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Chemical Composition And Larvicidal Activity Of Essential Oil From*Lippiajavanica*against*Rhipicephalusappendiculatus*larvae

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Abstract: *Animal ectoparasites particularly ticks contribute majorly to decreased productivity in the livestock industry globally, due to diseases they transmit. This study evaluated the larvicidal activity of essential oil of Lippiajavanicaleaves against larvae of Rhipicephalusappendiculatus. The oil was obtained by hydro-distillation of fresh leaves and oil components analysedusing Gas Chromatography-Mass Spectrometry (GC-MS). Major components in the oil were monoterpenes as shown by the high percentage of Artemisia ketone (49.52%), m-tert-Butylphenol (8.73%), Linalool (4.43%), beta-myrecene (3.13%), Targetone (2.85%) and Isopiperitenone (2.13%). Sesquiterpenes constituted only 6.06% of the total oil. Contact toxicity was used in the bioassay and mortality data obtained at 6, 12, 24 and 48 hrs. The data obtained during bioassay was then subjected to probit regression analysis to estimate concentration dependent mortality for LC_{50} and LC_{90} values in mg/ml. The LC_{50} in mg/ml were: 3.1(3.0-3.2), 3.1(3.0-3.2) 3.1 (3.0-3.2) 3.07(2.94-3.19) and LC_{90} were 3.9(3.7-4.2), 3.9(3.7-4.2), 3.9(3.7-4.2), 3.9(3.7-4.2) at 6, 12, 24 and 48 hrs respectively from the start of the experiment. The results of one way ANOVA 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 of the oil used with P>0.05.The findings showed that essential oil of L. javanicapossessed larvicidal properties and can be used to control tick larvae.*

Key words:*Cytotoxicity, Phytochemicals, Bioassay*

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I. Introduction

Ticks are blood-sucking ectoparasites majorly contributing to decreased productivity in the livestock industry globally due to diseases they transmit. They transmit diseases such as *anaplasmosis, babesiosis, theileriosis,* tick paralysis and *ehrlichiosis* among others (Piesman and Eisen, 2008). Ticks belong to phylum Arthropoda, class Arachnida and subclass Acari. It is a cosmopolitan group with an estimated 50,000 described tick species (Hallida*yet al.,* 2000). Among tick species, *R.appendiculatus* is of major economic importance in Africa since it transmits *Theileriaparva,* which causes *theileriosis* a threatening disease in the livestock industry in Eastern, Southeastern and Central Africa. It also transmits corridor disease in cattle, Nairobi sheep disease virus and thogoto virus to livestock (Walker *et al.,* 2000).Synthetic acaricidal agents currently used are associated with resistance, high costs, presence of chemical residues in animal products and environmental pollution. The concerns about negative effects associated with current acaricidal agents drive efforts in bioprospecting for novel compounds that possessacaricidal activity and are safe. Essential oils from plants have been reported to have various bio-efficacies, which include; antibacterial, antifungal, antiviral, antiparasitic and insecticidal (Plaza *et al.,* 2004). The enormous bioactivity of the essential oils has been attributed to low molecular weight of the volatile components present in the oil enabling them to diffuse rapidly through the skin membranes and thus reaching target sites quickly (Gutierrez *et al.,*2009). Among plant essential oils that have been reported to exhibit acaricidal properties is the essential oil from *Eucalyptus citriodora*which achieved 85% mortality against *Dermanyssusgallinae* mite (David*et al.,* 2009). Similarly, essential oil from *Origanumonites*exhibited acaricidalactivityagainst*Rhipicephalusturanicus* (Coskun*et al.,* 2008) while essential oil of *Lippiagracilis*Schauer showed acitivity against *Rhipicephalus (Boophilus) microplus*(de Oliveira *et al.,* 2013).The plant *L. javanica*belong to *Verbenaceae*family that has about 200 species. These plants are often aromatic due to presence of essential oils and are distributed throughout Central and South Americas as well as in tropical Africa (Arthur *et al.,* 2011). Previous studies on *L. javanica*essetial oilshowed it had strong and lasting repellent activity against adult*Hyalommamarginatum* cattle tick (Maganoe*t al.,* 2011). *L. javanica* aqueous leaf extracts at 10% and 20% w/v were found to be effective at controlling *R. appendiculatus, R. evertsi, B. decoloratus* and *Hyalomma* species (bont-legged ticks) found at study site in Zimbabwe (Madzimure*et al.,* 2011). Tea infusions of the leaves of *L. javanica*are used against common symptoms of HIV and AIDS, treatment of lung infections, dysentery and diarrhea (Palgrave *et al.,* 2003).The aim of this study was thus to determine the chemical components present in *L. javanica* oil and its larvicidal properties against *R. appendiculatus.*

II. Materials And Methods

Sample collection

Leaves of *L. javanica*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, EgertonUniversity.

