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Larvicidal and IGR activity of extract of Tanzanian plants against malaria vector mosquitoes

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Abstract

Background & objectives: This paper reports the larvicidal activity of seventeen Tanzanian plant species against the malaria vector, *Anopheles gambiae* s.s. Giles larvae. Some of the plants are used traditionally as sources of insecticidal materials.

Methods: The crude extracts from the leaves, stem and root barks of the investigated plants were obtained by solvent extraction and then bio-assayed following WHO protocols showed LC_{50} values 10 to 400 ppm after 24 h exposure. The structures were determined on interpretation of spectroscopic data.

Results: The most active extracts were those from the stem and root barks of *Annona squamosa, Uvaria faulknerae, U. kirkii* and *Uvariodendron pycnophyllum,* all of which had LC_{50} values between 10 and 100 ppm. Long-term exposure beyond 24 h also showed more susceptibility of the larvae to the extracts. Larvae deformities by forming tail-like structures were observed for the methanol extracts of *Tessmannia martiniana* var *pauloi*.

Interpretation & conclusion: The results suggest that the investigated plant extracts are promising as larvicides against *An. gambiae* s.s. Giles mosquitoes and could be useful leads in the search for new and biodegradable plant derived larvicide products.

Keywords *Anopheles gambiae* s.s. – botanical mosquitocides – IGR activity

Introduction

It has long been known that in Africa *Anopheles gambiae* s.s. Giles mosquitoes are the vectors that transmit malaria parasites. The disease's prevalence in Africa is estimated to constitute 300–500 million clinical cases every year, with a mortality rate of 1.4–2.6 million people as the disease is fatal if untreated[1]. Although malaria fevers have been reasonably brought under control by the application of synthetic insecticides to kill the vector mosquitoes, there is currently recrudescence of the disease. This has been ascribed to the emergence of many of breeding places brought about by human activities, and the ever-increasing resistance of mosquitoes to currently available commercial insecticides. It is still believed that the only way to decrease the incidence of malaria is by combating the vector mosquitoes. Experience has shown that aerial toxicants for mosquito control are not effective, since mosquitoes are now highly domesticated and many adults rest indoors in hidden places such as closets. Therefore, it is envisioned that the only successful method of reducing mosquito densities to an appreciable level for which malaria

epidemics can be controlled is by attacking the larval breeding places through the use of larvicides[2].

Chemical insecticides have continued to be commonly used for controlling mosquitoes in many parts of the world. Initially their use was focused on the control of mosquitoes, either by killing or repelling them. However, the appearance of mosquito resistance to conventional insecticides, together with public concern about the safety and availability of the insecticides have prompted the necessity to search for alternative insecticides that would be environmentally acceptable and less costly. Therefore, in recent years the use of environmentally friendly and easily biodegradable natural insecticides of plant origin has received renewed importance for malaria and other diseases control. Interest in this field is based on the fact that these substances are least phytotoxic and do not lead to the accumulation of chemical residues in flora, fauna, soil and the entire environment in general.

The above facts prompted us to undertake investigations of some plant species traditionally used as insecticidal agents, as well as other endangered Tanzanian plant species, with the aim of identifying lead compounds for the development of new plant based insecticidal agents. We now report results from these investigations.

Material & Methods

Plant materials: The investigated plant species are summarised in Table 1, which include parts investigated, locality and criteria used for their collection. All the plant species were identified on site and their identities confirmed at Herbarium of the Department of Botany, University of Dar es Salaam, where voucher specimens are deposited for future reference.

Extraction and isolation: Air-dried and pulverized root and stem barks, leaves, fruits or whole plant were extracted sequentially with pet ether, $CHCl_3$ and MeOH, 2 x 48 h for each solvent. The extracts were stored at –18°C until further analysis or assay. Bioassay-guided isolation using the brine shrimp lethality test (BST)[3] yielded the active compounds from the relevant extracts. Fractionation of the concentrated extracts was carried out by VLC, followed by repeated column chromatography on silica gel and/or Sephadex® LH-20, eluting with pet ether and then pet ether containing increasing amounts of EtOAc, and mixtures of MeOH and $CHCl_3$ (1:1, v/v) respectively. Structural determination was carried out based on interpretation of ^1H and ^{13}C NMR, and MS spectra, and upon comparison with the reported spectra data.

