SCREENING FOR LARVICIDAL ACTIVITY IN SOME THAI PLANTS AGAINST FOUR MOSQUITO VECTOR SPECIES

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Abstract. Ninety-six ethanolic extracts from various parts of 84 Thai plant species were tested for their larvicidal activity against *Aedes aegypti* mosquitoes. Extracts from *Rhinacanthus nasutus*, *Derris elliptica, Trigonostemon reidioides, Homalomena aromatica, Stemona tuberosa* and *Acorus calamus* possessed high larvicidal activity, with LC_{50} values between 16.0 and 48.2 mg/l. Petroleum ether (PE) and methanol (MeOH) extracts were tested for their larvicidal activity against 4 mosquito vector species. The PE extract of *R. nasutus* exhibited larvicidal effects against *Ae. aegypti, Culex quinquefasciatus, Anopheles dirus* and *Mansonia uniformis* with LC_{50} values between 3.9 and 11.5 mg/l, while the MeOH extract gave LC_{50} values of between 8.1 and 14.7 mg/l. *D. elliptica* PE extract showed LC_{50} values of between 11.2 and 18.84 mg/l and the MeOH extract exhibited LC_{50} values between 13.2 and 45.2 mg/l.

INTRODUCTION

Mosquito-borne diseases, such as malaria, filariasis and dengue hemorrhagic fever (DHF) are still major public health problems in the Southeast Asian countries because of their tropical or subtropical climate, the frequently poor drainage system especially during rainy seasons, and the presence of many fish ponds, irrigation ditches and rice fields which provide abundant mosquito breeding places. Although chemical vector control programs have been carried on for long time, these mosquito-vector diseases remain because of the refusal by householders to house spraying with synthetic insecticides, of changes in biting habits of vectors, and evolution of mosquito resistance to conventional insecticides (Nontananda, 1972; Ismail *et al*, 1978; Curtis and Pasteur, 1980; Wattal *et al*, 1981; WHO, 1992) and even to biopesticides such as *Bacillus sphaericus* (Rodcharoen and Mulla, 1994; Tabashnik, 1994).

Searching for new control agents from natural products such as plant secondary metabolites has gained popularity among researchers in countries with a strong herbal tradition and large numbers of plants have been reported to possess insecticidal activity (Yang and Tang, 1988; Curtis *et al*, 1989; Sukumar *et al*, 1991). Before the discovery of synthetic insecticides, natural insecticides such as pyrethrum, rotenone, nicotine, sabadilla, ryania, among others, have been extensively used for insect control (Balandrin, 1985; Pedigo, 1996). Currently, limonoids such as azadirachtin and gedunin, present in species of Meliaceae and Rutaceae, are recognized for their toxic effects on insects and are used in several insecticide formulations in many part of the world (Nagpal *et al*, 1996). Azadirachtin from *Azadirachto indica* has been reported to affect the neuro-endocrine control system in mosquito immature stages. Its mode of action by controlling insect hormones, its favorable toxicology and selective properties from the ecological perspective, provides a basis for emergence of a promising phytochemical for mosquito control (Zebitz, 1984).

Most of the problems of mosquito vector borne diseases occure in low-income tropical communities but these communities have the advantage of access to thousands of species of plants which may contain useful phytochemicals for control of both agriculturally and medically

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important insects. For example, in Argentina, 8 of 11 screened plant extracts showed toxicity against *Aedes aegypti* larvae with LC_{50}<500 mg/l, and among 8 extracts the dichloromethane extract of *Abuta grandifolia* and *Minthostachys setosa* demonstrated high larvicidal activity with LC_{50} of 2.6 and 9.2 mg/l, respectively (Ciccia *et al*, 2000). In Thailand, 10 species of plants which have been reported to possess carminative properties, were screened for larvicidal potential against early 4th instar larvae of *Culex quinquefasciatus*. Marked larvicidal effects were seen with *Kaempferia galanga*, *Illicum vernum* and *Spilanthes acmella* having LC_{50} values of 50.5, 54.1 and 61.4 mg/l, respectively (Pitasawat *et al,* 1998).