Extraction of essential oil

Fresh leaves of *L. javanica*were subjected to hydro-distillation in a modified clevenger-type apparatus for at least 4 hrsaccording 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

The extracted essential oil was first diluted with methyl-t-butyl ether (MTBE) (1:100) and analyzed on Agilent GC-MSD apparatus that was outfitted with a Rtx-5SIL MS (‘Restek') (30 mm × 0.25 mm,0.25 µm film thickness) . The GC column temperature was maintained at 50°C for 2 min, and then programmed at a temperature of 260°C at the rate of 5°C/min and was held at 260°C for 10 min. Helium was used as the carrier gas (at 0.8 ml/min). The samples were injected in split mode at a ratio of 1:10 to 1:100. The temperature was maintained at 250°C while the transfer line was kept at the temperature of 280°C. The operation of MS was done in the electron impact ionization (EI) mode set at 70 eV, in m/z that ranged from 42 to 350. Compound identification was achieved by comparing the mass spectra and retention indices with ones from the literature (Adams, 2007). It was also supplemented by Wiley and QuadLib 1607 GC-MS libraries. The relative proportions of the essential oil constituents were expressed as percentages obtained by peak area normalization while all relative response factors being taken as one.

Larval bioassay

The larvae used for the bioassay were reared according to Bailey (1960). The oil wasfirst dissolved in 2% DimethlySulfoxide (DMSO) and a stock solution of 4 mg/ml made. The stock solution was obtained after conducting preliminary screening. Serial dilution of 4 mg/ml resulted in 12 concentrations ranging from 1.5 to 4 mg/ml. The bioassay was done using contacttoxicity 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®.

III. Results

Table 1 shows the results of the GC-MS analysis of *L. javanica*essential oil. Major compounds weremonoterpenes and these were artemisia ketone (49.52%), m-tert-Butylphenol (8.73%), Linalool (4.43%), beta-myrecene (3.13%), Targetone (2.85%) and Isopiperitenone (2.13%). Table 2 shows mean percentage larval mortalities obtained at 6, 12, 24, and 48 hrs after treatment. LC_{50} in mg/ml were: 3.1(3.0-3.2), 3.1(3.0-3.2) 3.1 (3.0-3.2) 3.1(2.9-3.2) and LC_{90} were 3.9(3.7-4.2), 3.9(3.7-4.2), 3.9(3.7-4.2), 3.9(3.7-4.2) at , 6, 12, 24 and 48 hrs respectively from the start of the experiment. 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 of the oil against the larvae between 6, 12, 24 and 48 hrs in all the concentrations of the oil used with P>0.05.

Table 1: GC-MS analysis of *L. javanica*essential oil

S/N	R.T (min)	Compound name	% Concentration	Detection method
1	8.45	β – Myrcene	3.13	GC/MS
2	9.18	Menthatriene	0.51	GC/MS
3	10.12	Tagetone	2.85	GC/MS
4	11.23	Bicyclo[3.1.0]hexane,6-ethylene-	0.44	GC/MS
5	11.46	Linalool	4.43	GC/MS
6	12.67	Camphor	0.90	GC/MS
7	13.11	Artemisia Ketone A21	49.52	GC/MS
8	15.21	Phenol, M-tert butyl (Ocimenone)	8.73	GC/MS
9	16.28	Isopiperitenone	2.13	GC/MS

10	20.06	Z-caryophyllene	1.99	GC/MS
11	20.91	4,7,10-Cycloundecatriene,1,1,4,8-etramethyl-, cis, cis, cis-	0.92	GC/MS
12	24.05	Caryophellene oxide	1.31	GC/MS

