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Full Length Research Paper

Aloe plant extracts as alternative larvicides for mosquito control

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The larvicidal activity of extracts from *Aloe turkanensis*, *Aloe ngongensis* and *Aloe fibrosa* against the common malaria vector, *Anopheles gambie*, was determined. Ground *Aloe* leaves from the three plants were sequentially extracted with hexane, ethyl acetate, chloroform, acetone and methanol. Only the ethyl acetate extract of *A. turkanensis*, hexane, ethyl acetate, acetone, chloroform and methanol extracts of *A. ngongensis* and the hexane, acetone and methanol extracts of *A. fibrosa* showed activity. A series of concentrations of the extracts ranging from 0.05-2 mg/ml (0.005-0.2% w/v) were tested against third instar larvae and their percentage mortalities, LC_{50} values determined. The ethyl acetate soluble extract of *A. turkanensis* showed very high larvicidal activity where 100% mortality was achieved at a concentration of 0.2 mg/ml and it had an LC_{50} of 0.11 mg/ml. All the extracts of *A. ngongensis* showed larvicidal activity to *A. gambie* larvae, but at higher concentration showing LC_{50}´s of 0.84 (0.55 – 1.27), 1.14 (0.72 – 2.28), 0.98 (0.78 – 1.27), 1.08 (0.90 – 1.28), 2.0 (1.85 – 2.36) for the hexane, ethyl acetate, chloroform, acetone and methanol, respectively. The three active fractions of *A. fibrosa* had very close LC_{50}´s ranging from 1.76 – 1.90 mg/ml. Thin layer chromatographic analysis (TLC) showed the presence of chromones and anthrones in the chloroform and ethyl acetate extracts. Application of these extracts to larval habitats may lead to promising results in malaria and mosquito management programmes.

Key words: *Aloe*, *anopheles gambie*, larvicidal activity.

INTRODUCTION

Extracts from plants in the genus *Aloe* (Aloeaceae) have been widely used by pharmaceutical and cosmetic industries. *Aloe* species have long been known as medicinal plants (Cheney, 1970) and *Aloe vera* species is most widely used. The compositions of *Aloe* leaf exudates have been extensively investigated (Reynolds, 1985). The compounds that have been identified can generally be classified into two main groups, namely, chromones and anthraquinones or specifically anthrones.

Interest in the control of *Anopheles gambie* lies in the fact that it acts as a vector of malaria, which is a serious public health problem in Africa and many developing countries. Although some diseases such as yellow fever have been reasonably brought under control by vaccination, no effective vaccine is available for malaria. Therefore, the only efficacious approach of minimizing the incidence of this disease is to eradicate and control mosquito vectors mainly by application of insecticides to larval habitats. The plant-derived natural products as larvicides have the advantage of being harmless to beneficial non-target organisms and environment when compared to synthetic ones (Pitasawat et al., 2007). The synthetic insecticides do not only affect non-target organisms but also constantly increase resistance to the insecticides by the vector (Wattal et al., 1981).

In recent years, the emphasis to control the mosquito populations has shifted steadily from the use conventional chemicals towards more specific and environmentally friendly materials, which are generally of

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Table 1. Yield of extracts from 500 g of plant material.

Plant	Yield (g)				
	Hexane	Ethyl acetate	Acetone	Chloroform	Methanol
A. turkanensis	2.1	4.2	3.1	4.5	7.2
A. fibrosa	3.0	3.8	2.6	3.5	6.1
A. ngongensis	2.5	3.6	2.7	4.2	6.5

botanical origin. For this purpose, a lot of phytochemicals extracted from various plant species have been tested for their larvicidal and repellent actions against mosquitoes (Ciccia et al., 2000; Ansari and Razdan, 2000). One of the strategies of the WHO in combating tropical diseases is to destroy their vectors or intermediate hosts. Malaria is a parasitic disease from which more than 300 million people suffer yearly throughout the world. It is one of the main causes of infant and young child mortality (WHO, 1995). As part of our continued search for the biodiversity resource available in Kenya for natural products with utilisable bioactivity, we have assayed larvicidal activity towards *A. gambie* of extracts from three *Aloe* species growing in Kenya, namely *Aloe turkanensis*, *Aloe ngongensis* and *Aloe fibrosa*.

