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MOSQUITO-LARVICIDAL ACTIVITY OF SOME SRI LANKAN PLANTS

S.S. RANAWEERA
Department of Botany, University of Sri Jayewardenepura, Nugegoda.

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Abstract: Screening of plants of Sri Lanka for mosquito-larvicidal activity, revealed that 18 plant species out of 53 tested were lethal to *Culex quinquefasciatus* larvae. Methanol extracts of *Camellia sinensis*, methanol and petroleum ether extracts of *Acorus calamus* and petroleum ether extracts of *Cymbopogon nardus, Languas galanga, Canarium zeylanicum* and *Curcuma domestica* displayed significant mosquito-larvicidal activity (LC_{50} < 10.0 mg/l) against late 3rd instar *Culex* larvae. High mosquito-larvicidal activity was observed in the steam distillate of *Acorus calamus* (3.6-7.7 mg/l) against late 3rd instar mosquito larvae of *C. quinquefasciatus, Aedes aegypti, Aedes albopictus, Anopheles tessellatus* and *Anopheles subpictus*. The essential oil of *C. nardus* (Ceylon citronella) too displayed a significant activity (6.3 mg/l) against *C. quinquefasciatus* and lesser activity against *A. aegypti* (9.3 mg/l). Petroleum ether extract of the rhizome of *L. galanga* was similarly effective against *C. quinquefasciatus* (8.3 mg/l) and *A. albopictus* (9.3 mg/l). The essential oil of *C. domestica* had high larvicidal activity (4.5 mg/l) against *Anopheles culicifacies* which was resistant to other plant extracts. The results suggest that potent mosquito-larvicidal compounds may be isolated from *A. calamus, C. nardus* and *C. domestica*.

Key words: Mosquito-larvicidal activity, essential oils, mosquitoes, plants.

INTRODUCTION

The long term use of synthetic insecticides has created a number of ecological and medicinal problems such as the development of resistant insect strains, ecological imbalance and high toxicity to mammals.[1] Plants are known to contain compounds of insecticidal[2-4], insect-repelling[2,5] and insect anti-juvenile[6] properties. Most of these compounds are biodegradable and less harmful to mammals than synthetic insecticides. Therefore, there is a possibility of replacing synthetic insecticides with insecticides of plant origin.

Pyrethrins obtained from *Chrysanthemum cinerafolium* are still widely used as insecticides and insect-repellents in Africa and South America.[4] The insecticidal activity and photostability of natural pyrethrin have been increased by chemical synthetic methods. Apart from pyrethrins, the terminal epoxy derivative of geranylgeraniol obtained from *Pterodon pubescens* has been reported to be highly lethal to the trematode *Schistosoma mansoni*.[3] The insecticidal activity of *Acorus calamus* against many insects has been reported.[7] Steam volatile principle of the rhizomes of *A. calamus* L. has been shown to be toxic to adult *Culex* mosquitoes.[8] However, there is no recorded information on the availability of compounds of plant origin, which possess mosquito-larvicidal activity.

It was, therefore, considered important to screen the plants grown in Sri Lanka, for mosquito-larvicidal activity. The present study describes the mosquito-larvicidal activity of some local plant extracts of Sri Lanka against several species of *Culex, Aedes* and *Anopheles*.

METHODS AND MATERIALS

Plant material: Plants for screening for mosquito-larvicidal activity (Table 1) were selected randomly and also on the basis of the information gathered from native people living in rural areas of Sri Lanka. Plant specimens were identified by comparison with reference material at the National Herbarium, Royal Botanic Gardens, Peradeniya, Sri Lanka. Plants were washed in running water and were separated into plant parts (leaves, stem, root, bark etc.) and were air-dried.

Preparation of plant extracts: For preliminary screening for mosquito-larvicidal activity, air dried plant material (10 g) were chopped and macerated with 100 ml solvent for extraction (light petroleum ether, methanol or water). The extracts were transferred to conical flasks (250 ml) and were shaken in an orbital shaker for 1 h. After filtration methanol and light petroleum ether were evaporated to dryness under reduced pressure and the residues were redissolved in 5% aqueous ethanol (100 ml) and later used for the bioassay. The water extracts were directly used for the bioassay.

The plant specimens which showed considerable larvicidal activity (Table 2) were further extracted with each solvent (hexane, light petroleum (40-60%) and methanol) separately and sequentially (in order: hexane, light petroleum ether and methanol) by refluxing in a Soxhlet apparatus for 24 h. Extracts obtained were evaporated to dryness under reduced pressure and the residues were redissolved in 5% aqueous ethanol (100 ml) and later used for the bioassay.

