EFFECT OF CRUDE EXTRACT OF *SOLANUM XANTHOCARPUM* AGAINST SNAILS AND MOSQUITO LARVAE

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Abstract. The ethanolic crude extract from *Solanum xanthocarpum* was investigated for its molluscicidal activity against *Biomphalaria glabrata*, the snail vector of *Schistosoma mansoni*, and *Indoplanorbis exustus*, the snail vector of intestinal echinostomiasis and *Schistosoma spindale*, together with the larvicidal activity against the larvae of *Aedes aegypti*, mosquito vector of dengue hemorrhagic fever and *Culex quinquefasciatus*, the mosquito vector of urban bancroftian filariasis. The bioassays were carried out following the methods recommended by the World Health Organization. For molluscicidal activity, the LC_{50} against *Bi. glabrata* and *I. exustus* were reported at 163.85 and 198.00 mg/l while the LC_{90} were 219.33 and 236.80 mg/l, respectively. Regarding mosquito larvicidal activity, the LC_{50} against the larvae of *Ae. aegypti* and *Cx. quinquefasciatus* were 788.10 and 573.20 mg/l, while the LC_{90} were 1,288.91 and 1,066.93 mg/l, respectively. These results suggest a preparation of ingredients from this plant may be used as a biological larvicide for these vectors in the field.

Key words: *Solanum xanthocarpum*, crude extract, molluscicidal activity, larvicidal activity

INTRODUCTION

Mosquito and snail-borne diseases are among the most common causes of illness and death in tropical and subtropical countries, and to a lesser extent in temperate countries. These diseases include malaria, lymphatic filariasis, dengue/dengue hemorrhagic fever and schistosomiasis (WHO, 1997). Chemical measures to control mosquito populations were initially considered in most public health programs. However, these have failed because constant use of chemical insecticides has often led to disruption of natural biological control systems and outbreaks of insect species. Moreover, problems created by using synthetic insecticides include development of mosquito resistance, environmental pollution and undesirable effects on humans, mammals, and other non-target organisms (Brown, 1986; Lee *et al*,

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2001). In an attempt to resolve these problems, attention to insecticides of natural origin, particularly plant derived products, has been recently preferred. A considerable number of studies have emphasized the research and development of herbal pesticides for controlling mosquitoes (Sukumar *et al*, 1991; Tsao *et al*, 2002; Jeyabalan *et al*, 2003). For snail control, economic and ecological considerations increasingly favor the use of molluscicides that are selectively active, biodegradable, inexpensive, and readily available in effected areas. The high cost of imported synthetic compounds, along with increasing concern over the possible buildup of snail resistance to these compounds and their toxicity to non-target organisms, has given new impetus to the study of plant molluscicides (Kloos and McCullough, 1987). Several plants, such as *Tetraplura tetraptera* (Adewunmi, 1991), the well studied *Phytolacca dodecandra* (Endod) (Lemma, 1970) and *Swartzia madagascariensis* (Sarda *et al*, 1986), have already been identified as potentially useful in control of the intermediate hosts of schistosomes.

Solanum xanthocarpum (family Solanacea) is an indigenous herb that grows abundantly in Thailand. It is known in Thai as "*Makhua Khurn*". The plant has been used traditionally for curing various ailmants (Ghani, 1998; Govindan *et al*, 1999) and extracts of various parts have been used as a larvicide (Singh and Bansal, 2003; Mohan *et al*, 2005) and molluscicide (Wei *et al*, 2002; Li *et al*, 2005). This plant is known to produce a great variety of alkaloids in the from of glycoalkaloids, which are important natural resistance agents to several pests (Wink, 1998). WHO guidelines were used to assess the toxicity of crude ethanol extracts of *Solanum xanthocarpum* fruit on the snail intermediate hosts of human and animal schistosomes, *Biomphalaria glabrata* and *Indoplanorbis exustus* (WHO, 1965) and on the larvae of *Aedes aegypti* and *Culex quinquefasciatus* (WHO, 2005).

MATERIALS AND METHODS

Snails and mosquitoes

In this study, *Biomphalaria glabrata* snails were obtained from the Applied Malacology Center, Department of Social and Environmental Medicine, Faculty of Tropical Medicine, Mahidol University. *Indoplanorbis exustus* snails were collected from the field and acclimatized to the temperature (25-27ºC) and humidity (75±5%) of the laboratory for 10 days in the Malacology Laboratory, Department of Social and Environmental Medicine, Faculty of Tropical Medicine, Mahidol University before being used in the toxicity test.