MATERIALS AND METHODS

Plant Material

The plants *A. turkanensis*, *A. ngongensis* and *A. fibrosa* were collected from Turkana, Ngong and Kajiado regions of Kenya respectively, identified and cultivated at the Botanical Garden of Egerton University, Kenya. Voucher specimens (number Sk61, sk65, sk69) were deposited at the department of Biological Sciences, Egerton University.

Extraction

500 g of *Aloe* leaves for each plant were cut into segments prior to grinding in a blender. The resulting slurry was sequentially extracted with hexane, ethyl acetate, chloroform, acetone and methanol. The solvents were removed by rotor evaporation under vacuum to give five extracts for each species. The yields are given in Table 1.

TLC analysis

The extracts that showed bioactivity were subjected to thin layer chromatographic analysis. This was done on silica gel plates (Merck, $60F_{254}$) using the solvent system MeOH-$CHCl_3$, 1:4. The solvents were distilled before use. The visualization and identification of spots of the compounds was done using an ultra violet lamp at a wavelength of 254 nm. The retention factor (R_f) values were determined and compared to those of literature where similar conditions and reagents were used (Holzapfel et al., 1997; Dagne et al., 1996, 1997, 1998).

Tested material

The extracts were tested against third instar larvae of *A. gambie* at 2 mg/ml; fractions showing over 60% mortality after 24 h were selected for detailed assays at different concentrations.

Larvicidal assays

The extracts were solubilized in dimethyl-sulphoxide (DMSO) an analytical reagent obtained from Lobarchemi and diluted to give 2 mg/ml of stock solution with DMSO kept at a concentration of 1%. The bioassay experiments were conducted mainly according to standard WHO procedure (1981) with slight modifications. The bioassays were conducted at the Kenya Medical Research Institute (KEMRI), Centre for Disease Control (CDC), 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 to 75%. The bioassays were performed with third instar larvae of *A. gambie* and carried out in triplicate using 20 larvae for each replicate assay. The larvae were placed in 50 ml disposable plastic cups containing 15 ml of test solution and fed on tetramin fish feed during all testing. Larvae were considered dead if they were unrousable within a period of time, even when gently prodded. The dead larvae in the three replicates were combined and expressed as the percentage mortality for each concentration. The negative control was spring river water while the positive control was the pyrethrum based larvicide, pylarvex.

Statistical analysis

Probit analysis (Finney, 1971) of concentration mortality data was conducted to estimate the LC_{50} values and associated 95% confidence limits.

RESULTS AND DISCUSSION

The results obtained in the preliminary assays of fifteen extracts from *A. turkanensis*, *A. ngongensis* and *A. fibrosa* against third instar larvae of *A. gambie* showed that only nine were active according to our norm (60% mortality at 2 mg/ml or 0.2% w/v). Only the ethyl acetate extract of *A. turkanensis*, hexane, ethyl acetate, acetone, chloroform and methanol extracts of *A. ngongensis* and the hexane, acetone and methanol extracts of *A. fibrosa* showed activity. The larvicidal activities of the extracts against the mosquito larvae under laboratories conditions are given in Table 2. To the best of our knowledge this is the first time larvicidal activity of *Aloe* extracts is reported.

There is no vaccine to prevent infection caused by *A. gambie* mosquito and the malaria parasite is continually developing resistance to the available drugs, so vector control is the best option. A considerable number of plant derivatives have shown to be effective against mosquetoes with a safe manner. However due to the continuous increase in resistance of mosquitoes to familiar chemicals better alternative means are sought (El Hag et al., 1999).

Table 2. Larvicidal activity of extracts from *A. turkanensis*, *A. fibrosa* and *A. ngongensis* on *A. gambie.*

