FORMULATION OF TABLETS FROM THE CRUDE EXTRACT OF *RHINACANTHUS NASUTUS* (THAI LOCAL PLANT) AGAINST *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS* LARVAE: A PRELIMINARY STUDY

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Abstract. Dried root powder of *Rhinacanthus nasutus*, Thong Phan Chang (Thai name) were extracted with methanol (MeOH) in a Soxhlet apparatus and made into 2 formulations of tablet containing the extract at 5% and 10% concentration. Due to the viscous and poor flow properties of the crude MeOH extract obtained, a wet granulation method was conducted in developing the tablets. Lactose was used as a filler. Polyvinyl pyrrolidone (PVP) K30 (15% w/w solution in alcohol) was used as the binding agent, while stearic acid (2% w/w) was used as a lubricant. Both formulas of prepared tablets had a smooth shiny surface with a round shape. Other physical properties of the tablets, such as weight variation, friability and disintegration time, met the requirements of the USP XX standard. The mosquito larvicidal activity of prepared tablets containing 5% and 10% *R. nasutus* extract against *Aedes aegypti* were not significantly different from each other ($p>0.05$), with 48-hour LC_{50} values of 13.6 and 14.2 mg/l for the 5% and 10% tablets, respectively, while their activities against *Culex quinquefasciatus* were similar ($p>0.05$) with LC_{50} values of 18.7 and 17.3, respectively. The larvicidal activity levels against *Ae. aegypti* and *Cx. quinquefasciatus* were also not significantly different from each other ($p>0.05$). No larval mortality was observed in the two control groups: lactose solution and dechlorinated water. Toxicity to female and male fish (*Poecilia reticulata*) was tested with the prepared tablets. The toxicity of tablets containing 5% and 10% extracts were not significantly different from each other for the *P. reticulata* females with 48-hour LC_{50} values of 105.2 and 110.8 mg/l, respectively, and for *P. reticulata* males with LC_{50} values of 99.1 and 103.4 mg/l, respectively. Female and male *P. reticulata* were sensitive to the same dose of the extract. No fish died in the two control groups, with lactose solution and dechlorinated water. Acute-toxicity bioassay with fish showed that with an exposure of 48 hours the LC_{50} values of the tablets containing 5% and 10% were 5- to 10-fold higher than the LC_{50} of *R. nasutus* against mosquito larvae. These prepared tablets could possibly used to control mosquito vectors and be introduced into the mosquito control program.

INTRODUCTION

The effectiveness of vector control has declined because of the reduced effectiveness of insecticides caused by the emergence of resistance in mosquitoes against the currently used insecticides (Chandre *et al*, 1998). It is important to recognize that only a limited number of insecticides are available for use in public health. Therefore, an effort to find alternatives for the currently used insecticides in relation to mosquito control is needed. The application of easily degradable plant compounds is considered to be one of the safest methods to control insect pests and vectors (Alkofahi *et al*, 1989).

From many parts of the world, a number of crude extracts from various plants have been studied regarding their larvicidal activities. Some have shown high activity with remarkable LC_{50} values less than 15 mg/l against mosquito vectors. For example, in India the petroleum ether extract of the seeds of *Argemone maxicana* (Ver-

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nacular name: Piramathandu) showed larvicidal activity against 2nd instar larvae of *Culex quinquefasciatus* with an LC_{50} value of 8.8 mg/l (Sakthivadivel, 2001). The larvicidal activity of the extract of the seaweed plant, *Cleome viscosa*, obtained from India has been reported to have an LC_{50} of 10.7 mg/l against *Cx. quinquefasciatus* (Kalyanasundaram and Babu, 1982; Thangam and Kathiresan, 1988). The ethyl acetate soluble fraction of the seed extract of *Calophyllum inophyllum* and the leaf extract of *Rhinacanthus nasutus*, which are local Indian plants, showed very high toxicity ranging from a LC_{50} of 3.9 to 13.2 m/l against mosquito larvae (Pushpalatha and Muthukrishnan, 1999). In the USA, a butanol extract of the soapberry plant, *Phytolacca dodecandra* (endod), was toxic against the 3rd instar larvae of *Ae. aegypti*, *Cx. pipiens* and *Anopheles quadrimaculatus* with LC_{50} values ranging from 0.3 to 0.4 mg/l (Spielman and Lemma, 1973). In Brazil, the ethanolic extracts of *Tagetes minuta* and *Eclipta paniculata* had high larvicidal activity with LC_{50} of 1.0 and 3.3 mg/l, respectively against *Ae. fluviatilis* (Green *et al*, 1991; Macedo *et al*, 1997). From Argentina, the dichloromethane extract of *Abuta grandifolia* and the methanol (MeOH) extract of *Minthostachys setosa* were significantly active with LC_{50} of 2.6 and 9.2 mg/l, respectively, against *Ae. aegypti* larvae (Ciccia *et al*, 2000).