Larvicidal assay: The assay was performed by exposing the *An. gambiae* mosquito larvae in distilled water treated with a series of at least five concentrations of each test sample in DMSO, kept in beakers according to WHO protocols[4]. Twenty late III or young IV instar larvae were used per beaker with three beakers per concentration (the water temperature being 25 ± 1°C) and for each test three beakers containing distilled water and test larvae but without sample were used as controls. Observation on mortality and deformities of the larvae was recorded after every 24 h of continuous exposure and this was expressed as percent mortality[4], the lethal concentration at which 50% of the test larvae were killed (LC_{50}) being worked out using a POLO PLUS computer package.

Results & Discussion

Results of investigations for susceptibility of the *An. gambiae* larvae to crude extracts of seventeen plant species are shown in Table 2. All the extracts showed activity against the late III and/or early IV instar *An. gambiae* s.s. Giles larvae. The larvae mortality was observed to increase as the concentration of the test samples was raised. This activity trend was also observed in the case of time elapsed mortality. Furthermore, some of the treated larvae showed sluggish movements and peculiar coiling. This suggested either neural or muscular effects being exerted by some of the active principles being the cause of the acute lethal effects exhibited by the extracts. Delayed le-

Table 1. Investigated plant species

Name of the plant	Family	Parts	Locality	Criteria for selection
Uvaria lungonyana Vollesen	AN	SB, RB	Selous Game Reserve	Family activity*, Endemic plant species**
U. scheffleri Diels.	AN	SB, RB	Maramba, Muheza district	Family activity*
U. faulknerae Verdc	AN	SB, RB	Handeni, Pangani district	–do–
U. leptocladon Oliv.	AN	SB	Korogwe district	–do–
U. kirkii Hook. F.	AN	SB, RB	UDSM Main campus	–do–
Uvariodendron usambaranse Diels.	AN	SB, RB	Amani Nature Reserve	Family activity*
U. pycnophyllum (Diels) R.E Fr.	AN	SB, RB	Amani Nature Reserve	Family activity*, Endemic plant species**
Lettowianthus stellatus Diels.	AN	SB	UDSM Main campus (Planted)	Family activity*, Literature reports
Annona squamosa L.	AN	SB, RB	Kibanda village, Muheza district	–do–
Polyalthia tanganyikensis Vollesen	AN	RB	Kichi hills, Utete, Rufiji district	–do–
Asteranthe asterias (S. Moore) Engl. & Diels.	AN	LS, SB	Chalinze, Coast region	Literature reports
A. lutea Vollesen	AN	RB, SM	Kwamngumi, Muheza district	Literature reports
Tessmannia densiflora Harms	LG	SB, RB	Selous Game Reserve	Family activity, Endemic plant species**
T. martiniana var *pauloi* Harms	LG	SB, RB	Pugu forest, Coast region	–do–
T. martiniana var *martiniana* Harms	LG	SB, RB	Zaraninge Forest Reserve	–do–
Croton sylvaticus L	EB	SB, RB	Selous Game Reserve	Family and genus activity
Hoslundia opposita Vahl	LM	LS, RB	UDSM Main campus	Ethnobotanical information

*The family Annonaceae is known to have compounds with pharmacological, insecticidal, antimicrobial and antiprotozoal activities; **The plant is endemic to Tanzania and neither chemical nor biological investigations have been carried out; AN—Annonaceae; LG—Leguminosae; EB—Euphorbiaceae; LM—Lamiaceae; SB—Stem Bark; RB—Root Bark; LS—Leaves; UDSM—University of Dar es Salaam.

thal effects related to the duration of exposure to the test samples were also observed. These were attributed to the likely disturbance of the endocrine mechanisms that regulate moulting and metamorphosis, as it was previously postulated for the neem seed kernel extracts[5].