Plant	Extract	% Mortality (mean ± SD)								[a]LC_{50}
		0.05	0.1	0.2	0.3	0.5	1.0	1.5	2.0 (mg/ml)	
A. turkanensis	Ethyl acetate	0.0 ± 0.0	35 ± 27.2	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	0.11 (0.09 – 0.13)
A. fibrosa	Hexane	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.4 ± 2.4	15 ± 17.8	35 ± 8.2	63.4 ± 22.5	1.76 (1.45 – 2.46)
	Acetone	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.4 ± 2.4	10 ± 7.1	78.4 ± 6.2	1.79 (1.67 – 1.94)
	Methanol	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.4 ± 4.7	5 ± 4.1	30 ± 12.2	30 ± 4.1	61.7 ± 8.5	1.80 (1.38 – 2.83)
A. ngongensis	Hexane	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	45 ± 7.1	46.7 ± 29.5	70 ± 0.0	100 ± 0.0	0.84 (0.55 – 1.27)
	Ethyl acetate	0.0 ± 0.0	0.0 ± 0.0	6.7 ± 4.7	0.0 ± 0.0	0.0 ± 0.0	48.4 ± 12.5	65 ± 0.0	80 ± 4.1	1.14 (0.72 – 2.28)
	Acetone	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	66.7 ± 8.5	70 ± 4.1	81.7 ± 4.7	1.08 (0.90 – 1.28)
	Chloroform	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	5 ± 4.1	25 ± 8.2	40 ± 14.7	80 ± 4.1	81.7 ± 4.7	0.98 (0.78 – 1.27)
	Methanol	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.4 ± 4.7	50 ± 10.8	2.0 (1.85 – 2.36)
Control (+)	Pylarvex (0.05 mg/ml)		100 ± 0.0							
Control (-)	Spring water		0.0 ± 0.0							

[a] The lethal concentrations with the corresponding 95% confidence intervals are shown in parenthesis.

The most effective extract in this work was that of ethyl acetate from *A. turkanensis*. The results of this study (Table 2) clearly show that it demonstrated a high larval mortality. At a concentration of 0.2 mg/ml, this extract produced 100% mortality and had an LC_{50} of 0.11 mg/ml. The best lethal dosage (LC_{50}) for the *A. ngongensis* is that for the hexane extract with a value of 0.84 mg/ml. The extracts for this species showed mortalities that ranged from 50 to 100% at a concentration of 2 mg/ml (0.2% w/v) with corresponding LC_{50}'s of 2.0 to 0.84 mg/ml. Although the three active extracts of *A. fibrosa* (hexane, acetone, and methanol) did not show very high activity, they still caused mortalities of 61.7 to 78.4% at the same concentration.

As adult mosquitoes transmit diseases, the critical concentrations of the materials which inhibit 50% (LC_{50}) of the treated larval population from emerging adults are more meaningful (Bhakthratchagan et al., 1993; Moshen et al., 1995). The *Aloe* extracts have shown that they can kill up to 100% of the larvae population tested; this can certainly help reduce the mosquito population drastically. Considering that a large proportion of the human population living in malaria prone areas suffer from varying degrees of poverty, the discovery of plant extracts that could control the mosquito population could be of great value. In this context, the three plants under study grow widely in the rural parts of Kenya where *A. gambie* is a serious problem offer an opportunity for developing alternatives to rather expensive and environmentally hazardous inorganic insecticides. The results show that the extracts could be used in mosquito control instead of synthetic larvicides. Their active ingredients have no toxicities to humans since the *Aloe* plants from which the extracts are obtained have been used as traditional medicine for centuries without any reported illness or side effects resulting from their use (Cheney, 1970).

The major constituents identified in the members of Aloeceae family are, typically, chromones and anthroquinones or anthrones. Thin layer chromatographic analysis of the extracts showed that the chemical composition of the *Aloe* species under study has close similarities to those found in South Africa, namely, *Aloe littoralis, Aloe broomi* and *Aloe microstigma* (Dagne et al., 1996, 1997, 1998). Our investigation of the chloroform and ethyl acetate soluble part of the leaf extracts using TLC analysis and matching the experimental conditions with those found in literature (Dagne et al., 1996, 1997, 1998) showed three to five main constituents. The species showed the presence of mainly littoraloin, deacetyllitoraloin, 6'-O- caffeoyl-5-hydroxyaloin A, 5-hydroxyaloin A and (E)-2-Acetonyl-8-(2'-O-feruloyl)-β-D-glucopyranosyl-7-methoxy-5-methyl-chromone. It is important to note that our results relate to crude extracts and not purified active components, which when isolated, would be expected to show much lower LC_{50} values than those reported here. In conclusion, the results obtained from this study may be useful in the search for new, more selec-

tive and biodegradable larvicidal natural compounds. Application of these extracts to mosquito breeding habitats may leadto promising results in malaria and mosquito management programmes.

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