This present study was aimed to discover cost-effective alternatives for the control of mosquito vectors by evaluating the larvicidal activity of crude ethanolic extracts of 84 plant species from local Thai plants against late 3rd-early 4th instar larvae of *Ae. aegypti* mosquitoes. The promising plants, showing LC_{50} < 50 mg/l, were further selected for extraction with petroleum ether (PE) and methanol (MeOH) and tested for larvicidal activity against *Ae. aegypti*, *Cx. quinquefasciatus, Anopheles dirus* and *Mansonia uniformis* larvae.

MATERIALS AND METHODS

Mosquitoes

The mosquito species used for the tests were *Aedes aegypti, Culex quinquefasciatus, Anopheles dirus*, and *Mansonia uniformis*. These mosquitoes were uninfected laboratory strains and were reared for over 10 generations in the insectary of the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Thailand. The method for mass rearing was slightly modified from the procedure previously reported (Limsuwan *et al*, 1987).

Late 3rd-early 4th instar larvae of *Ae. aegypti* were used to screen the larvicidal activity of ethanolic extracts obtained from 84 candidate plants, while the larvae of *Cx. quinquefasciatus, An. dirus*, and *Ma. uniformis* were further evaluated with PE or MeOH extracts obtained from those plants whose ethanolic extracts showed LC_{50} values of less than 50 mg/l.

Plant collection

Plants of 84 species belonging to several families are shown in Table 1. Wild specimens of these plants were collected throughout Thailand, and as far as possible from cultivated areas to exclude possible contamination with insecticides. Some plants were purchased from medicinal plant shops in Bangkok. The identifications of the specimens were checked by Miss Nantawan Supuntee, Forest Herbarium National Park, Wildlife and Plant Conservation Department, Bangkok, Thailand.

Preparation of extracts

The aerial parts of 84 plants were dried in the shade, cut up and then ground in a homogenizer. The powder material from each species was macerated with 75% ethanol at room temperature for 3 days and then suction filtered through a Buchner funnel. The solvent was removed by rotary evaporation under reduced pressure at a temperature below 45°C. The resulting crude ethanolic extracts were kept at –20°C until screening for their larvicidal activity against *Ae. aegypti*.

The powder from each plant which showed an LC_{50} value of less than 50 mg/l was extracted in a Soxhlet extractor with PE, and then with MeOH. The solutions were concentrated under reduced pressure, below 45°C, to dryness. The resulting crude PE and MeOH extracts were kept at -20°C until testing for their larvicidal property against *Ae. aegypti, Cx. quinquefasciatus, An. dirus*, and *Ma. uniformis.*

Larvicidal test

Methods for testing larvicidal action of the crude extracts were slightly modified from those of World Health Organization (WHO, 1996). A stock solution was prepared by dissolving a known amount of the crude extract in an appropriate solvent and stored in a refrigerator at 15°C. Twenty healthy, late 3rd-4th instar larvae were introduced into each testing cup (sterilized plastic drinking cup of 150 ml capacity), which contained 100 ml of dechlorinated tap water. A measured volume of stock solution was added to obtain the desired concentrations. Experiments were carried out with a series of seven concen-

Table 1
Plants studied for their larvicidal effectiveness against *Ae. aegypti*.

