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Larvicidal Activity of Selected *Aloe* Species Against *Aedes aegypti* (Diptera: Culiciade)

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ABSTRACT. Management of mosquito vectors by current classes of mosquitocides is relatively ineffective and necessitates prospecting for novel insecticides with different modes of action. Larvicidal activities of 15 crude extracts from three geographically isolated *Aloe ngongensis* (Christian), *Aloe turkanensis* (Christian), and *Aloe fibrosa* (Lavranos & L.E.Newton) (Xanthorrhoeaceae) species (five each) were evaluated against *Aedes aegypti* (Linnaeus *in* Hasselquist) (Diptera: Culiciade L.) yellow fever mosquito. Freshly collected leaves were separately shade-dried to constant weight at room temperature (25 ± 2°C) and powdered. Each powder was macerated in solvents of increasing polarity (hexane, chloroform, ethyl acetate, acetone, and methanol) for 72 h and subsequently filtered. Third-instar larvae ($n = 25$) of the mosquito were exposed to the extracts at different concentrations for 24 h to establish dose response relationships. All the fractions of *A. ngongensis* were active below 1 mg/ml except *A. fibrosa* and *A. turkanensis*. The highest activity (LC_{50}) mg/ml was obtained with extracts of *A. fibrosa* hexane (0.05 [0.04–0.06]), followed by *A. ngongensis* hexane (0.11 [0.08–0.15]) and *A. turkanensis* ethyl acetate (0.11 [0.09–0.12]). The activities are apparently *Aloe* species specific and extraction solvent dependent. These findings suggest that extracts from selected *Aloe* species have mosquitocidal principles that can be exploited in development of new insecticides.

Key Words: phytochemicals, mosquitocidal, Diptera, Culicidae, yellow fever

Mosquitoes are primary vectors of vector-borne diseases and nuisance bitters that affect humans and their livestock (Peng et al. 2004). Malaria, dengue fever, chikungunya, lymphatic filariasis, and leishmaniasis account for thousands of human mortalities in Africa. Kenya has had multiple arbovirus outbreaks, yellow fever in 1992 and 1995, Chikungunya fever in 2004, and most recently Rift Valley fever in 1997 and 2006 (Bird et al. 2008, WHO 2011). Dengue epidemics are largely attributed to *Aedes aegypti* (Linnaeus *in* Hasselquist) mosquito whose populations appear to be driven by factors that include rapid urban population growth, travel, trade, and favorable climatic conditions in the tropics and subtropics.

About 2.5 billion people worldwide are at risk of contracting dengue fever, dengue hemorrhagic fever, and dengue shock syndrome (WHO 2010), with potentially serious implications due to absence of neither effective drug nor vaccine against these diseases. Physical and chemical methods are the only feasible alternatives to controlling the mosquito-borne diseases. Physical approaches are barrier to infection of humans by infected mosquitoes, achieved through repellents and insecticide-treated bed nets. However, resistance to pyrethroids present a real and immediate challenge to efficacy of these intervention methods (Etang et al. 2004). Mosquitocidal factors that interrupt vector ecology are ovicidal, larvicidal, pupicidal, and adulticidal and include organochlorides, organophosphates, and synthetic pyrethroids (WHO 2010, Panneerselvam et al. 2012). However, successive changes in insecticide regiments has now resulted in multiple resistance among vector populations in Sub-Saharan Africa (Chandre et al. 2000, Enayati and Hemingway 2010) and India (Govindarajan 2011), including in the larvae (Diabate et al. 2003, Amy et al. 2005, Wirth et al. 2005).