Essential oils in samples were extracted by steam distillation of plant samples using a Clevanger distillation apparatus as described previously.[9]

Test organisms: Late 3rd instar larvae of six mosquito species : *Culex quinquefasciatus* Say, *Aedes aegypti* L, *Aedes albopictus* Skuse, *Anopheles culicifacies* Giles, *Anopheles tessellatus* Theobald and *Anopheles subpictus* Grassi, were used for bioassay. For preliminary screening, *C. quinquefasciatus* was used as the test species.

Table 1: Mosquito-larvicidal actiivity of plant extracts against late 3rd instar larvae of *Culex quinquefasciatus*.

Plant Family/ Species	Plant[a] Part	Solvent[b]	Activity[c]
ARACEAE			
Acorus calamus L.	Rh;Lf	MeOH;Pet	++++
BURSERACEAE			
Canarium zeylanicum (Retz.)Bl.	Sd;Lf	MeOH;Pet	+++
POACEAE			
Cymbopogon citratus (DC.) Stapf	Lf	Pet	++
Cymbopogon confertiflorus Stapf	Lf	Pet	++
Cymbopogon nardus (L.) Rendle	Lf	Pet	+++
Cymbopogon winterianus Jowitt	Lf	Pet	++
LAURACEAE			
Cinnamomum camphora (L.) T. Nees & Eberm.	Lf	Pet	+
Cinnamomum litseifolium Thw.	Lf	Pet	+
Cinnamomum multifolium Wright.	Lf	Pet	+
Cinnamomum zeylanicum Bl.	Lf	Pet	+
FABACEAE			
Derris scandens (Roxb.) Benth.	Rt	MeOH	++
RUTACEAE			
Atalantia rotundifolia (Thw.) Tan.	Lf	Pet	+
RUBIACEAE			
Ophiorrhiza mungos L.	Lf	MeOH	++
THEACEAE			
Camellia sinensis (L.) Kuntz	Sd	MeOH	+++
ZINGIBERACEAE			
Curcuma domestica Valet.	Rh	Pet	+++
Curcuma zedoria (Berg) Roscoe	Rh	Pet	++
Languas galanga (L.) Stuntz	Rh	Pet	+++
Elettara repens (Sonner.) Baill	Rh	Pet	++
Zingiber zerumbet (L.) Sm.	Rh	Pet	++

[a] Lf = leaf, Rh = Rhizome, Rt = Root, Sd = Seed

[b] Pet = Light petroleum ether, MeOH = Methanol

[c] Larvicidal Activity,(LC_{50} value, mg/l)

++++ = < 5.0 ; +++ = 5.1-10.0

++ = 10.1-15.0 ; + = 15.1-20.0

Table 2: LC_{50} values (mg/l) of different plant extracts for late 3rd instar larvae of *Culex*, *Aedes* and *Anopheles* species.

Plant	Extract[a]	Mosquito species[b] / LC_{50}, mg/l					
		C.q.	A.e.	A.a.	A.c.	A.t.	A.s.
Acorus calamus	E.o	3.60	6.20	7.72	12.00	5.25	6.50
	Hex	4.00	7.93	9.31	13.63	8.30	-
	Pet	4.60	8.27	10.60	18.50	9.22	-
	MeOH	4.70	8.81	10.90	19.77	11.87	-
Canarium zeylanicum	Pet	9.60	10.40	11.10	-	-	-
Cymbopogon nardus	E.o	6.30	9.32	34.00	14.25	15.41	-
Camellia sinensis	MeOH	8.00	9.30	10.20	-	-	-
Curcuma domestica	E.o	9.20	12.50	11.20	4.50	12.67	-
	Pet	9.70	-	-	-	-	-
Languas galanga	Pet	8.25	10.86	9.30	12.22	13.85	-

[a] Pet = Light petroleum ether, MeOH = Methanol, Hex= Hexane, E.o = Essential oil

[b] C.q.= *Culex quinquefasciatus;* A.e.= *Aedes aegypti;* A.a.= *Aedes albopictus*; A.c.= *Anopheles culicifacies*; A.t.= *Anopheles tessellatus;* A.s.= *Anopheles subpictus*

- Not tested

Mosquito bioassay: LC_{50} values of plant extracts for mosquito larvae were determined by the procedure followed by The World Health Organization[10] with slight modifications. For bioassay, healthy late 3rd instar mosquito larvae were distributed in batches of 20 in beakers containing 25ml water. Test dispersions (25ml) were prepared in separate beakers by adding different amounts of the extract so that a series of ten final concentrations ranging from 20 to 200ppm was produced, when the contents with larvae in beakers were added to the latter (final volume, 50ml). Mortality counts were taken after 24 h and the bioassay was carried out at 29°C. Each bioassay was carried out with five replicates. LC_{50} values were estimated from a probit/log concentration graph.