Family and scientific name	Common name	Thai name
ACANTHACEAE		
Andrographis paniculata N.	-	Fa thalai chon
Rhinacanthus nasutus L.	-	Thong phan chang
AMARYLLIDACEAE		
Crinum asiaticum L.	cape lily, crinum lily	Phlap phlueng
ANACARDIACEAE		
Melanorrhoea usitata Wall	red zebra wood, vanish tree	Rak yai
ANNONACEAE		
Annona muricata L.	sour sop	Thurian thet
A. squamosa L.	custard apple, sugar apple,	Noina
APIACEAE		
Ocimum basilicum L.	Hoary basil	Maeng lak
APOCYNACEAE		
Allamanda cathartica L.	-	Banburi lueang
Cerbera odollum Gaertn.	-	Tin pet nam
APOCYNACEAE (cont)		
Cerbera peruviana Pers.	Trumpet flower	Ram phoei
Nerium oleander L.	Oleander	Yi tho
Strophanthus scaudatus L.	-	Yang nong kruea
ARACEAE		
Acorus calamus L.	myrtle grass	Wan nam
Dieffenbachia picta Schott.	-	Aai bai
Homalomena aromatica Schott.	-	Tao kiat
H. rubescens Kunth.	-	Sa ne chan daeng
Lasia spinosa L.	-	Phak hnam
ARISTOLOCHIACEAE		
Aristolochia indica L.	-	Kra chao sree da
ASCLEPIADACEAE		
Calotropis gigantean L.	crown flower, giant Indian	Rak dok
ASTERACEAE (COMPOSITAE)		
Ageratum conyzoides L.	Billy-goat weed, goat weed	Sap raeng sap ka
Artemisia annua L.	-	Kot chula lampha
Cosmos sulphureus Cav.	-	Dao krachai
Chromolaena odoratum L.	-	Sap suea
Melampodium divaricatum DC.	-	Kradum thong
Sphaeranthus africanus	-	Phak khart hua waen
Tagetes patula L.	marigold	Dao rueang noi
Thithonia diversiforia Grat.	Mexican sunflower weed	Bua tong
CAPPARIDACEAE		
Cleome viscose L.	-	Phak sian phi
COMBRETACEAE		
Combretum quadrangulare L.	-	Sakae na
CUCURBITACEAE		
Luffa acutangula L.	angled loofah	Buap liam
Momordica cochinchinensis L.	-	Fak khao
Trichosanthes tricuspidata L.	-	Khi ka daeng
CYPERACEAE		
Cyperus rotundus L.	nut grass	Ya haeo mu

Table 1 (continued).

Family and scientific name	Common name	Thai name
EUPHORBIACEAE	-	
Baccaurea ramiflora L.	croton oil plant	Mafia
Croton tiglium L.	Malayan spurge tree	Salot
Euphorbia antiquorum L.	Painted spurge	Salat dai pa
E. heterophylla L.	Poinsettia	Luk khoei tai mae yai tham sop
E. pulcherrima L.	-	Khritsamat
E. tirucalli L.	-	Phaya roi bai
Excoecaria cochinchinensis L.	-	Kra bue chet tua
Jatropha gossypifolia L.	-	Sabu lueat
J. multifida L.	Caston bean	Fin ton
Ricinus communis L.	-	Lahung
Trigonostemon reidioides Craib.		Lot thanong
FABACEAE		
Abrus precatorius L.	crab's eye vine	Maklam tanu
Acacia concinna Dc.	-	Sompoi
Adenanthera pavonina L.	red sandalwood tree	Ma klam ta chang
Cassia fistula L.	common pink cassia	Chaiya phruek
C. siamea Lam.	Thai copper pod	Khi lek
Derris elliptica Benth.	Tuba root, derris	Hang lai daeng
D. scandens Benth.	-	Thao wan priang
Phyllodium longipes Craib & Schindl	-	Klet lin yai
Pueraria candollei Graham.	-	Kwao khruea
ICACINACEAE		
Stemonurus secundiflorus Bl.	-	Ai bao phru
LAURACEAE		
Cinnamomum rhyncophyllum Miq.	-	Tae yor
Persea membranacea Kosterm	-	Kra thang thu
MALVACEAE		
Urena lobata L.	-	Khi khrok
MELIACEAE		
Aglaia rubiginosa Hein.	-	Chomphu samet
Azadirachta indica A. Juss.	neem	Sadao India
Melia azendarach L.	bastard cedar	Lian
MENISPEMACEAE		
Stephania venosa Spreng.	-	Bora phet phung chang
Tinospora crispa Beumee.	-	Bora phet
PALACEAE		
Caryota bacsonensis	Jaggery palm	Tao rang
PIPERACEAE		
Piper betle L.	Betel pepper	Phlu
PLUMBAGINACEAE		
Plumbago indica L.	-	Chetta mun phloeng daeng
POACEAE		
Cymbopogon nardus L. Rendle.	citronella grass	Ta khrai hom
Vetiveria zizanioides Nash.	vetiver	Ya faek hom
RUBIACEAE		
Ixora ebarbata Craib.	-	Khem khao
Morinda citrifolia L.	-	Yor
M. coreia Ham.	-	Yor pa
Clausena excavata	-	Mui yai

