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Chemical composition and larvicidal activity of *Zanthoxylum gilletii* essential oil against *Anopheles gambiae*

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Malaria is a serious health problem in many African countries. The *Anopheles gambiae* mosquito which is the major vector for this disease has developed resistance against synthetic pyrethroids which are the main stay of insecticide treated bed nets. The development of insecticide resistance and side effects associated with synthetic pesticides has triggered intense research efforts towards natural products (for vector control) such as essential oils because of their efficacy and safety. In this study, larvicidal potential of essential oil from *Zanthoxylum gilletii* was evaluated against malaria vector mosquito, *A. gambiae*. The essential oil was extracted by hydro-distillation, and its chemical compositions determined by gas chromatography mass spectrometry. The oil was dominated by sesquiterpenes and monoterpenes which accounted for 38.30 and 34.00%, respectively. The oil showed good activity against *A. gambiae* and recorded LC_{50} and LC_{90} values of 57.73 and 140.24 $\times 10^{-3}$ mg/ml, respectively. The results obtained show that the essential oil isolated from *Z. gilletii* is a promising mosquito larvicide.

Key words: Malaria, *Zanthoxylum gilletii*, essential oil, *Anopheles gambiae*, larvicidal activity

INTRODUCTION

Mosquitoes are known vectors of various diseases which are life threatening. *Anopheles gambiae* mosquitoes are known to transmit malaria (Cheng et al., 2003; Das and Ansari, 2003; Magalhaes et al., 2010). According to the latest WHO estimates, there were approximately 219 million cases of malaria globally in 2010 and 660,000 fatal cases: approximately 90% of these fatal cases occurred in Africa (WHO, 2012).

Currently there is no effective available vaccine for malaria (Matasyoh et al., 2008). Among the efforts that have been made in recent decades in seeking to reduce mosquito bites and transmission of malaria include the use of insecticide treated nets (ITNs) and larviciding. The insecticide treated nets rely solely on pyrethroids to enhance their protective utility (Chavasse et al., 1999). Larviciding has the greatest control impact on mosquito populations because the larvae are concentrated, immobile and accessible, and it employs the use of synthetic insecticides (Tiwary et al., 2007). However, the overreliance on these synthetic chemicals for mosquito control has resulted in the development of insecticide resistance over time (Hemingway and Ranson, 2000). The

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spread in resistance to majority of present synthetic insecticides by *A. gambiae* and the environmental pollution coupled with the safely risks for both human and domestic animals posed by these insecticides has highlighted the need for novel strategies for control of *A. gambiae* (Cheng et al., 2009b). Hence, there is a renewed interest in the exploration and use of plant products with insecticidal properties for mosquito control.

Aromatic plants and their essential oils are very important sources of many compounds that are used for different applications (Abduelrahman et al., 2009). Essential oils are simply volatile fractions obtained by either steam or water distillation of medicinal and aromatic plants (Rabha et al., 2012). Essential oils have received considerable renewed attention as potent bioactive compounds against various species of mosquitoes. They are potentially suitable for application in larval control management because they constitute a rich source of bioactive compounds that are effective and naturally biodegradable into non-toxic products (Lucia et al., 2007; Cheng et al., 2008; Cheng et al., 2009a).

Zanthoxylum gilletii is a tropical rainforest species, distributed between altitudes ranging from 900 to 2400 m. It is a valued forest tree that grows naturally but planted in Western Kenya for timber and medicinal properties (Kokwaro et al., 1976). The Luhya community; that is, a major habitat of this region uses the bark of *Z. gilletii* in traditional anti-malaria preparations (Nyunja et al., 2009).

The present study attempted to investigate the larvicidal efficacy of essential oils derived from *Z. gilletii* leaves against the medically important malaria vector mosquito, *A. gambiae* with the purpose of identifying effective indigenous bio-products to control the vector of mosquito-borne diseases, particularly in cases where the vector's susceptibility to conventional synthetics is decreasing.

MATERIALS AND METHODS

Sample collection

The leaves of *Z. gilletii* were collected from Kakamega forest, a tropical rain forest in Kenya which stretches from 0° 10′ to 0° 21′ N and longitude 34° 44′ to 34° 58′ E and an altitude of 1524 m above the sea level. The leaves were identified with the help of a taxonomist. Voucher specimens were deposited at the Department of Biological Sciences, Egerton University, Kenya.