RESULTS AND DISCUSSION

Preliminary screening of plant extracts

The results of screening plants for mosquito-larvicidal activity (Table 1) indicate that 18 plant species out of 53 tested were lethal to *Culex quinquefasciatus* larvae. The extracts of *Acorus calamus, Cymbopogon nardus, Languas galanga, Camellia sinensis, Canarium zeylanicum* and *Curcuma domestica* displayed significant larvicidal activity (LC_{50} < 10.0 mg/l) against late 3rd instar larvae of *C. quinquefasciatus*.

Among the species tested the following plant species had very low (LC_{50}>20.0 mg/l) or no activity : **AMARYLLIDACEAE**: *Pancratium zeylanicum* L., *Allium cepa* L., *Allium rubrum* L., *Allium sativum* L.; **APOCYNACEAE**: *Alstonia scholaris* (L.) R.Br., *Alstonia macrophylla* Wall. ex G. Don ; **ARACEAE**: *Alocasia macorrhiza* (L.) Schott, *Alocasia cucullata* (Lour.) Schott, *Lasia spinosa* (L.) Thw., *Amorphophallus campanulatus* (Roxb.) Bl.ex. Decne.; **ASTERACEAE**: *Anacyclus pyrethrum*, *Eupatorium ayapana*; **EUPHORBIACEAE**: *Balispormium montanum, Acalypha indica* L., *Antidesma alexiteria* L.; **FABACEAE**: *Atlosia rugosa* Wight and Arn., *Atylosia trinervia* (Spreng.) Gamble, *Canavalia ensiformis* (L.) DC; **MONOMIACEAE**: *Hortonia floribunda* Wight ex Arn.; **PIPERACEAE**: *Piper betle* L., *Piper longum* L., *Piper nigrum* L.; **RUTACEAE**: *Atalantia monophylla* DC., *Atalantia ceylanica* (Arn.) Oliv., *Citrus aurantium* L., *Citrus grandis* (L.) Osb., *Citrus hystrix* DC. ; **RUBIACEAE**: *Ixora coccinea* L., **SOLÅNACEAE**: *Datura suaveolens* Humb. & Bonpl.ex Willd., *Solanum jacquini* Thw., *Solanum indicum* L., *Solanum nigrum* L., *Solanum trilobatum* L., *Capsicum frutescens* L. ; **SPOTACEAE**: *Maduca longifolia* (L.) J.F.Machr.

Mosquito-larvicidal activity of essential oils and solvent extracts

Results presented in Table 2 indicate that essential oils of *A. calamus*, prepared by steam distillation had the highest mosquito-larvicidal activity (3.6-12.0 mg/l), against all mosquito species tested except *A. culicifacies*. *C. quinquefasciatus* was the most sensitive mosquito species for *A. calamus* oil (3.6 mg/l). The hexane extract of *A. calamus* was as effective (4.0 mg/l) as the steam distillate. Light petroleum ether and methanol extracts of *A.calamus* rhizome were less effective. When the *A. calamus* rhizome was sequentially extracted with hot hexane, light petroleum ether and methanol, mosquito-larvicidal activity was retained only in the hexane extract (Results are not presented). It has been reported that the toxicity of petroleum ether extracts of *A. calamus* against adult mosquitoes *(C. quinquefasciatus)* were almost two fold when compared to that of kerosene.[5]

The essential oil of *C. nardus* (Ceylon citronella) also displayed a significant activity (6.3 mg/l) against *C.quinquefasciatus* and lesser activity against *A.aegypti*. Citronella oil is well known to be a mosquito-repellant but its mosquito-larvicidal activity has not been tested before.

The petroleum ether extract obtained from the rhizome of *L. galanga* was effective against *C. quinquefasciatus* (8.3 mg/l) and *A. albopictus* (9.3 mg/l).

The essential oil of *C. domestica* had high larvicidal activity (4.5 mg/l) against *A. culicifacies* which was resistant to other plant extracts. However, it had very low activity against other mosquito species tested.

Our results suggest that the essential oils of *A. calamus, C. nardus, L.galanga* and *C.domestica* could be used as potent mosquito-larvicides. In order to use these essential oils as mosquito-larvicides it will be necessary to carry out further studies to determine their feasibilty of large scale use, stability of their active compounds under field conditions, and chemical identity of active compounds. The essential oils are complex mixtures of terpenes and there could be more than one compound responsible for the mosquito-larvicidal activity. Therefore, bioassay directed isolation of active components of essential oils of *A. calumus, C. nardus, L. galanga* and *C.domestica* should be undertaken.

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