สำนักงานกองทุนสนับสนุนการวิจัย
สกว TRF
THE THAILAND RESEARCH FUND

รายงานวิจัยฉบับสมบูรณ์

โครงการวิจัยหลังปริญญาเอก

เรื่อง

การคัดกรองพืชสมุนไพร เพื่อควบคุมลูกน้ำยุงลายบ้าน (Aedes aegypti) และความเป็นพิษต่อปลา

โดย ผศ.ดร. อมรา นาคสถิตย์ และคณะ

กันยายน 2546

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กิตติกรรมประกาศ

คณะผู้วิจัยขอขอบคุณอาจารย์จำลอง เพ็งคล้าย และ ดร.ก่องกานดา ชยามฤต ผู้เชี่ยวชาญด้านพันธุ์ไม้ แห่งหอพันธุ์ไม้ กรมอุทยานแห่งชาติ สัตว์ป่า และพันธุ์พืชแห่งประเทศไทย ที่ได้กรุณาตรวจสอบพันธุ์ไม้ให้กับคณะวิจัยเป็นอย่างดี

สัญญาเลขที่ PDF/59/2540

รายงานวิจัยฉบับสมบูรณ์

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เรื่อง
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คณะผู้วิจัย	สังกัด
1. ผศ.ดร. อมรา นาคสถิตย์	ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

พืชสมุนไพร

ตัดเป็นชิ้นเล็ก ๆ

↓

อบแห้งที่ 45°C

↓

บดเป็นผง

↓

นำมาแช่ใน 80% เอธิลแอลกอฮอล์

↓

กรอง

↓

ปล่อยให้แห้ง

↓

ได้สารสกัด (crude extracts)

↓

นำไปคัดกรองเบื้องต้น (preliminary screening)

↓

นำไปทดสอบความเป็นพิษต่อลูกน้ำยุงลายบ้าน (larvicidal test) → หาค่า LC_{50} และ LC_{90}

ส่งไป Herbarium

↓

นำไปจำแนกชนิดที่ถูกต้อง (identification)

4. การคัดกรองเบื้องต้น (Preliminary screening)

นำสารสกัดจากพืชที่ความเข้มข้น 100 mg ต่อลิตรใน absolute ethyl alcohol มาทดสอบกับลูกน้ำยุงลายบ้านระยะที่สามและสี่ที่เพาะเลี้ยงได้จากห้องปฏิบัติการกีฏวิทยาทางการแพทย์ของสถาบันวิจัย NIH ของกรมวิทยาศาสตร์การแพทย์ กระทรวงสาธารณสุข จังหวัดนนทบุรี โดยใช้มาตรฐานของการคัดกรองของห้องปฏิบัติการของ WHO (1981) โดยทำทั้งหมด 5 replicates พร้อม control ในช่วงทดสอบไม่มีการให้อาหารลูกน้ำยุงแต่อย่างใด ทำการเก็บข้อมูลหลัง 48 ชั่วโมง นับจำนวนลูกน้ำที่ตาย

5. การทดสอบความเป็นพิษต่อลูกน้ำยุง (larvicidal test)

วิธีทดสอบความเป็นพิษต่อลูกน้ำยุงที่ใช้ดัดแปลงมาจากวิธีทดสอบของ WHO (1981) ที่อธิบายไว้โดย Insunt *et al* (1999) แต่ละการทดสอบทำ 5 replicates พร้อม control สารสกัดพืชสมุนไพรที่ฆ่าลูกน้ำยุงได้ 50% ในการคัดกรองเบื้องต้น (preliminary screening) จะนำมาใช้ในการทดสอบทั้งหมด สารสกัดจากพืชสมุนไพรที่ความเข้มข้นต่ำที่สุดที่สามารถฆ่าลูกน้ำยุงตายได้มากกว่า 50% จะนำไปใช้ทดสอบความเป็นพิษต่อ non target organism คือ ปลาหางนกยูงต่อไป

6. ใช้โปรแกรมคอมพิวเตอร์ SPSS/PCT กับ probit analysis เพื่อหาค่า LC_{50} และ LC_{90}

ผลการทดลอง

1. การคัดกรองพืชสมุนไพรเบื้องต้น

สารสกัดจากพืชสมุนไพร 121 species อยู่ใน 112 จีนัส 50 สกุล ตารางที่ 1

ตารางที่ 1 พืชสมุนไพรที่นำมาใช้ทดสอบความเป็นพิษต่อลูกน้ำยุงลายบ้าน ระยะที่ 3 และระยะที่4

Family and Scientific name	Plant habit	Vernacular name Thai (T), English (E)	Parts use	Place obtained
Acanthaceae				
Andrographis paniculata (Burm.f.) Wall. Ex Nees	Herb	Fa thalai chon (T) Kreat (E)	Leaves	Yala
Justicia adhatoda L.	Shrub	Sa niat (T) Adhatoda (E)	Leaves	Pattani
Rhinacanthus nasutus (L.) Kurz	Shrub	Thong khan chang (T) - (E)	Leaves	Songkhla
Amaranthaceae				
Amaranthus spinosus L.	Exotic Herb	Phak khom nam (T) Spiny pigweed (E)	Leaves & Twings	Yala
Achyranthes aspera L.	Herb	Phan ngu (T) Pricklychaff-flower (E)	Leaves	Satun
Araceae				
Acorus calamus L.	Herb	Wan nam (T) Sweet flag (E)	Roots	Phatthalung
Alocasia macrorrhizos L.	Herb	Kradat daeng (T) Elephant ear (E)	Roots	Songkhla
Colocasia giganted Hook.f.	Herb	Kradat Khao Giant tard (E)	Roots	Songkhla
Anacardiaceae				
Anacardium occidentale L	Exotic Shrubby Tree	Mamuang himmaphan (T) Cashew nut tree (E)	Seeds	Songkhla
Annonaceae				
Annona muricata L.	Exotic Shrubby Tree	Thurian thet (T) Durian belanda (E)	Seeds	Songkhla
Apocynaceae				
Alsonia macrophylla Wall. Ex G. Don	Tree	Thungfa (T) Botino (E)	Fruits	Nokhon Si Thammarat

Family and Scientific name	Plant habit	Vernacular name Thai (T), English (E)	Parts use	Place obtained
Nerium indicum Mill	Exotic Shrubby Tree	Yitho (T) Rosebay (E)	Twigs	Pattani
Rauvolfia serpentine (L.) Benth. Ex Kurz.	Shrub	Rayom (T) Rauwolfia (E)	Stems	Surat thani
Thevetia peruviana (Pers.) K. Schum	Exotic Shrubby Tree	Ram phoei (T) Trumpet flower (E)	Leaves	Yala
Araliaceae				
Ployscias fruticosa (L.) Harms.	Herb	Lep khrut (T) Ming aralia (E)	Leaves	Narathiwat
Asparagaceae				
Asparagus racemosus Willd.	Climber	Sam sip (T) - (E)	Roots	Pattani
Cactaceae				
Opuntia elatior Mill	Exotic Shrub	Sema (T) - (E)	Stems	Yala
Capparaceae				
Cleome viscose L.	Herb	Phak sian phi (T) Polanisia vicosa (E)	Leaves & Twigs	Yala
Cecroplaceae				
Celastrus paniculata willd.	Climber	Kurapia (T) - (E)	Twigs	Songkhla
Celastraceae				
Celastrus paniculata Willd.	Climber	Krathong lai (T) - (E)	Twigs	Narathiwat
Salacia chinensis L.	Scandent Shrub	Kam phaeng Chet chan (T) - (E)	Stems	Yala
Colchicaceae				
Gloriosa superba L.	Herb-Climber	Dong dueng (T) Climbing lily (E)	Fruits	Pattani

Family and Scientific name	Plant habit	Vernacular name Thai (T), English (E)	Parts use	Place obtained
Compositae				
Blumea balsamifera (L.) DC.	Shrub / Shrubby Tree	Nat yai (T) Camphor tree (E)	Leaves	Yala
Eclipta prostrate (L.). L.	Herb	Ka meng (T) False daisy (E)	Leaves & Twigs	Pattani
Chromolaena odoratum (L.)	Exotic-Herb	Sap suea (T) - (E)	Leaves	Pattani
Gynura var. hispida Thwaites	Herb	Wan mahakan (T) - (E)	Stems	Surat Thani
Pluchea indica (L.) Less	Shrub	Khlu (T) Indian marsh fleabane (E)	Stems	Songkhla
Spilanthes acmella Murr.	Herb	Phak ksrat huawaen (T) Para cress (E)	Leaves Twigs	Yala
Tithonia diversifolia (Hemsl.) A. Gray	Exotic-Under Shrub	Bua tong (T) Mexican sunflower Weed (E)	Flowers	Chumporn
Connaraceae				
Connarus ferrugineus Jack.	Climber	Thop thaep (T) - (E)	Stems	Yala
Convolvlaceae				
Erycibe elliptilimba Merr& Chun	Climber	Change san sap man (T) - (E)	Stems	Trang
Costaceae				
Costus speciosus (Koen.) Sm.	Herb	Ueang maina (T) Spiral flag (E)	Roots	Krabi
Cucurbitanceae				
Bryonia laciniosa L.	Climber	Kiegakone (T) - (E)	Vines	Yala
Trichosanthes cucumerina L.	Herb-Climber	Buap knom (T) - (E)	Fruits	Yala
Cyperaceae				
Cyperus rotundus L.	Herb	Ya haeo mu (T) Nut grass (E)	Roots	Yala