Table 1 (continued).

Family and scientific name	Common name	Thai name
SAPINDACEAE		
Nephelium longan L. Hook.	Longan	Lamyai pa
N. hypoleucum Kurz.	-	Kho laen
Sapindus rarak Dc.	soap nut tree	Ma khamdi khwai
Zollingeria dongnaiensis Pierre.	-	Khi non
STEMINACEAE		
Cardiospermum halicacabum L.	wiwi, balloon vine	Khok kra om
Stemona tuberosa L.	-	Non tai yak
VERBENACEAE		
Duranta repens L.	golden dewdrop	Thian yot
ZINGIBERACEAE		
Boesenbergia pandurata Schltr.	-	Kra chai
Curcuma longa L.	tumeric	Khamin chan
C. zedoaria Roscoe.	-	Khamin oi
Hedyclium coronarium Roem	butterfly lily	Mahahong
Zingiber amaricans Blume	-	Kra thue
Z. officinale Roscoe.	ginger	Khing
Z. purpureum Roscoe.	-	phlai

trations, each with 5 replicates, with a final total number of 100 larvae for each concentration. Each batch of replicates contained one control of 100 ml of water alone and another of 100 ml of water containing a volume of solvent corresponding to the maximum volume of extract tested. As very few larvae succumbed within 24 hours of exposure to the test solutions, mortality was recorded after 48 hour of exposure, during which no food was offered to the larvae. The mortalities of mosquito larvae were recorded if moribund larvae were incapable of rising to the surface or of showing the characteristic diving reaction when the water was disturbed or they showed discoloration, unnatural position or rigor. The LC_{50} was determined by a Probit analysis program (Finney, 1971). Control mortality was accounted for by the formula of Abbott (1925).

RESULTS

Screening for larvicidal activity of ethanolic extracts against *Ae. aegypti*

From 96 ethanolic extracts, 44 extracts provided LC_{50} values < 750 mg/l and were considered effective against the larvae of *Ae. aegypti* (Table 2). Among these, only those from *Rhinacanthus nasutus* (roots), *Derris elliptica, Homalomena aromatica, Trigonostemon reidioides, Stemona tuberosa*, and *Acorus calamus* were found to have high larvicidal activity with 48 hours LC_{50} values of between 16 and 48 mg/l. Seven other extracts showed moderate larvicidal activity with LC_{50} ranging from 50 to 100 mg/l whereas 31 of the ethanolic extracts showed LC_{50} ranging from 100 to 800 mg/l and 52 showed no activity at doses as high as 1,600 mg/l.

Screening for larvicidal activity of PE and MeOH extracts against *Ae. aegypti, Cx. quinquefasciatus, An. dirus* and *Ma. uniformis*

The 48-hour LC_{50} and LC_{90} values of PE and MeOH extracts of the 6 most active plants against *Ae. aegypti, Cx. quinquefasciatus, An. dirus* and *Ma. uniformis* are shown in Tables 3-6. The PE extract of *R. nasutus* exhibited the highest activity against *Ae. aegypti, An. dirus* and *Ma. uniformis* while the PE extract of *D. elliptica* showed high activity against *Cx. quinquefasciatus* and *An. dirus*.