Table 2: Mean percentage larval mortalities obtained at 6, 12, 24, and 48 hrs

Concentration in mg/ml	**Mean % larval mortality at the hours shown below**			
mg/ml	**0-6**	**6-12**	**12-24**	**24-48**
1.50	0±0	0±0	0±0	0±0
2.00	0±0	0±0	1.7±2.9	5±5
2.22	8.3±7.6	8.3±7.6	8.3±7.6	10±5
2.50	13.3±5.8	15±5	15±5	16.7±2.9
2.80	28.3±5.8	33.3±2.9	36.7±2.9	38.3±2.9
3.00	38.3±2.9	38.3±2.9	43.3±2.9	48.3±2.9
3.20	50±8.7	51.7±5.8	56.7±7.6	60±5
3.45	61.7±7.6	63.3±7.6	66.7±7.6	73.3±5.8
3.65	70±5	71.7±7.6	76.7±2.9	80±5
3.70	85±5	86.7±2.9	90±5	96.7±5.8
3.85	96.7±2.9	100±0	100±0	100±0
4.00	100±0	100±0	100±0	100±0
Amitraz ®(0.2 % v/v) [P]	0±0	53.3±5.8	90±10	100±0
DMSO+distilled H20)[Q]	0±0	0±0	0±0	0±0

[P]positivecontrol,[Q]Negativecontrol

IV. Discussion

Major essential oil components presentwere monoterpenes with percentage yield of 70.79. This compares with essential oil of *Lippiapalmeri*which showed monoterpenes (70.35%) being dominant **(**Ortega-Nieblas*et al.,* 2011). Also essential oil of *Vitexagnus-castus*L was dorminated by high amount ofmonoterpenes and it constituted the predominantchemical class in the oils from the leaves and fruit. The similarity in dominace could be because the plants are of the same genus thus similar chemotype. Nonetheless, variation in essential oil components have been observed even within the same species. Variability in quantity and composition of essential oil in plants have been attributed to their developmental stage, climate factors, altitude, harvest season and plant handling (Dunford and Silva, 2005). The dominance ofmyrcene and linalool in this study compared with*L. javanica* oil in western Zimbabwe which showed the oil majorly contained myrcene (54.0 %) and linalool (68.8 %)(Chagonda and Chalchat, 2015), with the difference in concentration being brought about by difference in ecological conditions.Though Artemisia Ketone reported the highest concentration in this study (49.52), previous studies in Zimbabwe showed the plant had trace amount of this component i.e less than 0.05% indicating difference in climatic conditions (Chagonda and Chalchat, 2015). There is similarity in the major compounds observed with that of yegon*et al.,* (2015). This is because the plant was obtained from the same geographical locality.

The activity observed in the oil against the tick larvae could be due to synergistic action of major components present in the oil, whose acaricidalbioefficacy have been determined (Park *et al.,* 2008). Nevertheless, 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 the oil whose bioa-activity has been determined includeβ-myrcene,tagetone,linalool,camphor, and artemesia ketone.

Myrcenehas been found to be repellent against *R. appendiculatus* and *Sitophiluszeamais*Motschulsky (Ndungu*et al.,* 1 995). Linalool obtained from *ocimumcanum* has been reported to be toxic against *Zabratessubfasciatus* a coleopteran (Weaver *et al.,* 1991) while Linalool isolated from molasses grass *Melinisminutiflora*was found to be lethal to 100 % of *R. microplus* tick larvae in 15 min (Prates *et al.,* 1998). Commercial camphor and terpinen-4-ol were found to be 7 to 48 times more toxic against both male and female *A. obtectus*adults' mite than the less active monoterpenes tested (Papachristos*et al.,* 2004).Tagetonewas one of the major compound in *Tagetesminuta*essential oil that had over 95% efficacy against four species of ticks at a concentration of 20% (Garcia *et al.,*2012).

From the results of the bioassay, there was no significant difference in activity between 6, 12, 24 and 48 hrs in *L. javanica*with $P>0.05$. This is also similar to what was observed in *L. kituiensis*when it was exposed to the same larvae of *R. appendiculatus* (Kosgei*et al.,* 2014). This explains the fact that essential oils are volatile compounds, which are likely to evaporate, and are prone to rapid aerial oxidation and chemical re-arrangement hence loss of activity quickly. Therefore, further increase in time could not increase larval mortality (Birkett*et al.,* 2008).

Results of this study indicate that essential oils obtained from *L. javanica* planthave acaricidal properties and could be used as lead molecules for formulation of acaricidal drugs against larvae of *R. appendiculaus.*

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