Family and Scientific name	Plant habit	Vernacular name Thai (T), English (E)	Parts use	Place obtained
Dipterocarpaceae				
Cotylelobium lanceolatum Craib	Tree	Khiam (T) Resak tombaga (E)	Stem	Nakhon Si Thammarat
Euphorbiaceae				
Acalypha indica L.	Herb	Tamyae maeo (T) - (E)	Roots	Narathiwat
Aporosa dioica Muell. Arg.	Shrub / Shrubby Tree	Nuan sian (T) - (E)	Stem	Surat Thani
Croton tiglium L.	Exotic Shrubby Tree	Salot (T) Croton oil plant (E)	Twigs	Narathiwat
Euphorbia tirucalli L.	Exotic Shrubby Tree	Phaya raibai (T) - (E)	Stems	Krabi
E. ligularia Roxb.	Exotic Shrubby Tree	Som chao (T) - (E)	Stems	Yala
E. pulcherrima Willd. Ex Klotzsch	Exotic Shrubby	Song radu (T) Poinsettia (E)	Leaves	Yala
Excoecaria cochinchinensis Lour. Var. *Cochinchinensis*	Exotic Shrub	Kamlang kra bue (T) - (E)	Leaves	Yala
E. oppositifolia Griff	Shrub Tree	Fai duean ha (T) - (E)	Leaves	Yala
Suregada muttiflorum Bill	Shrub / Shrubby Tree	Khan thong Phayabat (T) - (E)	Leaves	Pathalung
Jatropha multifida L.	Exotic Shrubby Tree	Finton (T) - (E)	Leaves	Pattani
Mallotus repandus Mull. Arg.	Creeper	Makai khruea (T) Coral plants (E)	Twigs	Pattani
Phyllanthus pulcher Wall. Ex Mull. Arg	Shrub Shrub / Shrubby	Wan thoranisan (T) - (E)	Leaves	Narathiwat
Sauropus androgynus (L.) Merr.	Tree Shrub	Phak wan ban (T) - (E)	Leaves	Yala

Family and Scientific name	Plant habit	Vernacular name Thai (T), English (E)	Parts use	Place obtained
Labiatae				
Clerodendrum serratum (L.)	Under Shrub	Akkhi thawan (T) - (E)	Leaves	Songkhla
Moon var. *Wallichii* C.B.	Exotic	Khancha thet (T)	Leaves	Narathiwat
Leonurus sibiricus L.	Herb	Siberian motherwort (E)	Leaves	Yala
Ocimum sanctum L.	Under Shrub	Kaphroa (T) Thai basil (E)		
O. basilicum L.	Exotic Under Shrub	Horapha (T) Sweet basil (E)	Leaves	Yala
O. gratissimum L.	Exotic Shrub	Yira (T) - (E)	Leaves	Yala
Vitex rotundifolia L.f.	Creeper Shurb	Khon thi so thale (T) Chaste tree (E)	Leaves	Songkhla
V. negundo L.	Exotic Shurb / Shrubby Tree	Khon thi khamao (T) Chinese chaste tree (E)	Leaves	Chumporn
Coleus amboinicus (Lour.) Spreng	Exotic Herb	Niam husuea (T) Indian borage (E)	Leaves & Twigs	Chumporn
Lauraceae				
Cinnamomum porrectum (Roxb). Kosterm	Tree	Thep tharo (T) - (E)	Wings	Trang
C. iners Reinw. Ex Blume	Tree	Chiat (T) Cinnomorn (E)	Stem	Nakhon Si Thammarat
C. bejolghota (Buch-Ham.) Sweet	Tree	Op choei (T) - (E)	Leaves	Pattani
Temmodaphne Thailandica Kosterm	Tree	Samun la waeng (T) Tem's laurel (E)	Stems Bark	Nakhon Si Thammarat
Leguminosae – Mimosoideae				
Albizia procera (Roxb.) Benth	Tree	Thing thon (T) White siris (E)	Stems	Nakhon Si Thammarat
Caosalpinia pulcherrima (L.) Sw. Artz.	Exotic	Hang nokyung thai (T)	Leaves	Songkhla

Family and Scientific name	Plant habit	Vernacular name Thai (T), English (E)	Parts use	Place obtained
	Shurb	Peacock's orest (E)		
Delonix regia (Bojer ex Hook.) Raf.	Exotic	Hang nok yung farang (T)	Leaves	Songkhla
	Tree	Peacock flower (E)		
Derris scandens (Roxb.) Benth	Climber	Thao wan priang (T)	Steams	Songkhla
		Jewel Vine (E)		
Entada phaseoloides Dc.	Climber	Saba mon (T)	Seeds	Songkhla
		St. Thomas's Bean (E)		
Erythrina variegate L.	Tree	Thong lang lai (T)	Stem	Krabi
		Indian coral tree (E)	Bark	
Samanea saman (Jacq.) Merr.	Tree	Chamchuri (T)	Stem	Yala
		Rain tree (E)		
Leguminosae-Caesalpinio ideae				
Cassia alata L.	Exotic	Chumhet thet (T)	Leaves	Pattani
	Shrub	Golden Bush (E)		
C. tora (L.) Roxb	Under	Chumhet thai (T)	Roots	Yala
	Shurb	Foetid assia (E)		
Peltophorum terocarpum (DC.) Backer	Tree	Non si (T)	Stem	Yala
ex K. Heyne		Copper pod (E)	bark	
Senna siamea (Lam.) Irwin & Barneby	Tree	Khi lek (T)	Leaves	Pattani
		Thai copper pod (E)		
Leguminosae-Papilion oideae				
Sesbania grandiflora (L.) Desv	Exotic	Khae ban (T)	Stem	Narathiwat
	Shrub	Agasta (E)	bark	
Gentianaceae				
Fagraea Fragrans Roxb.	Tree	Kan krao (T)	Leaves	Pattani
Guttiferae				
Calophyllum inophyllum L.	Tree	Krathing (T)	Roots	Chumporn
		Borneo mahogany (E)		
Mammea siamensis Kostern	Tree	Saraphi (T)	Flowers	Pattani
		Negkassar (E)		

Family and Scientific name	Plant habit	Vernacular name Thai (T), English (E)	Parts use	Place obtained
Malvaceae				
Abelmoschus moschatus Medik.	Tree	Chamot ton (T) Musk mallow (E)	Leaves	Trang
Abutilon indicum (L.) Sweet.	Exotic Herb	Fan si (T) Country mallow (E)	Roots	Yala
Gossypium herbaceum L.	Under Shrub	Fai (T) Cotton plant (E)	Seeds	Songkhla
Meliaceae				
Lansium domesticum Correa	Tree	Langsat (T) Langsat (E)	Seeds	Yala
Menispermaceae				
Anamirta cocculus (L.) Wight & Arn.	Climber	Kho Khlan (T) Cocculus (E)	Twings	Songkhla
Arcangelisia flava (L.) Merr.	Climber	Khamin khruea (T) - (E)	Stems	Songkhla
Tinospora crispa (L.) Miers ex Hook. f. & Thomson	Climber	Boraphet (T) - (E)	Stems	Songkhla
Moraceae				
Streblus asper Lour	Tree	Khoi (T) Siamese rough bush (E)	Stem Bark	Surat Thani
Myristicaceae				
Knema globularia (L) Warb	Tree	Lueat raet (T) - (E)	Seeds	Krabi
Palmae				
Elaeis guineensis Jacq.	Exotic Palm	Palm namman (T) Aprican oil palm (E)	Flowers	Satun
Passifloraceae				
Passiflora foetida L.	Herb Climber	Ka thok rok (T) Stinking passion flower (E)	Stems	Satun
Plumbaginaceae				
Plumbago indica L.	Under Shurb	Chetta mub pholeng daeng (T) Rose coloured leadwort (E) Phak phai nam (T)	Roots	Songkhla