DISCUSSION

Much research has been conducted on

Table 2
LC_{50} and LC_{90} values of 75% ethanol plant extracts against late 3rd- early 4th instar larvae of *Ae. aegypti*.

Plant	LC values (mg/l)			
	LC_{50}	95% Cl of LC_{50}	LC_{90}	95%Cl of LC_{90}
R. nasutus (roots)	16.04	11.47 -22.42	47.08	27.91 -80.72
D. elliptica	20.49	18.55 -22.64	47.49	41.35 -56.35
H. aromatica	38.10	28.48 -51.02	131.5	82.01 -215.23
T. reidioides	40.89	36.92 -45.29	98.84	85.69 -117.87
S. tuberosa	43.48	38.98 -48.53	119.12	101.73 -144.62
A. calamus	48.24	43.42 -53.64	123.42	106.12 -148.73
P. membranacea	53.72	37.91 -76.13	191.33	109.98 -337.39
C. tiglium	60.87	43.03 -86.16	263.66	147.34 -481.23
Z. purpureum	64.02	39.31 -104.27	199.61	91.86 -437.48
M. azadarach (seed)	76.69	51.24 -114.83	260.89	134.83 -510.07
S. rarak	88.08	59.04 -131.63	375.98	185.22 -776.51
C. zedoaria	93.38	69.27 -125.92	152.92	98.32 -250.46
C. odollum (leaves)	96.16	86.90 -106.47	229.9	199.26 -274.39
A. squamosa	101.96	54.42 -191.34	557.02	164.29 -1,912.43
C. odollum (unripe fruits)	102.23	71.93 -145.45	312.42	174.37 -570.16
C. longa	106.38	78.31 -144.57	258.87	159.99 -423.67
D. scandens	122.90	75.73 -199.84	494.93	201.99 -1,229.63
C. peruviana	150.33	128.72 -178.62	757.92	562.95 -1,120.28
C. bacsonensis	155.65	115.47 -211.63	562.96	322.63 -1,036.50
A. concinna	162.59	140.86 -190.53	682.28	523.54 -964.00
P. betle	177.62	131.76 -239.66	557.06	343.07 -927.67
C. asiaticum	177.76	121.72 -259.89	664.02	347.71 -1,287.36
C. gigantean	183.07	151.84 -227.76	1,241.08	850.99 -2,047.38
Z. amaricans	188.08	147.17 -240.36	340.32	236.21 -502.98
C. rhyncophyllum	188.64	165.58 -217.91	635.49	501.12 -872.16
R. nasutus(leave and stem)	190.29	162.15 -228.74	914.07	668.48 -1,388.40
N. oleander (leaves)	197.97	165.89 -243.78	1,131.89	789.42 -1,845.57
P. indica	202.21	147.97 -276.87	717.55	420.66 -1,251.19
H. coronarium	241.73	219.03 -267.02	550.96	478.79 -656.72
S. africanus	260.66	188.30 -361.37	667.93	392.71 -1,171.84
Z. officinale	270.60	218.23 -335.52	427.47	311.09 -602.47
P. candollei	272.38	189.62 -391.54	635.72	355.28 -1,160.83
E. tirucalli	310.56	277.51 -349.44	864.88	722.01 -1,089.09
T. diversiforia	326.87	292.84 -365.17	912.08	778.42 -1,107.00
A. muricata	330.51	301.12 -363.30	685.74	599.61 -814.30
V. zizanioides	380.73	294.39 -492.96	1,106.88	735.54 -1,716.73
E. odoratum	433.88	392.68 -479.71	1,009.29	876.32 -1,203.16
R. communis	523.13	405.76 -675.00	1,146.81	777.38 -1,753.02
H. rubescens	542.88	501.83 -587.27	913.37	821.85 -1,048.30
C. halicacabum	543.19	396.23 -744.67	967.70	599.14 -1,586.57
E. pulcherrima	548.94	419.93 -718.38	1,316.51	856.01 -2,071.06
S. caudatus	573.80	524.26 -628.34	1,143.44	1,008.27 -1,341.63
E. antiquorum	599.74	538.97 -669.63	1,536.49	1,302.84 -1,896.08
C. quadrangulare	722.79	518.36 -1,014.40	1,991.22	1,092.81 -3,810.441

