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RESEARCH ARTICLE

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COMPARISON OF MOSQUITO LARVICIDAL ACTIVITY OF *ANNONA SQUAMOSA* LEAVES GROWING IN DIFFERENT ECO-ZONES IN TANZANIA

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ABSTRACT

Annona squamosa L. (Annoneceae) is a medicinal plant widely distributed in all eco-zones of Tanzania. The plant has a wide use as medicine, food and insecticide. A qualitative study of ethanolic extracts of *A. squamosa* collected from different eco-zones was done using HPTLC. Furthermore, each leaf ethanolic extract and leaf powders were screened for mosquito larvicidal activity against larvae of *Culex quinquefasciatus* Say and *An. gambiae* s.s. The results from HPTLC showed almost the same kind of compounds based on their retention factor as observed in both long and short wavelength. The leaf ethanolic extract and leaf powders from Brachystegia Julbernadia Savanna Woodlands eco-zone (T6) showed the highest larvicidal activity with LC_{50} value of 0.0066 mg/ml and 0.0860 mg/ml against *Culex quinquefasciatus* while activity of the same sample against *An. gambiae* was 0.0252 mg/ml and 0.0599 mg/ml respectively after 24 h of exposure. Phytochemical screening of DCM, EtoAc and ButOH fractions obtained from T6 leaf ethanolic extract indicated the presence of alkaloids, terpenoids and flavanoids. The results suggest that *A. squamosa* from T6 eco-zone is the most rich chemotype agent for mosquito control.

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KEY WORDS

Annona squamosa L., Annonaceae, HPTLC analysis, mosquito larvicide, eco-zones.

INTRODUCTION

Annona squamosa L. (Annoneceae) is a medicinal plant commonly known as sugar apple. It is a tropical fruit tree originating from Asia and America. In Tanzania, *A. squamosa* was introduced and is now cultivated in all regions but mostly along the coastal area and Zanzibar[1]. This plant species is traditionally used in treatment of different disorders such as constipation, fever, ulcers, cancer and tumor[2]. It is also reported to exhibit antimicrobial[3], antioxidant[4], antibacterial[5] and hepatoprotective[6]. The leaves and roots of *A. squamosa* are also well known for their delayed insect growth regulating activity in the form of larval-pupal intermediates and half ecdysed adults and as anti-larvae against *Culex quinquefasciatus*[7,8], *Anopheles stephens*[9], *An.gambiae*[10], *An. culicifacies*[9,11] and *Aedes albopictus*[7]. The plant is also effective against insects belonging to the genera *Lepidoptera*[12] and *Coleoptera*[13]. Therefore, the present study aimed at investigating if geographical location had any effect on larvicidal activity of leaves of *A. squamosa* growing in Tanzania.

MATERIAL AND METHODS

(i) Collection of Plant Materials

The leaves of *A. squamosa* were collected in September 2010 from five eco-zones in Tanzania. These are Igombe village in Mwanza region - The Moist Forest Mosaic zone (T1), Mbezi-Makabe village in Dar es salaam region – The Coastal Forests and Thickets zone (T2), Newland village in Kilimanjaro region – The Acacia-Commiphora Thornbush zone (T4), Mkambalani B village in Morogoro region - The Acacia–Savanna and Grasslands zone (T5) and Mpuguso village in Mbeya region – The Brachystegia Julbernadia Savanna Woodlands (T6). Identification of the plant species was done with the aid of the taxonomist at the site. The voucher specimens are kept in the herbarium of Institute of Traditional Medicine (ITM), Muhimbili University of Health and Allied Science (MUHAS), Tanzania with code number ASS-T1, ASS-T2, ASS-T4, ASS-T5 and ASS-T6.