Family and Scientific name	Plant habit	Vernacular name Thai (T), English (E)	Parts use	Place obtained
Polygobaceae				
Ploygonum flaccidium Meissn	Herb	Cotton plant (E)	Leaves	Yala
Punicaceae				
Punica granatum L. var. *Granatum*	Exotic	Thap thim (T)	Roots	Yala
	Shrub	Pomegranate (E)		
Morinda citrifolia L.	Shrub	Yo (T)	Leaves	Yala
	Tree.	Indian mulberry (E)		
Xantonnea parvifolia Craib	Shurb	Khrop chakkawan (T)	Roots	Yala
		- (E)		
Rutaceae				
Aegle marmelos (L.) Correa ex Roxb.	Tree	Tum (T)	Twigs	Pattani
		Beal fruit tree (E)		
Clausena excavate Burm. F.	Shurb /	San sok (T)	Stems	Songkhla
	Shrubby	- (E)		
		Tree		
Micromelum minutum (G. Forst.)	Shrub /	Mui khao (T)	Leaves	Pattani
Wight & Arn	Shrubby	Negkassar (E)		
Sapindaceae				
Cardiospermum halicacabum L,	Herb	Khok Kra om (T)	Leaves	Pattani
	Climber	Balloon vine (E)		
Quassia amara L.	Exotic	Pra that chin (T)	Roots	Yala
	Shrub	Stave-weed (E)		
Schizaeaceae				
Lygodium flexuosum (L.) Sw.	Climber	Ya yai phao (T)	Leaves	Narathiwat
	Fern	- (E)		
		-		
L. circinatum (Burm.f.) Sw.	Climber	Liphao hang kai (T)	Leaves	Narathiwat
	Fern	- (E)		

Family and Scientific name	Plant habit	Vernacular name Thai (T), English (E)	Parts use	Place obtained
Simaroubaceae				
Brucea jaranica (L.) Merr.	Shrub	Ratchadat (T) - (E)	Stems	Pathalung
Eurycoma longifolia Jack	Shrub / Shrubby	Pl alai phueak (T) - (E)	Leaves	Phangnga
Harrisonia perforate (Blanco) Merr.	Tree	Khontha (T)	Leaves	Songkhla
	Scan Shrub	Clitorea hanceana (E)		
Quassia amara L.	Exotic Shrub	Pra that chin (T) Stave weed (E)	Leaves	Yala
Solanaceae				
Datura metel L. var. *Metel*	Under Shrub	Lam phong (T) Thorn apple (E)	Leaves	Yala
Physalis angulata L.	Herb Climber	Thong theng (T) Cut leaf groung cherry (E)	Leaves & Branch	Pattani
Solanum sanitwongsei Craib	Herb	Ma waeng ton (T)	Fruits	Songkhla
Stemonaceae				
Stemona tuberose Lour.	Shrub	Non tai yak (T) Stemona (E)	Roots	Songkhla
Strychnaceae				
Strychnos nux-vomica L.	Tree	Salaeng Chai (T) Snake wood (E)	Seeds	Nakhon si Thammarat
Umbelliferae				
Anethum graveolens L.	Exotic Herb	Thian khao plueak (T) Dill (E)	Leaves & Twigs	Yala
		Bua bok (T)		
Centella asiatica (L.) Urb.	Exotic Herb	Asiatic pennywort (E)	Stems	Yala
Coriandrum sativum L.	Exotic Herb	Phak chi (T) Coriander (E)	Flowers	Yala
Urticaceae				
Pouzolzia pentandra Benn.	Herb	Ya non tai (T) - (E)	Leaves & Twigs	Songkhla
Verbenacea				
Lantana aculeate L.	Exotic Climber	Phak a krong (T) Hedge flower (E)	Leaves	Yala

Family and Scientific name	Plant habit	Vernacular name Thai (T), English (E)	Parts use	Place obtained
Vitaceae				
Parthenocissus quinguefolia (L.) Planch	Exotic	Thao khan daeng (T)	Leaves	Narathiwat
	Climber	True virginiacreeper (E)		
Zingiberaceae				
Alpinia officinarum Hance	Exotic	Kha lek (T)	Roots	Yala
	Herb	Galangal minor (E)		
Boesenbegia rotunda (L.) Mansf.	Herb	Kra chai (T)	Roots	Yala
		- (E)		
		-		
Curcuma xanthorrhiza Roxb.	Exotic	Wan chak motluk (T)	Roots	Yala
	Herb	- (E)		
Elettaria cardamomum (L.) Maton	Exotic	Krawan thet (T)	Fruits	Yala
	Herb	Cardamom (E)		
Etlingera elatior (Jack) R.M. Sm.	Exotic	Kala (T)	Flowers	Yala
	Herb	Torch ginger (E)		
Kaempferia galanga L.	Herb	Pro hom (T)	Roots	Songkhla
		- (E)		
Zingiber zerumbet (L.) Sm.	Herb	Kra thue (T)	Flowers	Yala
		Wild Ginger (E)		

เมื่อนำสารสกัดมาคัดกรองเบื้องต้น พบว่าพืช 14 species มีความเป็นพิษสูงมากกว่า 50% ต่อลูกน้ำยุงลายบ้าน พืชทั้ง 14 species นี้ได้แก่ *Abutilon indium*, *Samanea saman*, *Costus speciosus*, *Acorus calamus*, *Knema globularia*, *Stemona tuberosa*, *Strychons nux-vomica*, *Kaempferia galanga*, *Cinnamomum porrectum*, *Phyllanthus pulcher*, *Ancardium occidentale*, *Mammea siamensis*, *Anethum graveolens* และ *Annona muricata*.

2. ผลการทดสอบความเป็นพิษต่อลูกน้ำยุง (Larvicidal test)

พืชสมุนไพร 8 species จาก 14 species สามารถฆ่าลูกน้ำยุงได้ 100% เมื่อใช้ความเข้มข้นของสารสกัดที่ 100 mg/l และมีเพียง 1 ชนิด ที่ฆ่าลูกน้ำยุงได้ 100% เมื่อใช้สารสกัดที่ความเข้มข้น 100, 50 และ 25 mg/l และสารสกัดจากพืช 3 ชนิด ได้แก่ *Kaempferia galanga*, *Anacardium occidentale* และ *Mammea siamensis* ที่สามารถฆ่าลูกน้ำยุงได้ 100% ภายใน 24 ชั่วโมง (ดูตารางที่ 2)

ตารางที่ 2 สมุนไพร 14 ชนิด (species) ที่ให้ผลความเป็นพิษสูงต่อลูกน้ำยุงลายบ้าน (*Ae. aegypti*) ระยะที่ 3 และระยะที่ 4 ภายหลัง 24 ชั่วโมง และ 48 ชั่วโมง

Scientific	Conc. (mg/l)	100		50		25		12.5		6.3		3.2		1.6		0.8		0.4		0.2	
name	Hrs.	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48	24	48
Abutilon indicum		52	57	19	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Samanea saman		63	78	40	45	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Costus speciosus		38	100	33	82	27	32	13	13	3	8	1	2	0	0	0	0	0	0	0	0
Acorus calamus		80	100	45	79	5	7	4	5	1	2	0	1	0	0	0	0	0	0	0	0
Knema globularia		64	88	60	77	8	8	1	3	0	0	0	0	0	0	0	0	0	0	0	0
Stemona tuberosa		72	80	33	44	8	13	2	2	0	0	0	0	0	0	0	0	0	0	0	0
Strychnos nux-vomica.		55	93	20	27	12	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kaempferia galanga		100	100	98	99	23	24	5	10	3	3	0	1	1	1	0	1	0	0	0	0
Clinnamomum porrectum		92	100	71	82	42	48	18	18	1	1	0	0	0	0	0	0	0	0	0	0
Phyllanthus pulcher		94	100	90	92	80	91	73	87	7	10	1	1	0	0	0	0	0	0	0	0
Anacardium occidentale		100	100	100	100	98	100	70	82	58	74	9	13	5	10	0	0	0	0	0	0
Mammea siamensis		100	100	90	97	77	89	84	86	47	77	48	51	12	17	1	1	0	1	0	0
Anethum graveolens		97	100	85	91	72	76	51	54	9	14	0	0	0	0	0	0	0	0	0	0
Annona muricata		67	69	51	56	23	23	1	3	0	0	0	0	0	0	0	0	0	0	0	0
Control		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Mammea siamensis สามารถฆ่าลูกน้ำยุงได้ 50% เมื่อใช้สารสกัดที่ความเข้มข้นต่ำที่สุด (3.2 mg/l) ภายใน 48 ชั่วโมง