The larvicidal constituents were mostly less or moderately polar pet ether and chloroform soluble compounds. This activity trend was similar to the previously reported phenomenon for mosquitocidal constituents of *Neorautanenia mitis*[6]. The root bark extracts were more active than those from the stem barks for most of the investigated plant species. This activity trend was rationalized by considering the fact that the more confined root-soil environment makes the roots to be more susceptible to pathogenic and/or predatory soil organism attacks. Therefore, this would

Table 2. Activity of crude extracts against III/IV instar larvae of *Anopheles gambiae* s.s. Giles after 24 h exposure (ppm)

Botanical name	Plant part	PE		CH		ME	
		LC_{50}	95% CL	LC_{50}	95%CL	LC_{50}	95%CL
Uvaria lungonyana	SB	ne	ne	245	185–337	373	260–781
	RB	ne	ne	93	22–155	161	116–227
U. scheffleri	SB	130	84–189	224	150–355	250	153–545
	RB	209	135–339	363	243–880	164	104–252
U. faulknerae	SB	162	109–239	33	23–48	82	61–111
	RB	27	17–46	24	17–34	165	122–222
U. leptocladon	SB	153	98–228	88	26–142	393	263–1085
U. kirkii	SB	48	34–66	52	38–75	70	52–97
	RB	76	51–113	95	64–139	129	83–209
Uvariadendron usambaranse	SB	188	137–262	188	29–318	357	258–554
	RB	439	334–707	150	106–207	494	394–775
U. pycnophyllum	SB	ne	ne	56	34–87	109	66–192
	RB	ne	ne	56	34–86	56	35–86
Lettowianthus stellatus	SB	93	65–127	256	149–708	355	265–500
Annona squamosa	SB	50	38–67	17	9–25	24	10–40
	RB	44	29–66	13	8–18	21	38–48
Polyalthia tanganyikensis	RB	96	69–132	133	90–199	70	50–100
Asteranthe lutea	RB	59	32–95	326	214–616	488	334–707
	SB	335	246–553	212	138–342	582	482–802
A. asterias	LS	444	319–898	267	186–384	494	394–775
	SB	238	150 – 349	439	334 – 707	294	220–405
Tessmannia densiflora	SB	ne	ne	104	60–150	192	130–285
	RB	ne	ne	162	113–232	383	243–402
T. martiniana var *pauloi*	SB	ne	ne	83	40–120	122	82–173
	RB	ne	ne	ne	ne	114	44–186
T. martiniana var *martiniana*	SB	ne	ne	256	149–708	353	213–402
	RB	ne	ne	204	133–340	148	74–254
Croton sylvaticus	SB	246	195–372	232	170–342	238	184–354
	RB	110	76–157	163	117–232	164	115–239
Hoslundia opposita	LS	171	6–288	369	257–659	191	69–301
	RB	375	276–583	439	334–707	368	276–537

SB—Stem bark; RB—Root bark; LS—Leaves; PE—Pet ether; CH—Chloroform; ME—Methanol; ne—Not extracted.

compel the roots to produce more viable metabolites for self-defence as opposed to the aerial parts.

Table 2 shows that the *Annona squamosa* root and stem bark extracts were the most active among all the investigated plant extracts. In our investigations the root barks yielded the two *ent*-kaurane diterpenoids kaur-16-en-19-oic acid (**1**) (Structure depicted in Fig. 1) and 17-acetoxy-*ent*-kauran-19-al (**2**), all of which displayed larvicidal activity (Table 3). The fact that

no further bioactive metabolites were isolated from the extracts led us to conclude that the *ent*-kaurane diterpenoids **(1**) and **(2**)were the major active principles of the root bark extract, probably other active metabolites not having been isolated because of their minute concentrations in the extract.

The stem bark extracts of *A. squamosa* are known to accumulate pesticidal annonaceous acetogenins[7]. Therefore, the observed larvicidal activity of the stem bark extract was considered to have been exerted by such latter compounds, which however, were not isolated during these investigations, probably due to their low abundance in the investigated plant samples.

Extracts from the *Uvaria* (Annonaceae) species *U. faulknerae, U. kirkii, U. leptocladon, U. lungonyana* and *U. scheffleri* displayed larvicidal activity at varying levels (Table 2). Since both insecticidal and antitumour activities are known to exert similar modes of cell action by blocking the cellular oxygen transport system[8], the larvicidal efficacy of the *Uvaria* species were considered to be due to the *C*-benzyl dihydrochalcones and flavanones metabolized by *Uvaria* species[9]. Furthermore, *U. lungonyana,* which is endemic to Tanzania was first analysed chemically in these investigations and the root bark extract yielded polycarpol (**3**), chamanetin (**4**) and dichamanetin (**5**), melodorinol (**6**), acetylmelodorinol (**7**), benzyl benzoate (**8**), 2-methoxybenzyl benzoate (**9**), pinocembrin (**10**) and 5-hydroxy-7-methoxyflavanone (**11**). Some of these compounds exhibited larvicidal activity (Table 3). The metabolites **(6)** and **(7**) are hereby being reported for the first time from the genus *Uvaria*.