Extraction of essential oil

Fresh leaves of *Z. gilletii* were cut into pieces less than 2 × 2 cm within 12 h after collection and 1000 g hydro-distilled in a modified type-Clevenger apparatus for 4 h. The essential oil obtained was dried over anhydrous Na_2SO_4 and kept in glass vial under refrigeration at 4°C.

Essential oil analysis

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 × 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 to 1:100. The injector was kept at 250°C and the transfer line at 280°C. The 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 MS was operated in the electron impact ionization (EI) mode at 70 eV, in m/z ranging from 42 to 350. The identification of the 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.

Mosquito larvicidal bioassays

The larval toxicity tests were carried out following the standard World Health Organization larval bioassay method (WHO, 2005), with slight modifications. Since oil does not dissolve in water, it was first solubilized in dimethyl-sulphoxide (DMSO, analytical reagent, Lobarchemi) and diluted with spring river water to make a stock solution of 1000 mg/L. Serial dilutions of the stock solution were done at different concentrations which included 500, 250, 200, 150, 125, 100, 62.5, 55, 45, 40, 31.25, 15.6 and 7.8 $\times 10^{-3}$ mg/ml. The concentration of DMSO was kept below 1% since at this level it does not affect larval mortality. The bioassays were conducted at the Kenya Medical Research Institute (KEMRI), Centre for Global Health Research (CGHR), Kisumu, Kenya, where the insects were reared in plastic and enamel trays in spring river water. They were maintained and all experiments were carried out at 26 ± 3°C and the humidity ranged between 70 and 75%. The bioassays were performed with third instar larvae of *A. gambiae* and carried out in triplicate using 20 larvae for each replicate assay. The replicates were run simultaneously yielding a final total of 60 larvae for each concentration. The larvae were collected by direct pipetting from the enamel trays and transferred to 25 ml disposable plastic cups containing 10 ml of test solution and fed on tetramin fish feed during all testing. Mortality and survival was established after 24 h of exposure. Larvae were considered dead if they were unrousable within a period of time, even when gently prodded with a micro-pipette. The dead larvae in the three replicates were combined and expressed as the percentage mortality for each concentration. The negative control was 1% DMSO in spring river water while the positive control was the pyrethrum based larvicide, pylarvex.

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50} and LC_{90} at 95% fiducial limits of upper confidence limit and lower confidence limit (Finney, 1971) using IBM SPSS software version 15.

RESULTS AND DISCUSSION

The essential oil of *Z. gilletii* was dominated by monoterpenes and sesquiterpenes which accounted for 34.00 and 38.30%, respectively. The major monoterpene components included γ-terpinene (10.62%), β-myrcene (5.16%), sabinene (4.89%), β-ocimene (3.12%) and camphene (2.56%). The main sesquiterpene components were *trans*-caryophyllene (9.82%), caryophyllene oxide (4.4%), α-cadinol (2.71%), 1, 1, 4, 8-tetramethyl-4, 7, 10-cycloundecatriene (2.62%), δ-cadinene (2.52%) and τ-cadinol (2.29%) (Table 1).

