food Chem.*, **127**: 581–588.

Bailey KP. 1960. Note on the rearing of *Rhipicephalus appendiculatus* and their infection with *Theileria parva* for experimental transmission. *Bull Epizoot Dis Afr.*, **8**: 33-43.

Birkett MAA, Abassi S, Kröber T, Chamberlain K, Hooper AM, Guerin PM, Pettersson J, Pickett AJ, Slade R, Wadham LJ. 2008. Antiectoparasitic activity of the gum resin, gum haggar, from the East African plant, *Commiphora holtziana*. *Phytochemistry.*, **69**: 1710-1715.

Bruce TJA, Brikett MA, Blande J, Prosser L, Smart LE, Wadhame LJ. 2005. Response of economically important aphids to components of Hemizygia petiolata essential oil. Pest *Manag Sci.*, **61**: 1115-1121.

Catalan CAN, lampasona MEP. 2002. The chemistry of the genus *Lippia* (*Verbenaceae*). In *Oregano: The genera Origanum and Lippia* (1st edn), Kintzios SE (ed). Taylor and Francis: London.

Chagas ACS, Passos WM, Prates HT, Leite RC, Furlong J, Fortes ICP. 2002. Acaricide effect of Eucalyptus spp essential oils and concentrated emulsion on *Boophilus microplus*, *Braz. J. Vet. Res. Anim. Sci.*, **39**: 247–253.

Chena AC, Heb H, Davey R B. 2007. Mutations in a putative octopamine receptor gene in amitraz-resistant cattle ticks. *Vet Parasitol.*, **148**: 379–383.

Chiasson H, Bostanian NJ, Vincent C. 2004. Acaricidal properties of a Chenopodium-based botanical. *J. Econ. Ent.,* **97**: 1373–1377.

Chogo J, Crank G. 1982. Essential Oil and Leaf Constituents of *Lippia ukambensis* From Tanzania. *J. Nat. Prod*., **45**:186–188.

Coskun S, Girisgin O, Kürkcüoglu M, Malyer H, Girisgin AO, Kırımer N, Baser KH. 2008. Acaricidal efficacy of *Origanum onites* L. essential oil against *Rhipicephalus turanicus (Ixodidae). Parasitol Research.*, **103**: 259-261.

Habeeb SM. 2010. Ethno-veterinary and medical knowledge of plant extracts and its methods of application (Traditional and Modern) for tick control. *World Appl. Sci. J.*, **11**: 1047-1054.

Iacobellis NS, Lo Cantore P, Capasso F, Senatore F. 2005. Antibacterial activity of *Cuminum cyminum* and *Carum carvi* essential oils. *J. Agric. Food Chem.,* **53**: 57–61.

Jigam AA, Akanya HO, Ogbadoyi EO, Dauda BEN, Egwim CE. 2009. In vivo antiplasmodial, analgesic and anti-inflammatory activities of the leaf extract of *Lippia multiflora* mold. *J. Med. Plants Res.,* **3**: 148-154.

Macchioni F, Perrucci S, Cioni P, Morelli I, Castilho P, Portugal F, Cecchi F.2006. Composition and Acaricidal Activity of *Laurus novocanariensis* and *Laurus nobilis* essential Oils against *Psoroptes cuniculi*. *J. Essent. Oil Res.*, **18**: 111-114.

Magano RS, Nchu F, Eloff JN. 2011. In vitro investigation of the repellent effects of the essential oil of *Lippia javanica* on adults of *Hyalomma marginatum rufipes*. *Afr. J. Biotechnol.*, **10**: 8970-8975.

Martinez-Velazquez M, Rosario-Cruz R, Castillo-Herrera G, Flores-Fernandez JM, Alvarez AH, Lugo-Cervantes E. 2011. Acaricidal effect of essential oils from *Lippia graveolens* (Lamiales: *Verbenaceae*), *Rosmarinus officinalis* (Lamiales: *Lamiaceae*), and *Allium sativum* (Liliales: *Liliaceae*) against *Rhipicephalus (Boophilus) microplus* (Acari: *Ixodidae*). *J. Med. Entomol*., **48**: 822-827.