Aedes aegypti and *Culex quinquefasciatus* were raised in the insectarium of the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University.

Alcoholic plant extracts

Solanum xanthocarpum was home grown and free from pesticides. The species was confirmed by the Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University. Mature unripe *Solanum* fruits were washed with tap water, cut into small pieces and left to dry in a shaded place. After the plant was completely dried, it was grounded into powder using an electric blender. Dried and powdered plant material (250 g) was macerated in 95% ethanol at room temperature (25-27ºC) for 3 days. The crude extract was separated with a filtrating suction using No.1 Whatman filter paper. The filtrate was dried with a rotary evaporator at 45ºC until the solvent was completely evaporated. The ethanolic extract obtained was refrigerated at -20ºC until testing.

Molluscicidal activity test

Evaluation of molluscicidal activity of the plant extract against adult snails was done as recommended by the World Health Organization (1965) and modified by Duncan and Sturrock (1987). Ten adult snails were placed in a plastic cup, containing 200 ml of dechlorinated tap water and molluscicide to give a final concentration of 125, 150, 175, 200, 225 and 250 ppm. Each experiment was repeated in triplicate. Controls were prepared using the same concentration of solvent in water. Snails were exposed to the molluscicide suspension for 24 hours at room temperature (25-27ºC) and kept under normal diurnal lighting. After 24 hours, the suspension was decanted; the snails were rinsed twice with dechlorinated tap water and transferred to a new container filled with dechlorinated tap water. The numbers of dead and alive snails were recorded at 48 hours. Snails were considered dead if they did not move and were either retracted well into or hanging out of the shell, with discolored body and shell. Dead snails were removed as soon as possible.

Larvicidal activity test

Evaluation of the plant extract against *Ae. aegypti* and *Cx. quinquefasciatus* larvae was performed according to World Health Organization guidelines (WHO, 2005). Batches of 25 late third or early fourth instar larvae were transferred by means of strainers and droppers to disposable test cups, each containing 100 ml of water. Small, unhealthy or damaged larvae were removed and replaced. One mililiter of extract was added to a test cup and water added to give a total volume of 100 ml. Four replicates were carried out for each concentration and an equal number of replicates using controls were performed simultaneously with dechlorinated tap water, to which 1 ml ethyl alcohol was added. Each test was run three times on different days.

Twenty-four hours after exposure, larval mortality was recorded. Moribund larvae were counted and added to dead larvae for calculating percentage mortality. Dead larvae were those that could not be induced to move when they were probed with a needle in the siphon or cervical region. Moribund larvae were those incapable of rising to the surface or not showing the characteristic diving reaction when the water was disturbed.

Data analysis

Larvae that pupated during the test period were negated from the test. If more than 10% of control larvae pupated during the course of the experiment, the test was discarded and repeated. If the control mortality was between 5% and 20%, the mortalities of the treated groups were corrected according to Abbott's formula (Abbott, 1925).

$$\% M = \frac{\text{\% test mortality -\% control mortality}}{\text{100 - \% control mortality}} \times 100$$

The dose mortality was analyzed using a computerized log-probit analysis (Finney, 1971). Ninety-five percent confidence limits (CL) at lethal concentrations of 50% and 90% (LD_{50} and LD_{90}) were calculated.

RESULTS

Molluscicidal activity

The bioefficacy of *Solanum xanthocarpum* against *Biomphalaria glabrata* and *Indoplanorbis exustus* is shown in Table 1. The LC_{50} and LC_{90} of ethanolic extract against *Bi. glabrata* 24 hours and 48 hours after exposure were 163.85 and 219.33 ppm, whereas those against *I. exustus* were

Table 1
LC_{50} and LC_{90} with 24 hours of exposure of *Biomphalaria glabrata* and *Indoplanorbis exustus* to *Solanum xanthocarpum*.

Snail species	Time (in hours)	Lethal concentration (ppm)			
		LC_{50}	95%CL	LC_{90}	95% CL
Bi. glabrata	24	163.85	154.46-172.81	219.33	203.58-247.36
I. exustus	24	198.00	190.57-205.39	236.80	225.74-254.58

Table 2
LC_{50} and LC_{90} with 24 and 48 hours of exposure of the larvae of *Aedes aegypti* and *Culex quinquefasciatus* to *Solanum xanthocarpum*.