LC_{50} = median lethal concentration; Cl= confidence interval; LC_{90} = 90% lethal concentration.

Table 3
LC_{50} and LC_{90} values of 6 promising larvicidal plants against the late 3rd- early 4th instar larvae of *Ae. aegypti*.

Plant and extract	LC values (mg/l)			
	LC_{50}	95% CI of LC_{50}	LC_{90}	95%CI of LC_{90}
R. nasutus (roots):				
PE extract	3.93	3.05-4.77	18.51	14.99-24.59
MeOH extract	8.11	7.33-8.96	18.46	16.09-21.96
D. elliptica:				
PE extract	11.17	10.06-12.40	27.74	23.93-33.36
MeOH extract	13.17	11.88-14.60	32.22	27.92-38.42
H. aromatica:				
PE extract	40.36	23.56-69.18	97.35	39.01-245.65
MeOH extract	34.15	30.96-37.67	77.21	67.43-91.30
T. reidioides:				
PE extract	116.95	76.96-178.03	206.91	105.17-422.07
MeOH extract	36.38	32.98-40.13	82.48	72.00-97.58
S. tuberosa:				
PE extract	25.26	19.43-32.83	52.18	35.22-79.43
MeOH extract	41.50	37.39-46.08	104.41	90.11-125.15
A. calamus:				
PE extract	29.33	26.33-32.67	78.68	67.64-94.56
MeOH extract	29.29	22.85-37.53	73.01	50.27-108.50

LC_{50} = median lethal concentration; CI= confidence interval; LC_{90} = 90% lethal concentration.

Table 4
LC_{50} and LC_{90} values of 6 promising larvicidal plants against the late 3rd- early 4th instar larvae of *Cx. quinquefasciatus*.

Plant and extract	LC values (mg/l)			
	LC_{50}	95% CI of LC_{50}	LC_{90}	95%CI of LC_{90}
R. nasutus (roots):				
PE extract	9.98	5.44-18.28	43.08	16.76-111.99
MeOH extract	8.57	7.57-9.63	25.85	21.82-32.03
D. elliptica:				
PE extract	4.61	3.87-5.33	15.42	12.93-19.42
MeOH extract	18.53	14.22-24.15	54.4	36.05-84.33
H. aromatica:				
PE extract	22.87	20.29-25.75	75.32	63.54-92.47
MeOH extract	27.81	25.00-30.93	72.85	62.78-87.31
T. reidioides:				
PE extract	46.08	26.22-81.02	97.6	38.08-252.18
MeOH extract	40.61	36.75-44.88	94.78	82.47-112.55
S. tuberosa:				
PE extract	35.80	26.77-47.91	112.23	70.90-180.71
MeOH extract	15.15	11.58-19.81	36.34	24.21-55.55
A. calamus:				
PE extract	45.67	40.64-51.42	142.75	119.81-176.97
MeOH extract	58.57	38.29-90.28	282.89	110.63-760.41

LC_{50} = median lethal concentration; CI= confidence interval; LC_{90} = 90% lethal concentration.

Table 5
LC_{50} and LC_{90} values of 6 promising larvicidal plants against the late 3rd- early 4th instar larvae of *An. dirus*.

