RESEARCH NOTE

SCREENING FOR LARVICIDAL ACTIVITY OF TEN CARMINATIVE PLANTS

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Abstract. Ten species of plants, reported to possess carminative property, were screened for larvicidal potential against *Culex quinquefasciatus* by exposing early 4th instar larvae to a series of concentrations of the ethanolic extracts of the plants. Mortality counts were made after 24 hours exposure. Probit analysis using computerized Harvard Programming (Hg1, 2) was employed to determine the LC_{50}, LC_{95} and LC_{99} values in order to compare the larvicidal potency of the ten plants. Marked larvicidal effects were seen with *Kaempferia galanga, Illicium vernum* and *Spilanthes acmella* having LC_{50} values of 50.54, 54.11 and 61.43 ppm, respectively.

Various botanicals offer great promise as sources of phytochemicals for control of both agricultural pests and medically important insect species. Nowadays, the growing use of phytochemicals for control of these insects, may be attributed to the fact that populations throughout the world are coming to see the dangers inherent in conventional insecticides, particularly the detrimental effects on the environment.

Since the Second World War, the botanical pesticides used were pyrethrum, rotenone, nicotine, sabadilla, quassin, etc. Rotenone is presently used only on a limited number of crops due to its high toxicity to fish, and natural pyrethrum from chrysanthemum flowers is employed mainly as rapid knockdown agent for crawling and flying insects affecting man and animals. Nicotine, sabadilla and quassin are seldom used as pesticides today. Plant families with several representative species which appear to have the greatest potential for providing future mosquito control agents include Asteraceae, Canellaceae, Cladophoraceae, Labiatae, Malvaceae, Meliaceae, Oocystaceae, and Rutaceae (Arnason *et al,* 1988 ; Sukumar *et al,* 1991).

Plants which exhibit various degrees of toxicity to mosquitos such as *Piper longrum, Acorus calamus, Allium sativum, Ocimum basilicum* and *Ocimum sanctum* (Sukumar *et al,* 1991) are known to have carminative properties. Ten species of plants with carminative properties were selected for investigation, in an attempt to search and integrate traditional or new plants utilized in the development of effective mosquito control agents.

The selection of the plants to be used in the present study was focused mainly on plants with carminative properties. A total of 10 plants applied in this study is shown in Table 1. The larvae of *Culex quinquefasciatus* were collected from polluted street canals in Muang district, Chiang Mai province. The specimens were transported to the insectary of Department of Parasitology, Faculty of Medicine, Chiang Mai University. They were identified and reared in the insectary at the temperature of 25-30°C and 80-90% relative humidity. The mosquito larvae of at least F_8 progeny were used for investigations of larvicidal activity.

Ten plants (listed in Table 1) were collected and/or purchased in Chiang Mai. For each plant, 1.5 kg of dried and powdered material was macerated with 5 liters of 95% ethanol at room temperature for 2 days. The mixture was suction filtered through a Buchner funnel. The residue was reextracted with fresh 95% ethanol for 2 more times. The filtrate obtained was combined and evaporated by using a rotary evaporator at 60°C, and then lyophilized to yield an ethanolic extract. The ethanolic extract of each plant obtained was kept at -20°C until testing for the larvicidal screening. In preparing test concentrations, each plant extract was volumetrically diluted in a suitable solvent such as 50%, 70%, 95%, absolute ethanol or acetone at an appropriate test concentration. The control was prepared by addition of 1 ml of solvent (same as used for test substance) to the water in each container.

Larvicidal effects of plant extracts were tested according to the standard WHO method (WHO,

Table 1

List of plants used in the screening for larvicidal activity.

Thai name	English name	Botanical name	Part used
1. Krawann	Cardamom	*Amomum krervanh* Pierre	fruit
2. Kham foi	American saffron	*Carthamus tinctorius* L.	flower
3. Phak chee	Coriander	*Coriandrum sativum* L.	fruit
4. Kaan phluu	Clove	*Eugenia caryophyllata* Thunberg	flower buds
5. Pooi kak	Star anise	*Illicium vernum* Hooker	fruit
6. Proh hom	-	*Kaempferia galanga* L.	rhizome
7. Kaeo	Orange jasmine	*Murraya paniculata* L.	leaf
8. Chan thet	Mace	*Myristica fragrans* Houtt	arillode
9. Horaphaa chaang	Shrubby basil	*Ocimum gratissimum* L.	leaf
10. Phak khraat huawaen	Para-cress	*Spilanthes acmella* Murr	whole plant

Table 2

List of 10 plants with percent yield of ethanolic extracts.