Extracts from the two *Asteranthe* species occurring in Tanzania, *A. lutea* and *A. asterias*, upon analysis yielded the previously reported antifungal alkaloids 2',3'-epoxyasteranthine (**12**) and 2',3'-dihydroxyasteranthine (**13**)[10], all of which showed larvicidal activity (Table 3). These results demonstrate that compounds **(12)** and **(13)** that contain an indolepyranyl skeleton as a unique structural feature among the prenylated indoles from the family Annonaceae, have broad bioactivity spectra.

According to the *Flora of Tropical East Africa*, the four *Uvariodendron* species *U. kirkii, U. gorgonis,*

Table 3. Larvicidal activity of the compounds against III and/or IV instars larvae of *An. gambiae* s.s. Giles after 24 and 48 h exposure

Plant source	Compound	Duration (h)	Part/Extract	LC_{50} (ppm)	95% CL
Annona squamosa	1	24	RC	61	29–95
		48		20	5–33
	2	48	RC	173	123–247
		72		120	84–189
Uvaria lungonyana	3	24	RC	393	263–1085
		48		150	83–242
	4+5	24	RC	122	44–209
		48		50	38–67
	6	24	RC	80	52–191
		48		21	14–33
Asteranthe lutea	12	24	RC	2.3	
		48		0.5	
	13	24		77.5	
		48		33.8	

RC—Root bark chloroform extract; CL—Class limits.

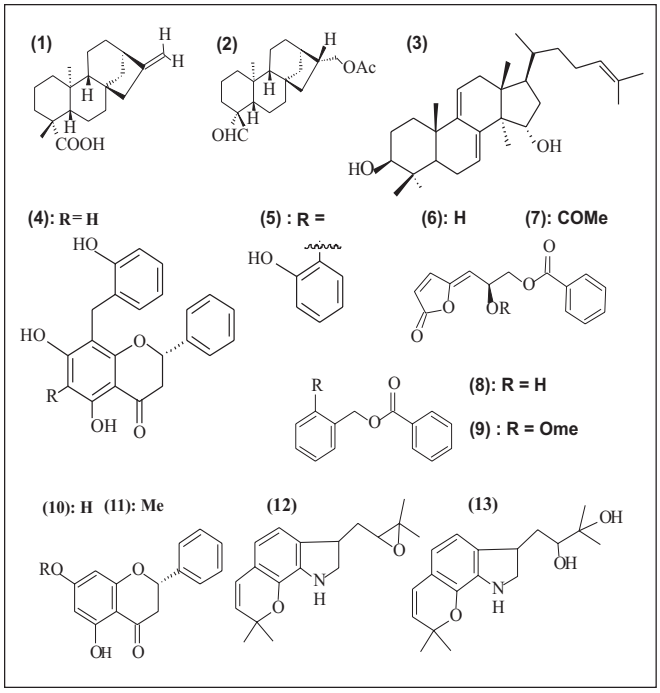

Fig. 1: Chemical structures of active compounds extracted from the investigated plants

U. pycnophyllum and *U. usambarensis* are endemic to Tanzania. So far there are no investigations describing the larvicidal or any biological activity of either of the plant species. Therefore, some of the *Uvariodendron* species were included in these investigations and crude extracts from *U. pycnophyllum* and *U. usambaranse* showed larvicidal activity (Table 2). The larvicidal activity was conceivably ascribed to the insecticidal phenylpropanoids such as eugenol and acetyl eugenol, which are the main constituents of some *Uvariodendron* species[11,12]. This indicates the genus to be a useful source of mosquito larvicides.