3. ค่า LC_{50} และ LC_{90} ของสารสกัดจากพืชสมุนไพรทั้ง 3 ชนิด แสดงให้เห็นในตารางที่ 3 และ 4 และรูปที่ 5 และ 6

ตารางที่ 3 ค่าความเข้มข้นของสมุนไพร 3 ชนิด (species) ที่มีฤทธิ์ฆ่าลูกน้ำยุงลายบ้าน (*Aedes aegypti*) ระยะที่ 3 และระยะที่ 4 ภายหลัง 24 ชั่วโมง

Scientific name	Lethal concentration (mg/l)		
	LC_{50} (95% CL*)	LC_{90} (95% CL*)	Slope ± SE
Mammea siamensis	6.2 (4.7 – 8.0)	24.1 (17.8 – 34.8)	1.7 ± 0.08
Anethum graveolens	16.6 (12.6 – 21.9)	64.7 (46.8 – 95.8)	2.6 ± 0.1
Annona muricata	60.9 (44.7 – 85.0)	237.7 (162.5 – 379.9)	3.8 ± 0.14

ตารางที่ 4 ค่าความเข้มข้นของสารสกัดจากพืชสมุนไพร 3 ชนิด (species) ที่มีฤทธิ์ฆ่าลูกน้ำยุงลายบ้าน (*Aedes aegypti*) ภายหลัง 48 ชั่วโมง

Scientific name	Lethal concentration (mg/l)		
	LC_{50} (95% CL*)	LC_{90} (95% CL*)	Slope ± SE
Mammea siamensis	4.1 (2.6 – 6.5)	14.0 (8.9 – 28.5)	1.4 ± 0.8
Anethum graveolens	13.7 (8.5 – 22.2)	48.8 (29.3 – 99.2)	2.6 ± 0.1
Annona muricata	53.9 (32.5 – 94.6)	192.3 (108 – 438)	4.0 ± 0.1

CL* = Confidence Limit

4. การทดสอบความเป็นพิษของสารสกัดต่อ non target fish

พบว่าสารสกัด 8 ชนิด ใน 14 ชนิด เป็นพิษต่อปลาหางนกยูง ขณะที่อีก 6 ชนิด ไม่เป็นพิษ พืชทั้ง 6 ชนิดได้แก่ *Cinnamomum porrectum*, *Phyllanthus pulcher*; *Anacardium occidentale*, *Mammea siamensis*, *Anethum graveolens* และ *Annona muricata* ดังแสดงในตารางที่ 5

ตารางที่ 5 ความเป็นพิษของสารสกัดสมุนไพร 14 ชนิด (species) ต่อปลาหางนกยูงที่ความเข้มข้นต่ำที่สุดที่จะสามารถฆ่าลูกน้ำยุงลายบ้าน (*Aedes aegypti*) ได้ 50%

Scientific name	Conc. At produced more than 50% larvae mortality rate (mg/l)	Mortality of guppy fish (N = 30) (%)
Abutilon indium	100	100
Samanea saman	100	100
Costus speciosus	50	100
Acorus calamus	50	100
Knema globularia	50	100
Stemona tuberose	100	80
Strychnos nux-vomica	100	100
Kaempferia galanga	50	80
Cinnamomum porrectum	50	0
Phyllanthus pulcher	12.5	0
Anacardium occidentale	6.3	0
Mammea siamensis	3.2	0
Anethum graveolens	12.5	0
Annona muricata	50	0
Control	0	0

สรุปและวิจารณ์ผลการทดลอง

จากการวิจัยครั้งนี้พบว่าสารสกัดจากส่วนต่างๆ ของพืชสมุนไพร 6 ชนิด ได้แก่ กิ่งของต้นเทพธาโร (*Cinnamomum porrectum*) ใบว่านธรณีสาร (*Phyllanthus pulcher*) เมล็ดมะม่วงหิมพานต์ (*Anacardium occidentale*) ดอกสารภี (*Mammea siamensis*) ต้นเทียนข้าวเปลือก (*Anethum graveoltens*) และเมล็ดทุเรียนเทศ (*Annona muricata*) มีความสามารถสูงในการกำจัดลูกน้ำยุงลายบ้าน และยิ่งไปกว่านั้น เราพบว่าสารภี เทียนข้าวเปลือก และทุเรียนเทศ มีผลทำให้ยุงตัวเต็มวัยที่รอดชีวิตวางไข่ได้จำนวนน้อยลงอีกด้วย ดังนั้นเราจึงนำสารสกัดจากพืชทั้ง 3 ชนิดมาทำการทดลองหาผลกระทบต่อวงจรชีวิตของยุงลายบ้านต่อไป

สารสกัดจากดอกสารภีมีความเป็นพิษสูงที่สุดโดยมีค่า LC_{50} เป็น 4.0 μg/ml ที่ 48 ชั่วโมง รองลงมาคือใบและกิ่งเทียนข้าวเปลือกที่มีค่า LC_{50} เป็น 14.2 μg/ml ที่ 48 ชั่วโมง และเมล็ดทุเรียนเทศที่มีค่า LC_{50} เป็น 53.2 μg/ml ที่ 48 ชั่วโมง

สารสกัดทั้ง 3 ชนิดไม่เป็นพิษต่อปลาหางนกยูงที่นำมาเป็นตัวแทนของ non-target organism ยกเว้นสารสกัดจากดอกสารภีที่ค่า LC_{90} ต่อลูกน้ำยุงลายบ้านมีความเป็นพิษเล็กน้อยต่อปลาหางนกยูง มีรายงานว่าสารภีมีสาร proanthocyanidin polymers ที่เป็นสารประกอบที่มีพิษต่อปลาได้ (Balza *et al* 1989) อย่างไรก็ดีดอกสารภีตากแห้งใช้เป็นยาสมุนไพรมาแต่โบราณและไม่เคยมีรายงานว่ามีพิษต่อมนุษย์แต่อย่างไร

นักวิจัยไม่สามารถนำสารสกัดจากพืชอีก 3 ชนิด คือ เทพธาโร ว่านธรณีสาร และมะม่วงหิมพานต์ มาทำการทดลองหาผลกระทบต่อวงจรชีวิตของยุงลายบ้านได้ เนื่องจากข้อจำกัดของเวลาโดยเฉพาะเมล็ดมะม่วงหิมพานต์ที่มีความเป็นพิษสูงต่อลูกน้ำยุงลาย แต่กระบวนการสกัดสารจากเมล็ดมะม่วงหิมพานต์มีความยุ่งยากและสลับซับซ้อนมาก เนื่องจากมีสารประกอบที่เป็นพิษและมีน้ำมันอยู่มาก

ข้อเสนอแนะสำหรับงานวิจัยในอนาคต

จากการศึกษาวิจัยเบื้องต้นพบว่าสิ่งที่น่าจะทำการวิจัยต่อไปคือ ศึกษาผลกระทบของสารสกัดจากพืช 3 ชนิดต่อวงจรชีวิตของยุงลายบ้านในแง่ของประชากรที่ลดลง (Population reduction) การเจริญเติบโตที่ช้าหรือหยุดชะงักไปของยุง (Growth retardation and prolongation of development period) ความผิดปกติของลักษณะทางสัณฐานวิทยาของยุงทุกๆ ระยะที่เป็นผลมาจากสารสกัด (Morphological aberration induced by medicinal plant extracts) การหาตำแหน่งที่สารสกัดจากพืชสมุนไพรมีผลทำให้ลูกน้ำยุงตาย (Possible site of action of plant extracts in killing larvae and pupae) การตรวจสอบลูกน้ำยุงที่ได้รับผลกระทบต่อสารสกัดจากภาพถ่ายที่ได้จากกล้องจุลทรรศน์อิเลคตรอนแบบสแกน (Scanning electron microscopic) และการตรวจสอบอาการของลูกน้ำยุงลายที่นำมาใช้ทดสอบกับสารสกัดสมุนไพร (Observations of symptoms of tested larvae)

Screening of medicinal plant extracts for larvicidal effects on *Aedes aegypti* (Diptera: Culicidae) and toxicity on a non target organism

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ABSTRACT. A preliminary study was conducted to find out the effects of the extracts of one hundred and twelve medicinal plant species, collected from the southern part of Thailand, on *Aedes aegypti*. Studies on larvicidal properties of plant extracts against the third and fourth instars larvae showed fourteen extracts with high toxicity. Six plant species, *Mammea siamensis, Anacardium occidentale, Anethum graveolens, Phyllanthus pulcher, Cinnamomum porrectum, and Annona muricata*, were comparatively more effective than others at very low concentration. The LC_{50} and LC_{90} values being 4.1 and 14.0 µg/ml for *Mammea siamensis*, 6.5 and 11.6 µg/ml for *Anacardium occidentale*, 13.7 and 48.8 µg/ml for *Anethum graveolens*, 15.6 and 44.4 µg/ml for *P. pulcher*, 51.2 and 59.8 µg/ml for *Cinnamomum porrectum* and 53.9 and 192.3 µg/ml for *Annona muricata*, respectively. These extracts demonstrated no or very low toxicity to guppy fish (*Poecilia reticulata*), which was selected to represent the most common non-target organism found in habitats of *Ae. aegypti*.