(ii) Extraction of Plant Material

The leaves from different eco-zones were separately dried at room temperature and ground to make fine powders which were soaked, each in a separate flask thrice with ethanol (99.5%) for 24 h and filtered. The filtrate were concentrated using rotary evaporator to obtain the leaf ethanolic extracts. A portion of the leaf ethanolic extracts from T6 was partitioned to obtain dichloromethane, ethyl acetate and butanol fraction while other leaf powder were sieved using a 2.0 mm sieves followed by 1.5 mm mesh size (ENDECOTTS, LTD, England) and kept in cold room for bioassay test.

(iii) Qualitative Determination of Chemical Constituents

The qualitative determination of chemical constituents was done by using CAMAG TLC scanner 3 (CAMAG, Switzerland). Stock solution was prepared by using 2mg of each leaf ethanolic extract dissolved in 1ml of methanol (HPLC grade). The volume of 50µl from each stock solution was loaded as 6 mm band length on silica gel plates ($60F_{254}$, 10 x 10 cm, Merck) using linomat 1V applicator (CAMAG, Switzerland). A solvent system consisting of toluene-acetone-methanol in a ratio of 9: 0.95:0.05 v/v/v were used as a mobile phase. Thereafter the TLC glass was dried at room temperature for about 10 min then scanned at different wavelength.

(iv) Phytochemical Screening of Compounds

Phytochemical analysis to assess possible classes of compound(s) presence in the dichloromethane, ethyl acetate and *n*-butanol fractions were carried out by using normal thin layer chromatography. After spraying the TLC plates with chemical reagents like vanilin-sulphuric acid, dragendorff and aluminium chloride, the colour changes observed indicated

either the presence of alkaloids, flavonoids or terpenoids[8,14].

(v) Mosquito Larvicidal Assay

This test was done by using *C. quinquefasciatus* Say and *An. gambiae s.s* (Kisumu strain) as per WHO protocol with modification[15, 16, 17]. For leaf ethanolic extracts 50 mg of each sample was dissolved in 1 ml of DMSO to make a stock solution while for the leaf powders, each experimental sample was introduced in distilled water 3 h prior to the time of introducing the larvae. Ten mosquitoes larvae at third late instar stage were then introduced in a 250 ml glass beaker containing 100 ml of distilled water and treated with 100, 50 and 10 µg/ml of the leaf ethanolic extract and 100, 50 and 10 mg of the leaf powders, both been replicated. Control experiment (blank) contained only DMSO, and distilled water was provided for the powders respectively. During the experiment, larvae were fed on Tetramin® fish food (Jinjiang Qimei Gifts & Favourite Industry Co. LTD, China) at 1 mg per beaker per day. All tests were conducted under controlled temperature (28°C± 2) and relative humidity of 75-85%. For all experiments mortality was observed after every 24 h of exposure for three days.

(vi) Data Analysis

The statistical calculation for LC_{50} and 95% Confidence interval (CI) were done using Fig. P-Computer software programme.[18]

RESULTS

1. Mosquito Larvicidal Activity.

Mosquito larvicidal activity of leaf ethanolic extracts and leaf powders against larvae of *C. quinquefasciatus* and *An. gambiae* showed an increase in mortality with time of exposure. The larvicidal activity for the leaf powders increased drastically from 24 h to 72 h showing that most of the active compounds could not yet been dissolved in water by 24h rather did progressively over time (Table 1)

Table 1

Larvicidal activity of leaf ethanolic extracts and powders of A. squamosa against C. quinquefasciatus.