Ae. aegypti is a cosmotropical species that proliferates in diverse habitats in domestic and peridomestic collections of fresh water that include flower vases, water storage jars, drums, tanks, broken coconut shells, old tyres, and roof gutters (Muir and Kay 1998, Harrington et al. 2005). This diversity limits application and efficacy of broad-spectrum synthetic insecticides with undesirable toxicity to nontarget organisms and has necessitated prospecting for novel environmentally friendly chemicals (of botanical or microbial origin) with desirable mosquitocidal efficacy (Ascher et al. 1995). The chemicals potentially have enhanced biological activity resulting from synergistic or additive effects of moderately active or individually inactive compounds (Berenbaum and Zangeri 1987; Isman et al. 1996, 2008; Bekele and Hassanali 2001) and have mitigating effects of structurally related or unrelated compounds that counter resistance development that characterizes most single-component bioactive compounds characteristic of current mosquitocide classes (Feng and Isman 1995, Isman et al. 1996). For these reasons, mosquitocidal botanicals are recognized as potent alternative insecticides to replace synthetic ones in mosquito control programs (Ascher et al. 1995).

This study explored effects of phytochemicals from three geographically isolated plant species belonging to the genus *Aloe* (*Aloe turkanensis* (Christian), *Aloe ngongensis* (Christian), and *Aloe fibrosa* (Lavranos & L.E.Newton)) against *Ae. aegypti*—the principal vector of arboviruses. Because of their overexploitation, plants in the genus *Aloe* are listed under Appendix II of Convention on International Trade in Endangered Species of Wild Fauna and Flora, which regulates international trade in endangered species (Lubia et al. 2008).

Materials and Methods

Plant Materials. *Aloe* (Xanthorrhoeaceae) species (*A. turkanensis*, *A. ngongensis*, and *A. fibrosa*) were collected from Turkana, Ngong, and Kajiado in Kenya. The plants were identified by taxonomy and voucher specimen deposited at Department of Biological Sciences of Egerton University Herbarium. Freshly collected leaves were washed in tap water, chopped into small pieces (~2 cm), and shade-dried at room temperature (23 ± 2°C). The dry plant material was coarsely ground into powder. From each plant species, about 1 kg each of powder was separately macerated in 3.0 liters of hexane, chloroform, ethyl acetate, acetone, and methanol for a period of 72 h and then filtered. The extracts were concentrated in a rotary vacuum evaporator until all solvent had evaporated. The resultant powder was stored in air-tight glass jar until when required.

Table 1. Dose response of third-stage *Ae. aegypti* larvae to *Aloe* plant extracts

Aloe species	Extract	LC_{50} (95CI)	LC_{90} (95CI)	LC_{99} (95CI)	Slope ($\beta \pm SE$)	χ^2
A. turkanensis	Ethyl acetate	0.11 (0.09–0.12)	0.19 (0.16–0.25)	0.31 (0.24–0.50)	4.99 ± 0.78	0.44
A. ngongensis	Hexane	0.11 (0.08–0.15)	0.48 (0.29–1.24)	1.67 (0.76–7.94)	1.00 ± 0.34	1.03
A. ngongensis	Ethyl acetate	0.15 (0.13–0.17)	0.32 (0.25–0.5)	0.62 (0.41–1.43)	3.70 ± 0.70	0.94
A. ngongensis	Chloroform	0.34 (0.29–0.38)	0.61 (0.51–0.81)	0.98 (0.75–1.61)	4.97 ± 0.79	1.52
A. ngongensis	Methanol	0.39 (0.34–0.45)	0.81 (0.64–1.25)	1.45 (1.01–3.05)	4.08 ± 0.73	1.73
A. ngongensis	Acetone	0.77 (0.67–0.89)	1.57 (1.26–2.42)	2.82 (1.97–5.82)	4.11 ± 0.73	0.09
A. fibrosa	Hexane	0.05 (0.04–0.06)	0.09 (0.07–0.14)	0.16 (0.11–0.32)	4.5 ± 0.075	5.88
A. fibrosa	Acetone	0.67 (0.54–1.07)	1.83 (1.12–7.15)	4.13 (1.96–34.68)	2.95 ± 0.73	0.13
A. fibrosa	Methanol	3.89 (3.38–4.47)	7.74 (6.31–11.27)	13.56 (9.75–25.81)	4.23 ± 0.73	2.05
Pyrethrum	Hexane	0.02 (0.01–0.02)	0.04 (0.03–0.06)	0.08 (0.05–0.15)	3.39 ± 0.51	0.84
Negative control	DMSO	3.32 (2.68–3.93)	8.13 (6.14–13.26)	17.23 (11.11–38.82)	3.20 ± 0.51	0.49
Positive control	Pyrlarvex			0.05 (100%)		

LCs of plant extract are expressed as mg/ml. 95 CI, 95% lower and upper confidence interval in parenthesis.