KEYWORDS : medicinal plants, *Aedes aegypti*, guppy fish, *Poecilia reticulata*

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KEYWORDS : medicinal plants, *Aedes aegypti*, guppy fish, *Poecilia reticulata*

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Evaluations of larvicidal activity of medicinal plant extracts to *Aedes aegypti* (Diptera: Culicidae) and other effects on a non target fish

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Abstract A preliminary study was conducted to investigate the effects of the extracts of 112 medicinal plant species, collected from the southern part of Thailand, on *Aedes aegypti*. Studies on larvicidal properties of plant extracts against the fourth instar larvae revealed that extracts of 14 species showed evidence of larvicidal activity. Eight out of the 14 plant species showed 100% mosquito larvae mortality. The LC_{50} values were less than 100 μg/mL (4.1 μg/mL−89.4 μg/mL). Six plant species were comparatively more effective against the fourth instar larvae at very low concentrations. These extracts demonstrated no or very low toxicity to guppy fish (*Poecilia reticulata*), which was selected to represent most common non-target organism found in habitats of *Ae. aegypti*, at concentrations active to mosquito larvae. Three medicinal plants with promising larvicidal activity, having LC_{50} and LC_{90} values being 4.1 and 16.4 μg/mL for *Mammea siamensis*, 20.2 and 34.7 μg/mL for *Anethum graveolens* and 67.4 and 110.3 μg/mL for *Annona muricata*, respectively, were used to study the impact of the extracts on the life cycle of *Ae. aegypti*. These plants affected pupal and adult mortality and also affected the reproductive potential of surviving adults by reducing the number of eggs laid and the percentage of egg hatchability. When each larval stage was treated with successive extracts at the LC_{50} value, then the first instar larvae were found to be very susceptible to *A. muricata* and the second instar larvae were found to be susceptible to *A. graveolens*, while the third and fourth instar larvae were found to be susceptible to *M. siamensis*. These extracts delayed larval development and inhibited adult emergence and had no adverse effects on *P. reticulata* at LC_{50} and LC_{90} values, except for the *M. siamensis* extract at its LC_{50} value

Key words *Aedes aegypti*, guppy fish, larvicidal activity, medicinal plants, *Poecilia reticulata*

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Introduction

One primary vector of yellow fever, chikungunya, dengue fever, dengue haemorrhagic fever (DHF) and dengue shock syndrome, is *Aedes aegypti* (Grantz, 1993). DHF is a major cause of child morbidity and hospitalization in Thailand as well as in other countries (Yasui, 1993). Control has been mainly affected by use of conventional insecticides but these have caused their own problems, such as adverse effects on the environment and the encouragement of pesticide resistance in some mosquitoes (Su & Mulla, 1998). These problems stimulated a search for safer alter-

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native anti-mosquito control.

It has been found that herbal extracts are one safer alternative method of control, especially the extracts of certain medicinal plants. One early report on the use of plant extracts against mosquito larvae was that of Campbell *et al.* (1933) where it was found that plant alkaloids like nicotine, anabasine, methyl anabasine and lumpinin extracted from the Russian weed, *Anabasis aphylla*, killed the larvae of *Culex* sp. Monzon *et al.* (1994) reported that some medicinal plants containing natural toxins were effective against mosquito larvae. Not only can medicinal plant extracts be effective but also they may greatly reduce the risk of adverse ecological effects and they do not induce pesticide resistance in mosquitoes. Since these chemicals are taken from medicinal plants, they are expected to have low human toxicity and a high degree of biodegradation (Choochote *et al.*, 1999).

Recently, many studies conducted in Thailand and around the world have shown that chemicals from medicinal plants have larvicidal, pupicidal and adulticidal effects on mosquitoes (Monzon *et al.*, 1994; Choochote *et al.*, 1999; Palakulk *et al.*, 1999; Rongsriyam & Baskoro, 1996; Shama & Shama, 1995). In the south of Thailand where many tropical rain forests are found, a vast number of medicinal plants have not had their mosquitocidal potential assessed. The identification and eventual use of indigenous medicinal plants in the control of mosquito larvae is beneficial to developing countries such as Thailand and its Southeast Asian neighbors. Thus, the principal objectives of this paper are, to screen the medicinal plants found in southern regions of Thailand for potential larvicidal effects, to test the effects of these extracts on the growth, survival, development and other life cycle aspects of the *Ae. aegypti*, and to study their impacts on fish, the most common group of non-target organisms in mosquito habitats.

Materials and methods

Plant collection

Samples of 112 medicinal plant species in 111 genera belonging to 50 families were collected from the southern part of Thailand from November 2000 to March 2002. Three methods of collection were used. Random sampling was the first method, *i.e.* taking any plant which could be collected in sufficient quantity and quality. The second method was to follow a chemotaxonomic approach, selecting the medicinal plants belonging to specific families which have been reported in scientific journals to have certain larvicidal or insecticidal properties. The third method was based on ethnopharmacological information (Martin, 1995): *i.e.* those plants already used as insecticides by local people. All parts of the plants were collected and kept separately in plastic bags and brought to the laboratory for extraction. Each herbarium specimen was prepared from each plant and was identified by: (i) comparing the morphological characters, habit and habitat with those described in the taxonomic literature; (ii) Dr Chamlong Pengklai and Dr Kongkanda Chayamarit, the Forest Botany Division, Forest Herbarium, National Park, Wildlife and Plant Conservation Department where voucher specimens were deposited.

Plant extraction

The method of plant extraction was modified from those of Satoto (1993) and Choochote *et al.* (1999). Five hundred grams of each plant (oven dried) was ground and filtered using a strainer silver number 60. The powder was macerated with 1.5 L of 80% ethanol solution and left to stand at room temperature for 3 days. The mixture was filtered through a Whatman no.1 filter paper by suction and the filtrate was evaporated under vacuum at 40℃ until completely dried, and kept at a constant 4℃ until needed for tests.

Rearing of Aedes aegypti

Ae. aegypti eggs were obtained from a colony maintained at the Medical Entomology Laboratory of the National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi Province, Thailand.

Filter paper with attached eggs was dipped into a plastic tray containing 500 mL of dechlorinated water for 30–40 min, time enough to allow for eggs to hatch into larvae. They were reared indoors at 26 ± 2℃, 70% ± 10% RH and a 14:10 light: dark photoperiod and they were fed daily with ground mouse feed until such time as they molt to become pupae. They were moved into a mosquito cage where the emergent adults were fed with a 10% sucrose solution. Five days after emergence, female mosquitoes were allowed to blood-feed on white mice for 2–3 hours. A few days after having a blood meal, the gravid mosquitoes laid their eggs.

Larvicidal test

Larvicidal tests were assessed by the standard method of WHO (1981) with some modifications. Medicinal plant extracts, which produced more than 50% larval mortality in preliminary screening, were serially diluted at concentrations of 100, 50, 25, 12.5, 6.3, 3.2, 1.6, 0.8, 0.4, and 0.2 μg/mL. One hundred milliliters of each test solution was placed in a plastic drinking cup along with (a standard group of) 25 fourth instar larvae. Each experiment was conducted with (alongside) four replicates and a concurrent control group. A control group consisted of 0.03 mL of absolute ethanol and 99.97 mL of distilled water and an untreated one, which contained only 100 mL of distilled water. No food was provided during the treatment. Observations were made 24 and 48 hours after treatment; dead larvae were counted and preserved in a 50% ethanol solution. Subsequently, the lowest concentration of crude extract that had successfully produced more than 50% larval mortality rate was used in a toxicity test on a non-target organism. A probit analysis using a computer program (SPSS/PC+, Finney, 1964) was employed on the results to determine LC_{50} and LC_{90} values.

Effects on growth and development of Ae. Aegypti

The effects of the three promising medicinal plant extracts, that were found to be effective as larvicides, were studied as follows:

Immature stages mortality and adult emergence Each cup from a larvicidal test (part 1 above), that was still holding living larvae after a 48-hour period, was placed into a separate mosquito cage. All larvae and emergent adults were fed daily. Larval, pupal and adult mortality as well as adult female and male emergence rates was determined.