The genus *Tessmannia* consists of 11 species that are either small or big trees and mainly found in the rain forests of some parts of Africa. In Tanzania, four *Tessmannia* species are reported, namely *T. densiflora, T. martiniana* var *pauloi, T. martiniana* var *martiniana* and *T. burttii*. Extracts from the three former species when assayed showed larvicidal activity. The methanol root bark extract of *T. martiniana* var *pauloi* also exhibited tail-like structural abnormalities for the larvae after 24 h exposure (Fig. 2). The latter structures emerged after 24 h of exposure and continued to grow until reaching the peak after 48 h, where the length of the larvae was equal to the length of the tail-like structures. The larvae also attained a dark brown colour and shaded during 48–72 h post exposure. Microscopic analysis suggested the tail-like structures to be part of the gut that had been elongated and extruded through the anal cavity.

The larvae that had shaded the tail and continued to survive were reared and monitored through their life cycle until adulthood. The morphological features of the emerged adults were normal as those from the control experiments. The males and females were allowed to mate and then fed with human blood. However, the females could not produce any batch of eggs. This suggested that factors that caused the larvae deformities had also interfered with the reproduction system in the adult mosquitoes. This process was repeated until the entire mosquito colony died. However, none of the isolated compounds could be assayed for this effect due to the paucity of the available samples. Furthermore, until now there is no literature describing the chemical constituents or any bioactivity information for the genus *Tessmannia*. In this regard, this study failed to associate the observed activity and the deformities with any class of compounds. As such there is great need to re-examine the extract and isolate the active compounds before the plants become extinct due to the on going deforestation process caused by harvesting the plant species for building poles and charcoal productions.

The crude pet ether, chloroform and methanol extracts from *L. stellatus* displayed larvicidal activity at varying levels, (Table 2). Previous investigations of *L. stellatus* indicated the crude extracts from the stem and root barks to have *in vitro* antimalarial activity, as well as weak toxicity against brine shrimp (BST) larvae[13]. Among the compounds isolated from the extracts was insect juvenile hormone III (JH III), which was previously isolated from this and other plant species[14]. The occurrence of JH III in plants has been quite intriguing since normally the compound

Fig. 2: Larvae deformities due to VLC fractions **(3)** and **(4)** of *T. martiniana* var *pauloi* root bark methanol extract— (a) normal larvae; (b) tailed larvae; and (c) closer look of larvae abdomen with tail connected

is metabolised by insects in order to regulate their developmental processes (metamorphosis). Therefore, the compound when produces by plants may have similar roles, suggesting that the plants would be producing the compound in order to deter insect accumulation, as the insects would not prefer to acquire additional JH III doses beyond what is normally required for metabolism. Accumulation of this compound beyond biochemically allowable levels would disrupt the insects' development process. Hence, the compound would act as a bio-insecticide. Therefore, the presence of this compound in plant extracts would make the extracts act as readily biodegradable environmental larvicides.

However, when assaying the *L. stellatus* extract no sign of growth disruption was noticed, either in the larvae or in the adult stage of the insect. This could have been attributed to either small amount of the compound present in the extract or due to absence of the compound in the investigated extract as a result of seasonal and/or geographical location.

Crude extracts from *H. opposita* also showed some larvicidal activity (Table 2). In previous studies, such extracts showed no larvicidal activity[15], probably due to seasonal fluctuations in the biosynthesis of the active components. The results could also have been due to different methods of extraction, photosensitivity of some of the compounds in the extract or geographical origin of the plant. In previous studies, *H. opposita* materials were collected from a locality (Kwamngumi Forest Reserve in Muheza district) different from in the present studies (University of Dar es Salaam, Main campus), and probably explaining the difference in the larvicidal results. Previous chemical investigations of *H. opposita* revealed the presence of various types of compounds, including flavanones[16] and 3-*O*-benzoyl or 3-*O*-cinnamoylabiatane diterpenoids[17], some of which possess antitumour, insect antifeedant, antimicrobial and allelochemical activities. Since the physiological effects of the crude extracts on the tested larvae were not investigated, at this stage it is difficult to relate the observed larval toxicity to effects of some of the constituent compounds acting as allelochemicals.

The crude extracts from *Croton sylvaticus* also displayed larvicidal activity (Table 2). The genus *Croton* is known to constitute toxic plant species, making some *Croton* species to be used as sources of poison for hunting and fishing[18]. As several antimicrobial cleorodane diterpenes and other compounds have previously been isolated from the genus[19]. Similar compounds could have been responsible for the observed larvicidal activity in these studies, but such compounds not having been isolated possibly due to their low abundance.

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