Eco-zones where *A.squamosa* was collected	Exposure time(h)	Leaf ethanolic extracts		Leaf powders	
		LC_{50} (mg/ml)	95%CI*	LC_{50} (mg/ml)	95%CI*
Moist Forest Mosaic zone-**T1**	24	0.1390	0.0414 – 0.4667	31.2052	9.3005 – 104.7008
	72	2.0×10^{-7}	$1.2 \times 10^{-11} – 3.7 \times 10^{-3}$	0.0954	0.0018 – 1.6210
Coastal Forests and Thickets zone-**T2**	24	0.0368	0.0213 – 0.0636	33.0630	15.0177 – 72.7920
	72	0.0045	0.0012 –0.0161	5.7165	1.8416 – 17.7444
Acacia-Commiphora Thornbush zone-**T4**	24	0.0799	0.0426 – 0.1496	13.8690	6.7990 – 28.3000
	72	0.0047	0.00071 – 0.0307	0.0542	0.00056 – 0.5222
Acacia–Savanna and Grasslands zone-**T5**	24	0.4160	0.1558 – 1.1112	36.8405	1.9330 – 70.20456
	72	0.1226	0.1191 – 2.1158	2.2518	0.2337 – 21.6967
Brachystegia Julbernadia Savanna Woodlands-**T6**	24	0.0066	0.0056 – 0.0077	0.0860	0.0045 – 1.6388
	72	0.0051	0.0043 –0.00 61	0.0017	0.0001 – 0.0229

LC= lethal concentration; * Confidence Interval (CI) values coinciding are not significantly different at ≥ 95% (by Fig P. Software Programme).

In general, the extracts performed better than powders at the concentration levels tested. This could be due to random selection of concentration which did not take into account the concentration of active ingredients which are present in each sample. However, it was evident from the test that, the Brachystegia Julbernadia Savanna Woodlands (T6) eco-zone plant species exhibited the highest larvicidal activity (Table 1 and 2). The LC_{50} value against *C. quinquefasciatus* was 0.0066 mg/ml and 0.0860 mg/ml in 24 h and 0.0051 mg/ml and 0.0017 mg/ml in 72 h for leaf ethanolic extracts and powders respectively (Table 1). The LC_{50} value for the same sample against *An. gambiae* was 0.0252 mg/ml and 0.0599 mg/ml in 24 h and 0.0187 mg/ml and 0.0017 mg/ml in 72 h for the extract and powder respectively (Table 2). Treatment of *An. gambiae* with samples from other eco-zones did not produce remarkable larvicidal results showing that, *Culex* mosquitoes are more susceptible to these treatments than *Anopheles* mosquitoes. The large number of larval mortality due to treatment with the plant specimen collected from Brachystegia Julbernadia Savanna Woodlands (T6) eco-zone suggest presence of large quantity of active ingredient(s) than the rest of the plants collected from other ecological zones.

Table 2

Larvicidal activity against larvae of An. gambiae of the leaf ethanolic extracts and powders of A. squamosa from the Brachystegia Julbernadia Savanna Woodlands (T6)

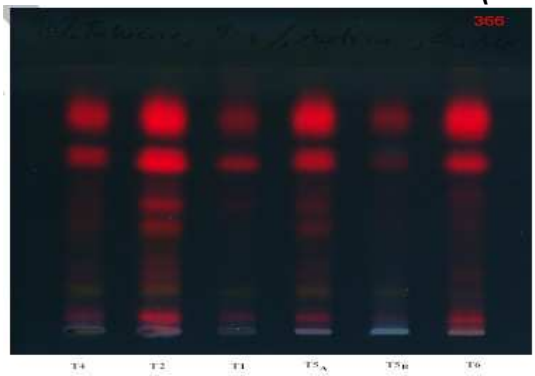
Exposure Time (h)	Leaf ethanolic extracts		Leaf powders	
	LC_{50} (mg/ml)	95%CI*	LC_{50} (mg/ml)	95%CI*
24	0.0252	0.0191 – 0.0332	0.0599	0.0106 – 0.3399
72	0.0187	0.0132 – 0.0266	0.0017	0.0001 – 0.0028

2. Qualitative Determination of Compounds

The HPTLC chromatograms using a CAMAG TLC scanner 3 showed the presence of compounds with different retention factor (Figure 1 and 2). This reveals that the plant species collected contained compounds with different polarity however the results indicated that most of the compounds were similar based on their retention factor, but having different intensity hence suggesting differences in quantity of constituents' compounds.