Egg and larval number reduction A determination was made of the extract impact on fertility and of fecundity in emerging adults. After blood feeding and mating, females were isolated in another cage and allowed to lay eggs. The eggs were collected, counted, and recorded daily until all females died, the eggs then being allowed to hatch after being dried for a 3-day period. The number of viable larvae was recorded at the fourth instar stage. The larval reduction in the F_1 generation due to exposure to medicinal plant extract was calculated using the following formula (Thangam & Kathiresan, 1992).

Larval reduction (%) = [(A−B/A)] 100%,

A = Average number of larvae that hatched per female per cage in the control (The average was calculated in relation to the total number of mosquitoes tested).

B = Average number of larvae hatched per female per cage for mosquitoes treated with a particular plant extract.

Growth retardation and prolongation of development Groups of 25 of the first, second, third and fourth instar larvae of *Ae. aegypti* were each exposed to LC_{50} doses of the three specific kinds of (selected) medicinal plant extract. There was no fixed duration of exposure: exposure was sustained from the onset of egg hatching until larval death or else of successful adult emergence. The larvae were fed with ground mouse feed for the duration of their exposure. Data on growth and survival percentage was collected and the Harley index (1967) was used for comparing the revelant effects of the various extracts, the Harley formula being

Mean index = Percentage of individuals pupation+ Percentage of individuals reaching adulthood/median day of pupation.

Toxicity to guppy fish

A test to determine the susceptibility of a selected non-target organism to plant extracts followed Mittal *et al.*' s approach (1994). The guppy fish, *Poecilia reticulata*, was selected as the non-target organism. The following stage test was conducted.

Stage one assessment of toxicity, at the lowest concentration of crude extract to produce a more than 50% larval mortality rate in a larvicidal test. This test was conducted on guppy fish. Thirty guppy fish were placed in a rectangular, glass aquarium containing 400 mL of plant extract water solution in three replicates. Each group of 30 fish was exposed to a test solution. A control, consisting of 30 fish in dechlorinated tap water, was studied at the same time. The number of dead fish was recorded first at a 24-hour point and then at a 48-hour point and the percentage mortality calculated. All of these bioassay tests were conducted at a room temperature of approximately 27−28 ℃, without aeration or renewal of water.

Stage two assessment of toxicity fist at a LC_{50} value (point) and then at a LC_{90} value (point), which showed no toxic effects (had suffered no illness) to fish in stage one, was conducted in the same manner.

Results

Larvicidal tests

Preliminary screening 14 species of plant extracts were found to show high toxicity with a more than 50% mortality rate at of the fourth instar larvae stage in a concentration of 100 μg/mL at the 48 h after treatment point (Table 1).

Larvicidal tests Eight out of fourteen plant species, *Anacardium occidentale*, *Mammea siamensis*, *Phyllanthus pulcher*, *Anethum graveolens*, *Kaempferia galanga*, *Cinnamomum porrectum*, *Costus speciosus*, and *Acorus calamus* at a concentration of 100 μg/mL showed 100% larval mortality after an exposure of 48 hours (Table 2). These plant extracts showed high larvicidal activity, their LC_{50} after 48 hours < 100 μg/mL, 13.9−56.2 μg/mL (Table

Table 1 Fourteen medicinal plants with high toxicity to the fourth instar larvae of *Aedes aegypti* in preliminary screening.

Family and sientific name	Plant habit	Thai common name	English common name	Part use
Malvaceae *Abutilon indicum*	Under shurb	Fan-si	Country mallow	Roots
Leguminosae-Mimosoideae *Samanea saman*	Tree	Chamchuri	Rain tree	Stem bark
Costaceae *Costus speciosus*	Herb	Ueang mai-na	Spiral flag	Roots
Araceae *Acorus calamus*	Herb	Wan-nam	Sweet flag	Roots
Myristicaceae *Knema globularia*	Tree	Lueat raet	–	Seeds
Stemonaceae *Stemona tuberosa*	Herb	Non tai yak	–	Roots
Strychnaceae *Strychnos nux-vomica*	Shrub	Salaeng chai	Snake wood	Seeds
Zingiberaceae *Kaempferia galanga*	Herb	Pro hom	–	Roots
Lauraceae *Cinnamomum porrectum*	Tree	Thep tharo	–	Wood
Euphorbiaceae *Phyllanthus pulcher*	Shrub	Wan-thorani-san	–	Leaves & twigs
Anacardiaceae *Anacardium occidentale*	Exotic shrub tree	Mamuang Him-ma-phan	Cashew nut tree	Seed shell
Guttiferae *Mammea siamensis*	Tree	Saraphi	Negkassar	Flower
Umbelliferae *Anethum graveolens*	Exotic herb	Thain khoa Pluea	Dill	Leaves & twigs
Annonaceae *Annona muricata*	Exotic Shrub tree	Thurian-thet	Durian belanda	Seeds

2). Six remaining medicinal plant species, *Strychnos nux-vomica, Knema globularia, Stemona tuberosa, Samanea saman, Annona muricata, Abutilon indicum* at a concentration of 100 μg/mL showed moderate percentage mortality of larvae (93%, 88%, 80%, 78%, 69% and 57%, respectively), their LC_{90} after 48 hours < 200 μg/mL, 82.6–130.3 μg/mL (Table 2).

Effects on growth and development of Ae. aegypti

Immature stages mortality and adult emergence The effects of the extracts on still living larvae were monitored continuously after an initial 48-hour period of exposure. It was found that slightly more pupae and also more adults died following exposure to solutions containing *A. muricata* and *A. graveolens* extracts than did larvae. Details of the comparative effects of three medicinal plant extracts on mortality of *Ae. aegypti* are shown in Table 3. It was found that three of the medicinal plant extracts, *M. siamensis*, *A. graveolens* and *A. muricata* caused very low mortality among larvae, actual rates of mortality being 0%–3%, 0%–6%, and 0%–10%, respectively. The mean mortality rates of larvae for all concentrations of these plant extracts were 1%, 2%, and 2%, respectively. Mortality of pupae ranged from 2%–7%, 2%–9%, and 0%–13%, respectively. The mean number of dead pupae listed in the same order, were 3%, 5%, and 7%, respectively. Similarly, mortality at the adult stage ranged from 2%–26%, 4%–35%, and 9%–20%, respectively. The mean numbers of dead adults were 10%, 18% and 13%, respectively. Adult emergence from pupae after permanent exposure to three medicinal plant extracts ranged from 2%–79%, 9%–81%, and 6%–87%, respectively, while the means were 34%, 42%, and 63%, respectively.

Number of eggs and larval reduction In comparison to the control, the three medicinal plant extracts re-

Table 2 Toxicity of 14 medicinal plant extracts on *Aedes aegypti* fourth instar larvae.

Scientific name	24 h		48 h	
	LC_{50}† (μg/mL)	LC_{90} (μg/mL)	LC_{50} (μg/mL)	LC_{90} (μg/mL)
Abutilon indicum	94.2 acg (81.3–112.1)	136.3 k (116.5–170.9)	89.4 a (78.0–106.3)	130.3 m (112.9–162.3)
Samanea saman	79.2 bf (68.5–92.8)	121.1l s (103.8–145.5)	69.2 bf (59.7–75.5)	104.5 n (90.0–121.9)
Costus speciosus	98.5 ac (83.1–164.8)	171.2 m (113.1–387.5)	33.7 ci (28.5–39.3)	53.6 os (45.4–64.1)
Acorus calamus	67.2 de (9.1–79.2)	103.6 ns (90.4–123.6)	40.15 d (34.6–46.00)	56.2 os (50.0–68.7)
Knema globularia	72.1 def (3.8–107.1)	114.4 ls (87.0–183.2)	53.2 e (39.9–76.4)	82.6 p (64.5–126.6)
Stemona tuberosa	75.2 bef (5.2–88.8)	114.9 ls (100.2–140.3)	65.8 bfg (56.4–78.0)	105.0 n (91.2–128.4)
Strychnos nux-vomica	90.0 ag (6.5–108.4)	137.7 k (115.7–171.7)	62.8 bfg (55.7–74.6)	92.2 q (82.3–112.7)
Kaempferia galanga	30.7 h (7.5–37.0)	44.9 o (39.8–55.1)	29.5 hi (24.9–34.5)	43.6 rs (36.3–52.3)
Cinnamomum porrectum	43.5 i (9.5–73.5)	73.6 p (53.0–136.7)	31.5 hi (26.7–36.3)	49.8 ors (42.1–58.3)
Phyllanthus pulcher	25.8 h (9.9–66.2)	50.7 o (21.2–76.1)	15.9 j (9.6–28.0)	28.0 t (22.7–68.1)
Anacardium occidentale	9.1 j (6.5–11.4)	15.3 q (10.8–19.9)	7.7 k (4.9–9.6)	13.9 u (8.7–17.3)
Mammea siamensis	5.9 j (4.53–7.6)	27.8 r (17.7–40.1)	4.1 k (3.0–5.1)	16.4 u (11.2–24.5)
Anethum graveolens	27.4 h (17.1–41.9)	50.0 o (31.6–79.4)	20.2 l (12.5–40.9)	34.7 t (24.2–81.9)
Annona muricata	69.25 def (52.1–101.0)	113.9 lns (86.0–175.1)	67.4 bf (50.8–98.8)	110.3 n (85.2–173.3)