Mosquito Rearing. The *Ae. aegypti* colony was obtained from The Pyrethrum Board of Kenya, Nakuru. At the time of the experiment, the colony was in the 35th filial generation postfield sampling. Feral individuals were collected from the field and added to the gene pool biannually. The mosquitoes were reared following the procedure of Foggie and Achee (2009). Mosquito life stages were maintained and experiments conducted under temperature (23 ± 2°C), relative humidity (75–85%) conditions, and at a 12:12 (L:D) h photoperiod. The eggs were placed in culture trays (18 by 13 by 4 cm) containing 500 ml of water for hatching. Once a day, the larvae were fed on about 0.2–0.3 mg of diet (pulverized Tetramin fish food-Tetra GmbH, Melle, Germany) sprinkled on the water surface until they pupated. The pupae were collected from the culture trays and transferred to breeding trays (12 by 12 cm) that provided 500 ml of water with the help of a dipper. The breeding trays were kept in an adult emergence cage (90 by 90 cm) whose sides were covered with fine net to prevent escape. Upon emergence, adult mosquitoes were provided a 10% sugar solution diet imbibed in cotton wool for a period of 2 d. On the third day, females were separated and allowed to feed on blood of a rabbit (one rabbit a day, exposed on the dorsal side) for another 2 d to ensure adequate feeding. After 2–3 d, oviposition trays containing water from culture trays were introduced into the cages. A 200-ml oviposition tray was half filled with distilled water to a depth of 2.0 cm. It was lined with a filter paper (3.5 cm in width) to keep eggs from getting stranded on the sides of the bowl. The setup was left for 48 h, after which the container was removed and water was drained out. The paper containing eggs was then air dried and kept in a dry place until next use.

Larvicidal Test. One gram of the extract was dissolved in 100 ml of dimethyl sulphoxide (DMSO) to form a 1% stock solution from which concentrations ranging from 0.05 to 2.0 mg/ml were prepared via dilution with distilled water. For the purpose of comparison, 1 ml of the desired concentration of plant extract was dissolved in 100-ml distilled water. DMSO (1% in distilled water) and Pylarvex (Pyrethrum Board of Kenya, Nakuru, Kenya) (a conventional pyrethrum based larvicide) at 0.05 mg/ml were used as negative and positive controls, respectively. In addition, a hexane extract of pyrethrum plants (the solvent and plant of choice in synthetic mosquitocidal chemicals) was made and its activity compared with those of *Aloe*. Larvicidal tests of the extracts were conducted following standard guidelines (WHO 2005). Twenty-five third-stage larvae were placed in 50-ml cups containing 15 ml of respective solvent extract. The setup was replicated three times for each species of *Aloe* and for each concentration of the plant extract. The larvae were checked daily until death or pupation. Larval mortality in treatment and control groups was recorded after 24 h exposure. From this data, the median (LC_{50}) and maximum (LC_{95}) lethal concentrations (LCs) ranges were established by Probit analysis (Finney 1971). Larvae were assumed dead when they did not respond to gentle probing by a pipette tip. The percentage mortality was then calculated for each concentration of the plant extract.

Data Analyses. The Abbot's formula (Abbott 1925) was used to correct for acute mortality among the treated and control groups, the data were then transformed into Probits (Finney 1971) for linear regression analyses and median (LC_{50}) and maximum (LC_{90}) LC determination at the associated 95% confidence limits. Data sets with more than 10% control mortality were excluded from the analyses (Finney 1971).