Mosquito species	Time (h)	Lethal concentration (ppm)			
		LC_{50}	95%CL	LC_{90}	95% CL
Ae. aegypti	24	788.10	710.73-873.58	1,288.91	1,094.88-1,524.86
	48	731.06	641.13-833.31	1,165.83	958.72-1,421.34
Cx. quinquefasciatus	24	573.20	481.02-682.81	1,066.93	793.19-1,439.64
	48	503.60	428.53-591.34	979.79	765.21-1,259.52

198.00 and 236.80 ppm, respectively.

Larvicidal activity

The effect of *S. xanthocarpum* against the larvae of *Aedes aegypti* and *Culex quinquefasciatus* is shown in Table 2. Twenty-four hours after exposure, the LC_{50} and LC_{90} against *Ae. aegypti* larvae were 788.10 and 1,288.91 ppm, respectively whereas an LC_{50} and LC_{90} of 573.20 and 1,066.93 ppm, respectively, were obtained against *Cx. quinquefasciatus*. When the exposure time was increased to 48 hours, the LC_{50} and LC_{90} were 731.06 and 1,165.83 ppm against the larvae of *Ae. aegypti* and 503.60 and 979.79 ppm against the larvae of *Cx. quinquefasciatus*, respectively.

DISCUSSION

The findings of this evaluation of the molluscicidal properties of *Solanum xanthocarpum* against *Biomphalaria glabrata* and *Indoplanorbis exustus* are in accordance with the study of Wei *et al* (2002) who used 95% ethanol for extraction and then purified the product with 1% acetic acid, 5% ammonia and ethyl acetate. The results from their study also showed a significant effect of the plant extract on *Oncomelania hupensis*, *Bi. glabrata* and *Lymnaea stagnalis* snails with LC_{50} values after 24 hours of 0.62, 1.35 and 1.25 mg/l, respectively. They found this plant was a promising molluscicide. A comparison was made between the molluscicidal activity of the crude ethanolic extract of the plant tested and the specific active ingredient from Wei's study. The results show the crude ethanolic extract was 264.3 time less effective than the specific active ingredient when testing the LC_{50} levels against *Bi. glabrata*. *S. xanthocarpum*'s molluscicidal activity was

seen in a study by Li *et al* (2005) who used 95% ethanol as a solvent for extraction and then further purified the active component. Solamagine, the active component, showed an excellent capability in killing *Oncomelania* snails. According to Mott (1987) for a plant to be considered as a molluscicide, the crude extract of the plant should be active at a concentration ≤100 mg/l killing 90% of the snails after 24 hours exposure. The results of our study showed the test solution caused 90% snail mortality after 24 hours exposure at concentrations of 219.33 and 236.80 mg/l for *Bi. glabrata* and *I. exustus*, respectively. These solutions are well above the concentration of 100 mg/l, indicating the ethanolic extract in this study has a low potency as a molluscicide and may fail in snail control.

S. xanthocarpum had larvicidal activity against the larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. This is similar to the findings of Singh and Bansal (2003) who investigated the larvicidal activity of *S. xanthocarpum* crude extract against the mosquito larvae of malaria and dengue vectors. They found larvicidal activity against *Anopheles culicifacies*, *An. stephensi* and *Ae. aegypti*. They also described the different degrees of toxicity of the crude extracts from different parts of the plant. The LC_{90} results of fruit extract against *An. culicifacies*, *An. stephensi* and *Ae. aegypti* were 0.258 (2,580 mg/l), 0.289 (2,890 mg/l) and 0.218% (2,180 mg/l), respectively, whereas the root extract gave results of 3.237 (32,370 mg/l), 2.789 (27,890 mg/l) and 3.581% (35,810 mg/l), respectively. Our findings are aslo in accordance with Mohan *et al* (2005) who studied the crude extracts of *S. xanthocarpum* against *An. stephensi* and *Cx. quinquefasciatus*. They found different solvents used during extraction resulted in different levels of larvicidal activity. Species susceptibility was described by Bansal and Singh (2005) who exposed the larvae of *An. culicifacies*, *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* to the methanolic fruit extract of *S. xanthocarpum* and found the larvae of *An. culicifacies* were 1.01, 2.29 and 3.04 times more susceptible than the larvae of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus*, respectively the LC_{50} and LC_{90} by 48 hours of exposure were lower than at 24 hours of exposure. This implies the tested plants are more potent after 48 hours exposure (Lapcharoen *et al*, 2005).

This investigation suggests the active ingredient of the plant extract responsible for causing mortality in snails and mosquito larvae should be identified and evaluated to avoid causing toxic effects to non-target organisms. It may be prepared as a commercial product for vector control.

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