Plants	Yields(%)
Eugenia caryophyllata Thunberg	28.15
Myristica fragrans Houtt	26.72
Illicium vernum Hooker	15.03
Murraya paniculata L.	10.26
Carthamus tinctorius L.	6.36
Kaempferia galanga L.	6.27
Spilanthes acmella Murr	5.90
Ocimum gratissimum L.	5.08
Coriandrum sativum L.	2.81
Amomum krervanh Pierre	1.97

1970) with slight modifications. For experimental treatment, 1 ml of the test solution was completely mixed with 224 ml of distilled water in an enamel bowl of 10 cm in diameter and 8 cm in depth. Then 25 early 4th instar larvae of *Cx. quinquefasciatus* in 25 ml of distilled water were transferred to that bowl. Each experiment was done in 4 replicates with the final total number of 100 larvae for each concentration. Each replicate set contained one control which consisted of 1 ml of solvent and 249 ml of distilled water and one untreated which contained only 250 ml of distilled water. After a period of 24 hours, the mortality counts were performed. The moribund and dead larvae in four replicates were combined and expressed as percentage mortalities of each concentration. Dead larvae were identified as unrousable larvae after probing with a needle in the siphon or the cervical region. Moribund larvae were those incapable of rising to the surface (within a reasonable period of time) or of showing the characteristic diving reaction when the water was disturbed. They might also show discoloration, unnatural positions, tremors, incoordination, or rigor. It was of importance to obtain not less than 3 mortality counts between 10% and 90%. The larvicidal effect was analyzed by means of computerized probit analysis (Harvard Programming; Hg1,2). The analysis for each test yield LC_{50}, LC_{95} and LC_{99}.

Ethanolic extracts of 10 plants with percent yield are shown in Table 2. *Eugenia caryophyllata* provided the maximum yield (28.15% w/w) whereas the minimum yield was obtained from *Amomum krervanh* (1.97% w/w). These ethanolic extracts thus obtained were used for larvicidal study. Larvicidal activity of 10 plants selected for the screening is shown in Table 3. It was apparent that potency of the 10 selected plants against *Cx. quinquefasciatus* larvae differed greatly. Quite marked larvicidal activities were seen with *Kaempferia galanga, Illicium vernum and Spilanthes acmella* having LC_{50} values of 50.54, 54.11 and 61.43 ppm, respectively. It could thus be postulated that the active larvicidal components present vary from one plant species to another. *K. galanga,* the most effective plant is a perennial herbaceous plant, wild growing in mountains and cultivated in some places for ethnomedical uses. The rhizomes of the plant, containing essential oil, are used in decoction or powder for indigestion, cold, pectoral and abdominal pains, headache and toothache. Its alcoholic maceration is applied as

Table 3

Larvicidal activity of ethanolic extracts of 10 plants against *Cx. quinquefasciatus*.

Plant	Larvicidal activity (ppm)		
	LC_{50}	LC_{95}	LC_{99}
Kaempferia galanga L.	50.54	73.61	90.88
Illicium vernum Hooker	54.11	159.00	290.95
Spilanthes acmella Murr	61.43	194.26	370.40
Myristica fragrans Houtt	139.03	291.51	441.48
Coriandrum sativum L.	181.23	655.32	1,347.12
Eugenia caryophyllata Thunberg	198.97	314.44	406.41
Amomum krervanh Pierre	235.79	323.33	385.95
Murraya paniculata L.	324.10	968.86	1,790.12
Carthamus tinctorius L.	445.93	916.35	1,372.20
Ocimum gratissimum L.	726.56	1,369.04	1,952.82

liniment for rheumatism (Keys, 1976; Lieu, 1990). Although never shown before to be effective against mosquito larvae, this plant's rhizome has been shown by Kiuchi *et al* (1988) to have larvicidal activity against the second stage larva of dog roundworm, *Toxocara canis,* which is a common pathogenic parasite in visceral larva migrans. The other effective plants, *I. vernum* and *S. acmella* are also used as medicinal herbs. *I. vernum* is used to promote digestion and appetite and to relieve flatulence. However, its potential as a larvicidal plant has never been reported. The larvicidal effect of *S. acmella* is supported by the study of Pendse *et al* (1945) which observed anti-anopheline activity in ether extract of *S. acmella.* In addition to insecticidal properties, *S. acmella* has been reported to have strong anti-microbial activity against some microorganisms such as *Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans* (Desta, 1993). Even though the screening for larvicidal activity was limited within one mosquito vector, *Culex quinquefasciatus,* these present results provide at least three additional promising botanical agents which might be used in purified form as alternative control strategies that are effective against target mosquito species.

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REFERENCES

Arnason JT, Philogene BJR, Morand P. Insecticides of plant origin. USA: American Chemical Society, 1989.

Desta B. Ethiopian traditional herbal drugs. Part II : Antimicrobial activity of 63 medicinal plants. *J Ethnopharmacol* 1993; 39: 129-39.

Keys JD. Chinese herbs (Their Botany, Chemistry, and Pharmacodynamics). Tokyo: Charles E, Tuttle , 1976:77.

Kiuchi F, Nakamura N, Tsuda Y, Kondo K, Yoshimura H. Studies on crude drugs effective on visceral larva migrans. II. Larvicidal principles in *Kaempferia rhizoma. Chem Pharm Bull* 1988; 36: 412-5.

Lieu VD. Medicinal plants in Vietnam, Ha Noi , 1990:239.

Pendse GS, Phalnikar NL, Bhide BV. Investigation of new plant-larvicides with special reference to *Spilanthes acmella. Curr Sci* 1945;14 :37-41.

Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control : a review. *J Am Mosq Control Assoc* 1991; 7: 210-36.

WHO. Insecticide resistance and vector control. 17th Report WHO Expert Committee on Insecticides. *WHO Tech Rep Ser* 1970; 443: 47-71.