†Value ranges in the brackets stand for LC estimated by probit analysis 95% confidence limit. Letters are indices to show similarities of concentrations at LC_{50} and LC_{90}. The pair or group of plants that have the same indices have similar concentration at $P \geq 0.05$ or different at $P < 0.05$.

duced the number of eggs laid, the hatching ability and the larvae in the first generation of *Ae. aegypti* after treatment as fourth instar larvae. The results are shown in Table 3. The mean numbers of eggs laid for the three medicinal plant extracts were 46 eggs, 31 eggs, and 27 eggs, respectively. The percentages of hatched eggs for the three medicinal plant extracts ranged from 47%–60%, 40%–62%, and 35%–63%, respectively, while the mean percentages of hatched eggs were 54%, 51%, and 49%, respectively. The percentage reductions in larvae ranged from 20%–53%, 42%–82%, and 60%–84%, respectively, while the mean reductions in larvae were 44%, 66%, and 70%, respectively.

Growth retardation and prolongation of developmental period The comparative effects of the three medicinal plant extracts, at their LC_{50} concentrations, on the growth, survival and prolongation of the various instar larvae of *Ae. aegypti* are shown in Table 4. It was found that the first instar larvae were more susceptible to *A. muricata* and the second instar larvae more susceptible to *A. graveolens*. The third and fourth instar larvae were more susceptible to *M. siamensis*. Survival percentage of pupae were reduced when treated at the first and second instar stage with *A. muricata* and at the third and fourth instar stage with *A. graveolens*. Percentage adult emergence was lower for first and second instar larvae treated with *A. muricata*, for third instar treated with *A. graveolens* and for fourth instar treated with *M. siamensis*, than that of the control. The larvae took more time to develop to pupae, *i.e.*, 30 days, 33 days, 35 days and 20 days, respectively, in developing to pupation compared to the control, when the first instar were treated with *A. graveolens* extract, the

Table 3 Effects of three medicinal plant extracts on immature stages mortality and adult emergence of *Aedes aegypti*.

Plant	Concentration (μg/mL)	Number of larvae after 48 h	Mortality (%) L	Mortality (%) P	Mortality (%) A	No. M	No. F	Alive (%) A	No. M	No. F	Average number of eggs laid/female	Hatched eggs (%)	Reduction in larvae over control
Mammea siamensis	100	–	–	–	–	–	–	–	–	–	–	–	–
	50	3	1	2	–	–	–	–	–	–	–	–	–
	25	11	1	2	6	5	1	2	1	1	40	53	53
	12.5	14	3	4	2	2	0	5	3	2	65	55	20
	6.3	23	2	3	6	5	1	12	8	4	40	58	49
	3.2	49	1	3	23	11	12	22	5	17	52	48	44
	1.6	83	3	4	15	14	1	61	18	43	60	47	38
	0.8	99	1	5	26	20	6	67	28	39	42	52	51
	0.4	99	2	7	13	8	5	77	45	32	39	56	51
	0.2	100	0	2	19	17	2	79	33	46	40	60	47
	Mean	48	1	3	10	8	2	34	14	20	46	54	44
	Control	100											
Anethum graveolens	100	–	–	–	–	–	–	–	–	–	–	–	–
	50	9	0	9	–	–	–	–	–	–	–	–	–
	25	24	5	6	4	4	0	9	5	4	59	44	42
	12.5	46	0	8	11	9	2	27	14	13	33	52	62
	6.3	86	6	5	27	24	3	48	24	24	18	61	76
	3.2	100	1	6	35	28	7	58	19	39	28	47	71
	1.6	100	1	2	31	27	4	66	20	46	16	50	82
	0.8	100	0	3	30	28	2	67	27	40	33	52	62
	0.4	100	3	9	27	20	7	61	7	54	21	62	71
	0.2	100	0	5	14	10	4	81	45	36	40	40	62
	Mean	67	2	5	18	15	3	42	16	26	31	51	66
	Control	–	–	–	–	–	–	–	–	–	–	–	–
Annona muricata	100	31	2	3	20	14	6	6	2	4	25	36	80
	50	44	10	12	9	7	2	13	7	6	23	61	69
	25	77	1	6	17	14	3	53	25	28	24	63	67
	12.5	97	3	12	16	15	1	66	18	48	39	46	60
	6.3	100	0	13	9	9	0	78	42	36	16	44	84
	3.2	100	0	6	12	8	4	82	31	51	22	59	71
	1.6	100	1	5	14	11	3	80	34	46	27	48	71
	0.8	100	0	4	11	8	3	85	48	37	29	62	60
	0.4	100	1	5	12	7	5	82	47	35	38	48	60
	0.2	100	0	0	13	10	3	87	44	43	23	35	82
	Mean	85	2	7	13	10	3	63	30	33	27	49	70
	Control	–	–	–	–	–	–	–	–	–			

L = larva, P = pupa, A = adult, M = male, F = female.

second and the fourth instars with *M. siamensis* extract, and the third instar larvae with *A. muricata* extract, respectively. *Anethum graveolens* produced a marked reduction in Harley' s index of 4, 5, 5, and 4 in the first, second, third and fourth instar larvae, respectively, while *M. siamensis* produced an index of 4 in the fourth instar stage in comparison with the control.

Toxicity to guppy fish

Toxicity at the lowest concentration of crude extracts that produced more than 50% larval mortality in the larvicide's test Out of 14 plant extracts found to be larvicidel, eight plant extracts were toxic to guppy fish and six extracts were found to be not so toxic.

Table 4 Effect of the three medicinal plant extracts at LC_{50} on survival and prolongation of different larvae instar of *Aedes aegypti*.

Scientific name	First instar (*n*)						Second instar (*n*)						Third instar (*n*)						Fourth instar (*n*)					
	L	P/ (%)	A/ (%)	LP	MD	MI	L	P/ (%)	A/ (%)	LP	MD	MI	L	P/ (%)	A/ (%)	LP	MD	MI	L	P/ (%)	A/ (%)	LP	MD	MI
M. siamensis	33	20 (61)	15 (46)	21	14	8	58	46 (79)	40 (69)	33	19	8	59	45 (76)	36 (61)	26	15	9	37	13 (35)	5 (14)	20	14	4
A. graveolens	85	36 (42)	26 (31)	30	19	4	45	19 (42)	16 (36)	22	15	5	80	23 (29)	35 (44)	26	15	5	64	20 (31)	11 (17)	18	11	4
A. muricata	28	11 (39)	8 (29)	27	15	5	64	24 (38)	18 (28)	16	11	6	60	37 (62)	33 (55)	35	17	7	55	34 (62)	23 (42)	17	14	7
Control	100	100	100	12	6	33	100	100	100	12	6	33	100	100	100	12	6	33	100	100	100	12	6	33

L = Larva; P = Pupa; A = Adult; LP = Larvae Period; MD = Median Day; MI = Mean Index.

Abutilon indicum, *Samanea saman*, *Costus speciosus*, *Acorus calamus*, *Knema globularia*, *Stemona tuberosa*, *Strychnos nux-vomica*, and *Kaempferia galanga* extracts were toxic to guppy fish at concentrations of 100, 100, 50, 50, 50, 100, 100, and 50 μg/mL, respectively. They all produced 100% mortality for guppy fish except for *S. tuberosa* and *K. galanga*, which resulted in 80% mortality. Six plant species, *C. porrectum*, *P. pulcher*, *A. occidentale*, *M. siamensis*, *A. graveolens*, and *A. muricata* were not toxic to guppy fish at concentrations of 50, 12.5, 6.3, 3.2, 12.5 and 50 μg/mL, respectively (Table 5).

Table 5 Toxicity of extracts of medicinal plants to guppy fish at the lowest concentration that produced more than 50% larval mortality in the larvicidal test.