Plant and extract	LC values (mg/l)			
	LC_{50}	95% CI of LC_{50}	LC_{90}	95%CI of LC_{90}
R. nasutus (roots):				
PE extract	7.91	5.30-11.73	30.11	16.97-54.69
MeOH extract	14.51	13.13-16.02	33.64	29.31-39.88
D. elliptica:				
PE extract	8.07	5.40-12.05	15.77	8.54-29.47
MeOH extract	16.17	14.31-18.26	54.23	45.37-67.51
H. aromatica:				
PE extract	43.32	25.89-72.50	168.51	68.29-421.34
MeOH extract	18.35	16.44-20.47	50.42	43.13-61.04
T. reidioides:				
PE extract	43.02	31.50-58.86	167.99	98.69-292.84
MeOH extract	36.67	18.31-73.48	249.77	63.36-996.84
S. tuberosa:				
PE extract	36.09	24.91-52.29	111.14	61.55-203.70
MeOH extract	174.45	102.34-305.30	749.34	243.96-2489.92
A. calamus:				
PE extract	30.36	27.39-33.64	74.29	64.38-88.58
MeOH extract	33.23	20.59-53.64	125.32	55.76-283.85

LC_{50} = median lethal concentration; CI= confidence interval; LC_{90} = 90% lethal concentration.

Table 6
LC_{50} and LC_{90} values of 6 promising larvicidal plants against the late 3rd- early 4th instar larvae of *Ma. uniformis*.

Plant and extract	LC values (mg/l)			
	LC_{50}	95% CI of LC_{50}	LC_{90}	95%CI of LC_{90}
R. nasutus (roots):				
PE extract	11.52	10.45-12.71	25.88	22.61-30.61
MeOH extract	14.73	13.36-16.25	33.21	29.01-39.26
D. elliptica:				
PE extract	18.84	16.99-20.91	46.95	40.59-56.15
MeOH extract	45.16	40.14-50.88	142.75	119.52-177.74
H. aromatica:				
PE extract	74.55	66.43-84.15	217.81	180.73-276.20
MeOH extract	60.22	53.67-67.80	179.65	150.21-224.75
T. reidioides:				
PE extract	51.06	34.99-74.55	137.53	74.94-255.50
MeOH extract	49.94	45.27-55.14	113.75	99.10-134.99
S. tuberosa:				
PE extract	27.56	25.10-30.27	57.79	50.78-67.97
MeOH extract	201.88	162.44-272.78	919.4	586.56-1827.00
A. calamus:				
PE extract	126.13	109.67-418.66	416.99	319.69-600.64
MeOH extract	51.82	46.30-58.10	149.55	126.35-184.28

LC_{50} = median lethal concentration; CI= confidence interval; LC_{90} = 90% lethal concentration.

plant derived chemicals which are non-toxic to man and domestic animals and serve as useful basis for the development of safer and more selective mosquito insecticides (Sukumar *et al*, 1991). Our screening showed that among 96 ethanolic extracts, 6 extracts [*R. nasutus* (roots), *D. elliptica, H. aromatica, T. redioides, S. tuberosa*, and *A. calamus*] gave high potency with low critical lethal concentrations (LC_{50} <50 mg/l) against late 3rd-early 4th instar larvae of *Ae. aegypti* (Table 2).

After subsequent extraction with PE and MeOH, our results showed that the PE extracts were always more effective than the MeOH and ethanol extracts. Similar effects were obtained by other researchers. The PE extract of *Allium sativum* was found to be highly effective with an LC_{50} of 12.4 mg/l while water and ethanolic extracts gave LC_{50} of 388.3 and 548.51 mg/l, respectively against *Cx. tritaeniorhynchus* (Satoto, 1993). However, with *Ervatamia coronaria* the ethanolic extracts was highly active against 4th instar larvae of *Ae. aegypti* whereas the PE extracts had no larvicidal activity (Qureshi *et al*, 1986).