Results

Larvicidal Results. Five, two, and one extract from *A. ngongensis*, *A. fibrosa*, and *A. turkanensis*, respectively, had activity at below 1 mg/ml concentration (Table 1). For *A. ngongensis*, observed activity of extracts was in decreasing order that included hexane and ethyl acetate followed by chloroform and methanol, while the least was acetone extracts. On the other hand, the highest activity among *A. fibrosa* extracts was hexane followed by acetone whose activity was mild, while methanol was inactive (comparable to negative control—DMSO). For *A. turkanensis,* only ethyl acetate extracts caused mortality below 1 mg/ml. The hexane extracts of pyrethrum plant had the highest activity, whereas Pylarvex caused 100% mortality at 0.05 mg/ml. The observed percentage mortality appeared to correlate with the concentration of the extracts used.

Discussion

The results demonstrate mosquitocidal activity of phytochemicals from *Aloe* against *Ae. aegypti.* It builds on a previous study by Matasyoh et al. (2008) using the same plant species against *Anopheles gambiae* sensu stricto (Giles). The larvicidal activities (LC_{50}) observed in the two studies are markedly different only sharing values of *A. turkanensis* (in ethyl acetate) and the observation of low activity of *A. fibrosa* against the tested mosquitoes. The differences in the observed LC values are likely to indicate that the two mosquito species (*Ae. aegypti* and *An. gambiae s.s*) experience different levels of susceptibility to plant extracts. This is expected to have implications on mosquito control programs as "a one-size fits all" approach cannot be employed for control of different mosquito species. The above findings are consistent with previous findings that insecticidal effects of plant extracts vary due to several factors: 1) plant species, 2) mosquito species, 3) geographical varieties, 4) plant parts used, 5) extraction methodology adopted, and 6) the polarity of the solvents used during extraction (for a review, see Ghosh et al. 2012).

In addition, activity of the plant extracts in this study showed variations not only according to species but also with organic solvent of extraction; from the highest to the lowest (*A. turkanensis*, *A. ngongensis*, and *A. fibrosa*), respectively. These plants though belonging to the same genus are geographically isolated, and the environment under which they grow in the wild is different. It is possible that the observed unique biological activity plants of the three members in the genus *Aloe* may either be species (Farnsworth and Bingel 1977) or even environment dependent (Berenbaum and Zangeri 1987). Similarly, Kovendan et al. (2012) observed that the choice of solvent used in phytochemical extraction has an impact on mosquito larval mortality. For example, the findings of this

study, the maximum activity (at 1 mg/ml) of methanolic extracts for the three species of *Aloe* (*A. turkanensis*, *A. ngongensis*, and *A. fibrosa*) ranged from: inactive, mild, and weak, respectively. This further corroborates earlier reports that even from the same plant sample larvicidal activity for each extract may be different depending on the choice of solvent (Sukumar et al. 1991, Zaridah et al. 2006, Govindarajan 2011).

Although the phytochemicals were detrimental to *Ae. Aegypti* developmental stages, it was not possible to account for the observed effects. This is mainly because their mode of action remains unknown. However, secondary metabolites from different plant species cause physiological and cellular disturbances that include inhibition of acetylcholinesterase, disruption of sodium and potassium ion exchange (by pyrethrin), and interference of mitochondrial respiration (Usta et al. 2002). Additionally, they affect midgut epithelium or gastric caecae and the malpighian tubules in mosquito larvae (Rey et al. 1999, David et al. 2000; for a review, see Rattan 2010). It would be of interest to decipher the mode of action and the precise developmental effects of these phytochemicals prior to the broad application in mosquito control. Prior to commercialization of these biopesticides, other factors must be put into consideration such as evaluation of their mode of action, safety of nontarget and beneficial organisms, indicator species, their performance in actual field conditions, and residual life span some of which are already underway in subsequent studies.

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