Scientific name	Concentration which caused more than 50% larval mortality (μg/mL)	Mortality of selected non-targe torganism (%)
Abutilon indicum	100	100
Samanea saman	100	100
Costus speciosus	50	100
Acorus calamus	50	100
Knema globularia	50	100
Stemona tuberosa	100	80
Strychnos nux-vomica	100	100
Kaempferia galanga	50	80
Cinnamomum porrectum	50	0
Phyllanthus pulcher	12.5	0
Anacardium occidentale	6.3	0
Mammea siamensis	3.2	10
Anethum graveolens	12.5	0
Annona muricata	50	0
Control	0	0

Toxicity of the three medicinal plant extracts to guppy fish at their LC_{50} and LC_{90} concentrations Of the three lavicidal plants *A. graveolens*, *A. muricata*, and *M. siamensis* did not exhibit any noticeable effect on guppy fish after either 24 or 48 hours of exposure at their LC_{50} and LC_{90} values. However, *M. siamensis* at LC_{50} value did affect guppy fish slightly, and produced 17% mortality at the LC_{50} value and 100% mortality at the LC_{90} value.

Discussion

Larvicidal tests

From the 112 species of medicinal plant collected from the southern part of Thailand, 14 species (12.5%) showed toxicity against the third and the fourth instar larvae of *Ae. aegypti* while eight out of these 14 species (7.1%) demonstrated toxicity to the selected non-target organism. Six of the fourteen species (5.4%) showed excellent larvicidal properties against the fourth instar larvae of *Ae. aegypti*. All had LC_{50} values at 48 hours after treatment under 100 μg/mL (4.1–67.4 μg/mL); therefore, these plants have potential as good mosquitocides.

Effects on growth and development of Ae. aegypti

The immature stages mortality and the stage of the emergent adult Low mortality rates of larvae were observed after permanent exposure to extracts of *M. siamensis*, *A. graveolens*, and *A. muricata*. It was found that some larvae successfully continue to develop up to the pupal stage. Of these, some failed at the pupal stage, but still some emerged as adults. *Mammea siamensis* demonstrated some inhibition of the growth of larvae more than any correspondent inhibition of pupa and of adults. Extracts of *A. graveolens*, and of *A. muricata* produced larvae mortality rates after 24 hours and 48 hours of relevant exposure, rates that were lower than these produced by *M. siamensis*, and yet these two extracts produced high mortality rates among adults and among pupae. Some of the larvae did not die within the 48 hour period, but instead they died at the pupal or at the adult stage, due to the chronic effects of chemical compounds attributable to the medicinal plant extract (Sukumar *et al.*, 1991). It has already been reported that some plant chemicals produced either larvicidal, pupicidal or adulticidal effects, and that at the various stages of their life cycle, mosquitoes differ markedly in their susceptibility to these phytochemicals (Rongsriyam & Baskoro, 1996).

Number of eggs and larval reduction It was found that the average number of eggs laid by females that emerged from medicinal plant treatment was lower than the number of eggs laid by the females of the control group. Hatchability of these eggs was also low and the size of the first generation was small when mosquitoes were treated at the third and the fourth instar larvae stage, including mosquitoes treated with *M. siamensis* extract. Mahidol *et al.* (2002) reported that plants of the genus *Mammea* were known to be rich sources of various coumarine and xanthones and coumarines were reported to exhibit diverse biological activities. It seemed possible that *M. coumarins* flowers might inhibit ecdysteriod hormone and juvenile hormone, or that while cytotoxic coumarines and insecticidal compounds stimulated ovaries to produce more eggs, these compounds still had an adverse effect on egg development of *Ae. aegypti* in that they decreased their hatchability. These findings have both biological and physiological significance in that they indicate that some compound had affected reproductive organ development, insinulated itself into the eggs and affected some vital physiological and biochemical processes associated with embryonic development. It would seem that this was the case with all those eggs that failed to hatch (Choochote *et al.*, 1999; Zebitz, 1984). Su and Mulla (1998) report coumarines capable of producing such multiple effects on insects as anti-feeding, growth inhibition, fecundity suppression, sterilization and changes in biological fitness.

Growth retardation and prolongation of developmental period There was a delay in the development of larvae to the pupal stage when the first, second, third or fourth instar larvae were exposed to all three medicinal plant extracts. This was especially noted in *A. graveolens*, followed by *A. muricata*. This may be due to the presence of high juvenile hormone levels in the larvae or else due to chemical compounds in the medicinal plant, preventing normal pupation and preventing adult emergence from occurring. Zebitz (1984) reports that azadirachtin may act as an anti-ecdysteroid or else otherwise affected the neuroendocrine control of ecdysteroids such that growth is inhibited and that the developmental period is prolonged. Mohtar *et al.* (1999) report the effect of a methanol-aqueous extract of *Nerium indicum* leaf at 100 mg/L on different larval instars of *Ae. aegypti* and show an elongation of the preimago period for all their larvae treated when compared to the control larvae. Many studies have drawn attention to the effects of plant extracts on adult eclosion (Yodbutra *et al.*, 1985; Schwartz *et al.*, 1998). The benefit of elongation is that mosquito larvae numbers are reduced due to the longer period needed for a new generation to complete the mosquito life cycle (Havertz & Curtin, 1967).

This study found that six medicinal plant extracts, *C. porrectum*, *P. pulcher*, *A. occidentale*, *M. siamensis*, *A. graveolens*, and *A. muricata* evinced high larvicidal activity against the fourth instar larvae of *Ae. aegypti*. Furthermore, *M. siamensis*, *A. graveolens*, and *A. muricata* were all found to have a chronic effect on the fourth instar larvae of *Ae. aegypti* after permanent exposure, leading to a reduction in the number of resultant eggs and subsequent larvae of the mosquitoes.

Mode of action of three medicinal plant extracts, *M. siamensis*, *A. graveolens* and *A. muricata* as it impacted on the life cycle of *Ae. aegypti* was studied.

Mammea siamensis crude extract was the most toxic of the three, with a 48-hour LC_{50} value of 4.1 μg/mL. Other workers have reported similar results. Issakul *et al.* (2004), who investigated insecticidal substances extracted from *M. siamensis*, report insecticidal affects on the eggs of the house fly, *Musca domestica*. Avirutnant and Pongpan (1983) reported that alcohol and water extracts of *M. siamensis* flowers shown inhibitory effects on microorganisms.

The second potential anti-*Ae. aegypti* plant extract was the dill plant, *A. graveolens*, having a 48-hour LC_{50} value of 20.2 μg/mL. Its leaves are used for their antimicrobial and nematocidal properties, as well as for their properties as insect repellent and insecticide. It has been found to contain insecticidal components and also is synergistic with carbamate and organophosphorous insecticides for some insect species. Carvone and myristicin in the aerial parts of dill, including seeds ("dill green") act as insecticides and synergists (Lichtenstein *et al.*, 1974). Supavarn *et al.* (1974) report a methanol extract of whole plants of *A. graveolens*, demonstrating low toxicity to the fourth instar larvae of *Ae. aegypti* but high inhibition of pupal development.

Annona muricata seed was found to be an active larvicide with a 48-hour LC_{50} value of 67.4 μg/mL. Jacobson

(1958) reports similar results for the seed extracts of another Annona species, namely *A. cherimola, A. glabra* and *A. squamosa*, which had lethal effects on larvae of *Aedes* sp. Its potential as a larvicidal plant was further supported by the results of a recent study by Satoto (1993), who found that *A. squamosa* seed was one of the most effective larvicides against both *Culex tritaeniorhynchus* and *Ae. aegypti*. Grainge and Ahmed (1988) report that the seed alkaloids from *A. muricata* were antifeedants to *Ae. aegypti*, known for about 10 years; they are characteristic of the Annonaceae, (Annona, Asimina, Goniothalamus, Rollinia, Uvaria), where they are mostly concentrated in the seeds. The potential applications of these molecules are due to their marked cytotoxic and antitumor (asimicin, bullatacine), antibacterial (cherimolin) and insecticidal (asimicin) properties.

Toxicity to guppy fish

They were non-toxic to the tested non-target organism, except *M. siamensis* at larval LC_{90} value for *Ae. aegypti* was slightly toxic to fish. *Mammea siamensis* has been reported to contain proanthocyanidin polymers as the active compounds producing piscicidal effects (Balza *et al.*, 1989). However, dried flowers from these plants have been traditionally used in herbal medicine (Soralum *et al.*, 2001) and they have not been found to be toxic to human beings. The other two plant extracts had no toxic effect on fish.

Investigators in this study were not able to investigate *C. porrectum*, *P. pulcher* and *A. occidentale* although these plants are worthy of further study. Furthermore, *A. occidentale* appears to a have strong potential as a larvicide. However, the extraction process, from cashew nuts, is complex as it contains a toxic astringent as well as complex corrosive oily substances.

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