We found that the PE extract of *R. nasutus* gave an LC_{50} value of 10 mg/l while Pushpalatha and Muthukrishman (1999) found that PE extract of *R. nasutus* leaf gave an LC_{50} value of 13.2 mg/l against *Cx. quinquefasciatus* larvae. In addition, the PE extract from leaf of *R. nasutus* has been reported to decrease the fecundity of mosquitoes and the hatchability of their eggs (Pushpalatha and Muthukrishnan, 1999; Muthukrishnan and Pushpalatha, 2001).

When roots of *D. elliptica* were extracted with water, PE, acetone or ethanol, the latter extract produced the highest mortality in *Ae. aegypti* (Ameen *et al*, 1985). With a different species of *Derris*, our results showed that the MeOH extract of *D. elliptica* gave LC_{50} value of 18.5 mg/l, similar to that of the ethanolic extract of *D. urucu* root with an LC_{50} value of 17.6 mg/l against 4th instar larvae *Ae. aegypti*.

No previous studies on the larvicidal activity of *H. aromaticum* have been reported. However, our results showed the PE extract of this plant was the third most effective among those tested against *Ae. aegypti, Cx. quinquefasciatus*, *An. dirus* and *Ma. uniformis*. *H. cordata* has been reported to reduce larval and adult emergence rates by approximately half when this plant was experimentally grown in freshwater swamp forest with the aim of controlling the *Brugia* filariasis vectors *Ma. bonneae* and *Ma. dives* (Chang *et al*, 1988; Seng *et al*, 1991).

There is no previous report of the mosquito-larvicidal activity of *T. redioides*; however, dichloromethane extracts from the roots of this species have shown a strong inhibition of feeding of agricultural pests (Chavasiri *et al*, 1999). The extracts of *T. redioides* have been used in traditional medicine as an expectorant and a laxative and in treatment of skin diseases (Smitinand, 1980).

Our results confirmed the larvicidal activity of *S. tuberosa* reported in a study in Malaysia (Lee and Chiang, 1994). For other species of *Stemona*, the crude aqueous extract of *S. collinsae* has an LC_{50} of 104 mg/l against *Ae. aegypti* larvae (Virachat, 1985), but our results with the PE extract showed an even higher effect. *S. tuberosa* has remarkable repellency against the agricultural pest *Spodoptera littoralis*, the tub erostemonine being the dominant alkaloid in the roots (Brem *et al*, 2002) In addition, these stemona alkaloids demonstrated antitussive activity in guinea pigs after induction of coughing by citric acid aerosol stimulation (Chung *et al*, 2003).

In agreement with our findings, the best result against mosquito larvae was obtained with the PE extract of *A. calamus* (Chavan *et al*, 1979; Sujatha *et al*, 1988). High mosquito-larvicidal activity has been observed in both the steam distillate and PE extract of *A. calamus* (Ranaweera, 1996). Phytochemical study with the rhizome extract of *A. calamus* reported the presence of phenyl indane derivative, 2,3-dihydro-4,5,7-trimethoxy-1-ethyl-2-methyl-3-(2′,4′,5′-trimethoxyphenyl) indane (Saxena and Sumithra, 1985). Insecticidal activity obtained by distillation of the PE extract of *A. calamus* appears to be due to the *trans*-isomer of asarone (Dixit *et al,* 1956).

Indigenous plants are perennially available in large quantities and the cost involved in the preparation of these extracts is minimal. More-

over, solvents used for the extraction can be recovered and recycled. The high larvicidal activity of *R. nasutus* and *D. elliptica* and the abundant availability of these plants in tropical and subtropical countries may make them economical for field use in mosquito control programs. Although these plant extracts are less toxic than the synthetic insecticides DDT and temephos, they may eventually replace the conventional synthetic materials if they can be shown to be less polluting to the environment and do not disrupt the balance of the ecosystem.

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