The essential oil of *Z. gilletii* was active against third

Table 1. Identified compounds of the essential oil from *Zanthoxylum gilletii.*

Compound name	Retention time (min)	Concentration (%)
Monoterpenes		
γ-terpinene	6.93	10.62
β- myrcene	8.54	5.16
Sabinene	7.96	4.89
β-Ocimene	10.19	3.12
Camphene	7.25	2.56
Alloocimene	12.37	1.35
Bornyl acetate	16.77	1.16
3,7-dimethyl-1,6-octadien-3-ol	11.53	0.84
n- Decanal	14.55	0.58
Terpine-4-ol	13.73	0.55
Cis-epoxyocimene	12.73	0.44
2-methyl-2-phenylpropanal	15.53	0.37
Tricyclene	8.81	0.32
(2-methylpropyl)-benzene	16.01	0.16
trans-Sabinene hydrate	10.61	0.13
trans-(+)-carveol	14.95	0.12
Total		34.00
Sesquiterpenes		
trans-Caryophyllene	20.46	9.82
Caryophyllene oxide	24.39	4.40
α-Cadinol	25.99	2.71
1,1,4,8-tetramethyl-4,7,10-cycloundecatriene	21.26	2.62
δ-Cadinene	22.84	2.52
τ -Cadinol	25.67	2.29
β-Cubebene	19.56	1.70
2-isopropyl-5-methyl-9-methylene-bicyclo [4.4.0] dec-1-ene	22.26	1.43
β-Selinene	21.99	1.35
Germacrene D	20.99	0.87
1a,2,3,4,4a,5,6,7b-octahydro-1,1,4,7-tetramethyl-1H-cycloprop (e) azulene	23.21	0.84
2,6,6-trimethyl-5-(3-methyl-2-butyl)-1-cyclohexene-1-methanol	23.87	0.77
α-Copaene	19.15	0.58
3-thujopsanone	30.22	0.39
Juniper camphor	26.86	0.31
β-oplopenone	29.05	0.30
Cis-nerolidol	33.43	0.29
α-Cubebene	18.46	0.23
Total		38.30
Diterpenes		
Phytol	35.02	1.51
5-(decahydro-5,5,8a-trimethyl-2-methylene-1-naphthalenyl)-3-methyl-2-pentenoic acid	33.96	0.28
2,6,10-trimethyl-13-(1-methylethenyl)-2,5,9-cyclotetradectrien-1-ol	38.53	0.04
n-eicosane	41.32	0.04
Total		8.50

Table 1. Contd.

Others		
2-Undecanone	17.44	3.64
Cryptone	14.06	1.00
4-ethenyl-cyclohexenemethanol	23.55	0.98
Octahydro-4a-methyl-7-(1-methylethyl)-2(1H)-naphthalenone	30.81	0.84
8-dodecenol	27.13	0.30
Palmitic acid	32.24	0.24
Nonadecane	44.30	0.24
Nonacosane	47.95	0.16
(1S,2S,5R)-(+)-4-isopropyl-7-methyl-1-oxaspiro(2,5) octane	50.25	0.03
Total		19.10
Total percentages		99.90

Table 2. Larvicidal activity of essential oil against third instar larvae of *An. gambiae* after 24 h of exposure.

Concentration (× 10^{-3} mg/mL)	% Mortality ± SD	LC_{50} (× 10^{-3} mg/ml)	LC_{90} (× 10^{-3} mg/ml)
7.80	0.00 ± 0.00		
15.60	1.67 ± 2.89		
31.25	3.33 ± 2.89		
40.00	13.33 ± 5.77		
45.00	33.33 ± 5.77		
55.00	46.67 ± 2.89		
62.50	68.33 ± 5.77		
100.00	76.67 ± 2.89	57.73 (45.40-73.05)	140.24 (105.73-217.05)
125.00	80.00 ± 10.00		
150.00	85.00 ± 8.66		
200.00	88.33 ± 2.89		
250.00	96.67 ± 5.77		
500.00	100.00 ± 0.00		
1000.00	100.00 ± 0.00		
Pylarvex (0.1 mg/mL)[X]	100.00 ± 0.00		
Spring water + DMSO[Y]	0.00 ± 0.00		

[X]Positive control, [Y]Negative control.

instar larvae of *A. gambiae* with LC_{50} and LC_{90} values of 57.73 and 140.24 ×10^{-3} mg/ml respectively (Table 2). The negative control showed no activity against third instar larvae of *A. gambiae*. At a concentration of 100×10^{-3} mg/ml, the positive control pyrethrin EC 0.5% w/v (Pylarvex™) recorded 100 % larval mortality (Table 2).

Compared to essential oils from other plants, the essential oil of *Z. gilletii* exhibits significant activity against malaria vector *A. gambiae*. Reports from earlier studies indicate that larvicidal activity of essential oil from *F. angolensis* (Rutaceae) against third instar larvae of *A. gambiae* exhibited LC_{50} and LC_{90} values of 83.7 and 324.0 mg/L, respectively (Mudalungu et al., 2013). Kweka et al. (2012) reported larvicidal activity of *P. amboinicus* essential oil against late third instar larvae of *A. gambiae* and observed LC_{50} and LC_{90} values of 67.53 and 107.60 ppm, respectively. The essential oil of *C. citratus* was observed to have an LC_{50} of 69 ppm against *Aedes aegypti* larvae (Cavalcanti et al., 2004). The same oil was reported to possess larvicidal activity against *Culex quinquefasciatus* larvae with LC_{50} of 165.7 ppm (Pushpanathan et al., 2008) (Table 3).