Moss M, Cook J, Wesnes K, Duckett P. 2003. Aromas of rosemary and lavender essential oils differentially affect cognition and mood in healthy adults. *Int. J. Neurosci*., **113**: 15-38.

Mwangi JW, Addae-Mensah I, Muriuki G, Munavu R., Lwande W, Hassanali A. 1992. Essential Oils of *Lippia* Species in Kenya. IV: Maize Weevil (*Sitophilus zeamais*) Repellancy and Larvicidal Activity. *Pharm. Biol.*, **30**: 9-16.

Olwoch JM, Reyers B, Engelbrecht FA, Erasmus BFN. 2008. Climate change and the tick-borne disease, Theileriosis (East Coast fever) in sub-Saharan Africa. *J Arid Environ*., **72**: 108–120.

Pamo ET, Tendonkeng F, Kana JR, Payne VK, Boukila B, Lemoufouet J, Miegoue E, Nanda AS. 2005. A study of the acaricidal properties of an essential oil extracted from the leaves of *Ageratum houstonianum*. *Vet. Parasitol*., **128**: 319-323

Pamo TE, Tendonkeng F, Kana JR, Tenekeu G, Tapondjou LA, Payne K. 2004. The acaricidal effect of the essential oil of *Ageratum houstonianum* Mill. Flowers on ticks (*Rhipicephalus lunulatus*) in Cameroon. *S. Afr. J. Anim Sci.*, **34**: 244–247.

Papachristos DP, Karamanoli KI, Stamopoulos DC, Menkissoglu-Spiroudi U. 2004. The relationship between the chemical composition of three essential oils and their insecticidal activity against *Acanthoscelidesobtectus*. *Pest. Manag. Sci.,* **60**: 514–520.

Papachristos DP, Stamopoulos DC. 2004. Fumigant toxicity of three essential oils

on the eggs of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). *J. Stored Prod. Res.*, **40**: 517-525.

Park IK, Kim JN, Lee YS, Lee SG, Ahn YJ, Shin SC. 2008. Toxicity of plant essential oils and their components against *Lycoriella ingenua* (*Diptera*: *Sciaridae*). *J. Econ Entomol.*, **39**: 275–279.

Pascual ME, Slowing K, Carretero E, Sánchez mata D, Villar, A. 2001. *Lippia:* traditional uses, chemistry and pharmacology: a review *J. Ethnopharmacol.*, **76**: 201-214.

Pattanayak P, Behera P, Das D, Panda SK. 2010. Ocimum sanctum Linn. A reservoir plant for therapeutic applications: An overview. *Pharmacogn Rev*., **4**: 95–105.

Picollo MI, Toloza AC, Mougabure CG, Zygadlo, J, Zerba, E. 2008. Anticholinesterase and pediculicidal activities of monoterpenoids. *Fitoterapia*., **79**: 271–278.

Ribeiro VL, Rolim V, Bordignon S, Henriques AT, Dorneles GG, Limberger RP, von Poser G. 2008. Chemical composition and larvicidal properties of the essential oils from *Drimys brasiliensis* Miers (Winteraceae) on the cattle tick *Rhipicephalus (Boophilus) microplus* and the brown dog tick *Rhipicephalus sanguineus*. *Parasitol Res.* **102**: 531-535.

Runyoro D, Ngassapa O, Vagionas K, Aligiannis N, Graikou K, Chinou I. 2010. Chemical composition and antimicrobial activity of the essential oils of four *Ocimum* species growing in and Tanzania. *Food Chem*, **119**: 311-316.

Tucker AO, DeBaggio T, DeBaggio F. 2009. *The Encyclopedia of Herbs: a Comprehensive Reference to Herbs of Flavor and Fragrance* (2nd edn). Timber, Portland, Or. : London.