Figure 1

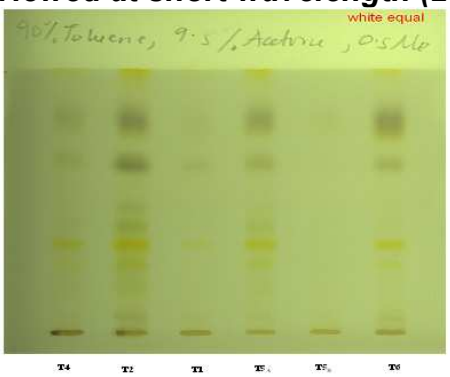
HPTLC chromatograms of leaf ethanolic extracts of A. squamosa from different eco-zones of Tanzania as viewed at fluorescence (366nm).



$T5_A$: Fresh leaves collected from T-5 eco-zone, $T5_B$: Dry leaves collected from T-5 eco-zone.

Figure 2

HPTLC chromatograms of leaf ethanolic extracts of A. squamosa from different eco-zones of Tanzania as viewed at short wavelength (254 nm).



$T5_A$: Fresh leaves collected from T5 eco-zone, $T5_B$: Dry leaves collected from T5 eco-zone.

Phytochemical screening of the most active leaf ethanolic extracts from T-6 eco-zone has shown positive results for alkaloids from butanol fraction terpenoids from ethyl acetate and dichloromethane fraction while flavanoids were in trace (Table 2).

Table 2

Phytochemical screening of compounds from three fractions of A. squamosa from T6 eco-zone.

Class of compound	Reagent used	Dichloromethane	Ethyl acetate	Butanol
Alkaloids	Dragendorff	_	_	+
Flavanoids	Aluminium chloride	+	_	_
Terpenoids	Vanilin- sulphuric acid	+	+	_

+ *Presence ; - Absence*

DISCUSSION

Vector control is the most useful method of reducing incidences of mosquito borne disease today. In this regards, herbal products are the best alternative due to their accessability, environmental safety and less toxic to humans[19]. *Annona squamosa* has been reported for its mosquito larvicidal activity from different country in the world[7-11,19-20]. However, the current investigation revealed that *A. squamosa* leaves collected from different ecological zones may possess different mosquito larvicidal effectiveness against *C. quinquefasciatus* (Table 1). This may be due to differences in kinds and/or concentration of the active compounds present in the plant species which may be caused by differences of soil and vegetation type among the ecological zones. Previous investigation that was done to validate the effect of geographical location on *Anoigeissus leiocarpus*[21], *Ficus bengalesis*[22] and *Harrisonia abyssinica*[23] revealed that, the medicinal activity of these plants differ in their activity from one location to another. Similarly, the larvicidal results against *C. quinquefasciatus* for *A. squamosa* leaves collected from Coastal Forests and Thickets eco-zone (T2) which was previously reported by Magadula et al 2009 seemed to have slight differences in LC_{50} value with the current results. This may be due to differences in plant chemical composition between seasons of collection. The qualitative based analysis of the leaf ethanolic extracts of *A. squamosa* showed almost similar compounds. Furthermore, phytochemical screening indicates the presence of alkaloid, terpenoids and flavanoids which was in agreement with other researchers who isolated such class of compounds as bioactive larvicides from the

plant species[3,8,10]. Although the present study suggests the Brachystegia Julbernadia Savanna Woodlands (T6) plant species to be the richest chemo-type, further studies are needed to isolate and quantify the compounds from leaves collected from each ecological zone.

CONCLUSION

Variation of mosquito larvicidal activity shown by the leaf ethanolic extracts and powders confirm that, differences in ecology had a strong effect on bioactivity of *A. squamosa* against immature mosquitoes. This information is pertinent to researchers aiming at discovery of novel larvicidal formulations from this plant species.

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