γ-Terpinene, the major component of *Z. gilletii* essential oil has been found to possess larvicidal activity against three mosquito species (Cheng et al., 2009c; Zhu and Tian., 2011). This compound had LC_{50} values of 26.8 µg/ml, 22.8 µg/ml and 29.21 mg/L against *A. aegypti*, *Aedes albopictus* and *A. anthropophagus* larvae,

Table 3. Comparison of relative toxicity of essential oil from *Zanthoxylum gilletii* with three previously isolated essential oils tested against *A. gambiae*, *A. aegypti* and *C. Quinquefasciatus.*

Essential oil	Mosquito species	LC_{50} (mg/ml)	LC_{90} (mg/ml)
Zanthoxylum gilletii	*Anopheles gambiae*	57.73	140.24
Plectranthus amboinicus	*Anopheles gambiae*	67.53	107.60
Cymbopogon citrates	*Aedes aegypti*	69.00	
	Culex quinquefasciatus	165.70	
Fagaropsis angolensis	*Anopheles gambiae*	83.70	324.00

respectively. It recorded an LC_{90} value of 63.1mg/L against *A. anthropophagus*. In a previous study, the same compound was isolated from the oil fractions of *Cymbopogon nardus* and had an excellent effect against third instar larvae of *C. quinquefasciatus* in 24 h, with LC_{50} value of 0.8 mg/L (Ranaweera and Dayananda, 1996).

Sabinene which was also in appreciable amount in the oil has been reported to exhibit larvicidal activity against third instar larvae of *C. quinquefasciatus*, *A. aegypti* and *Anopheles stephensi* (Govindarajan, 2010). The LC_{50} values recorded against the three mosquito species were 25.01, 21.20 and 19.67 ppm, respectively. The compound also recorded LC_{90} values of 45.15 ppm against *C. quinquefasciatus*, 39.22 ppm against *A. aegypti* and 36.45 ppm against *A. stephensi*. Sabinene was also isolated from the essential oil of *Clausena dentata* and found to be active against *Spodoptera litura* with LC_{50} and LC_{90} values of 21.42 and 40.39 ppm respectively (Krishnappa et al., 2010). Another monoterpene also present in appreciable amount in the oil was β-myrcene. Previous studies have documented the activity of β-Myrcene against the larvae of *A. aegypti* and *A. albopictus* (Cheng et al., 2009c). The LC_{50} values recorded were 27.9 and 23.5 µg/ml respectively.

Trans-Caryophyllene, which occurs in appreciable amounts in this oil, is also reported to show activity against *A. aegypti* larvae with LC_{50} of 104 ppm (Morais et al., 2006). Its oxygenated form caryophyllene oxide is known to exhibit larvicidal activity against the fourth instar larvae of *A. anthropophagus* (Zhu and Tian, 2013) with LC_{50} and LC_{90} values of 49.46 and 115.38 mg/L, respectively. α-Cadinol although in small quantity in *Z. gilletii* oil, is known to possess larvicidal activity against *A. aegypti* with LC_{50} value of 76.1 ppm (Chun et al., 2008). Germacrene D is known to be effective against larvae of *A. aegypti* and *A. stephensi* (Kiran et al., 2006). This sesquiterpene hydrocarbon, isolated from the essential oils of *Chloroxylon swietenia*, had LC_{50} values of 63.6 and 59.5 µg/ml against *A. aegypti* and *A. stephensi* respectively. The LC_{90} values recorded for this compound were 100.7 µg/ml against *A. aegypti* and 96.4 µg/ml against *A. stephensi.*

The high larvicidal activity of *Z. gilletii* can therefore be attributed to the presence of γ-terpinene, β-myrcene, sabinene, *trans*-caryophyllene, caryophyllene oxide, α-cadinol and germacrene D which have been documented to possess larvicidal activity against different species of mosquito.

Conclusion

Plants are rich source of bioactive organic chemicals and offer an advantage over synthetic pesticides as they are less toxic, less prone to development of resistance, and easily biodegradable. The findings of this study show that the essential oil isolated from *Z. gilletii* holds great promise as potential mosquito larvicides. Furthermore these outcomes could be useful in the search for newer more selective, biodegradable and natural larvicidal compounds. These findings also offer an opportunity for developing alternatives to inorganic insecticides.

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