We can see that efforts have been made to fractionate, isolate, and identify promising extracts to be used as botanical insecticides. The various results of these studies have been different due to the plants, environment, and synergism of extracts in the compound (Chiu, 1989). A program of new agent discovery (mainly for pharmaceutical purposes) is a costly process and may take 10-20 years to product development (Cragg *et al*, 1993). In this study, we present a new approach to the development of a tablet containing the crude plant extract. In crude extracts, pure chemical components are unstable plant products. The development of appropriate formulations is necessary to increase their stability and efficiency and to make them suitable for field use.

From our studies of mosquito larvicidal activity screening in 84 Thai plants, using either petroleum ether or MeOH, extracts of *R. nasutus* showed significant toxicity, with LC_{50} values less than 15 mg/l against *Ae. aegypti*, *Cx. quinquefasciatus*, *An. dirus* and *Ma. uniformis*. These results are supported by Pushpalatha and Muthukrishnan (1999). Based on its water solubility and dispersibility, the MeOH extract of *R. nasutus* was selected as the active ingredient for preparing a tablet formulation. The tablets were prepared using wet granulation, a versatile process, because the MeOH extract contained high humidity and poor flow (Lachman, 1976).

The aims of the present, preliminary study were the formulation of tablets from the MeOH extract of *R. nasutus* roots and investigation of the tablet's toxicity in fish *Poecilia reticulata* (common guppy).

MATERIALS AND METHODS

Plant extract preparation

The roots of *R. nasutus* were collected from Chaiyaphum Province, Thailand. They were cleaned with tap water, dried in the shade, ground first with a knife then with a homogenizer. The powder material was extracted in a Soxhlet extractor with MeOH. The MeOH solution was concentrated under reduced pressure, below 45ºC, to dryness. The resulting crude MeOH extracts were used for the tablet formulation (Redwane *et al*, 2002).

Tablet formulation by wet granulation

The tablet were prepared in 2 dosage forms, containing 5% and 10% MeOH extracts of *R. nasutus*. Lactose was used as a soluble filler-binder for the tablets. Lactose tablets were also prepared as a control. Percentages of each ingredient and the preparation method are shown in Table 1. All the steps of preparation were carried out at the Department of Industrial Pharmacy, Faculty of Pharmacy, Mahidol University.

According to quality control of product development, the physical properties that consisted of shape, color, weight, thickness, diameter, hardness, friability and disintegration were identified. Biological properties were investigated by testing against *Ae. aegypti* and *Cx. quin-*

Table 1
Tablet formulation by wet granulation.

Ingredients	Percent
R. nasutus, MeOH extract	q.s.
Lactose	q.s.
PVP K30	6
Stearic acid	4

Method:

1. Mixed the extract and lactose in an appropriate mixer.
2. Prepared 15% w/w PVP K30 in absolute ethanol.
3. In a planetary mixer, slowly and continually added 15% PVP K30 to the mixture from no.1 over 15 minutes.
4. Passed the wet mass through a 14-mesh screen and dried in an incubator at 40ºC for 2 hours.
5. Reduced the dry granules by passing through an 18-mesh screen.
6. Added stearic acid powder to the mixture from no.5 in a rotary mixture for 5 minutes.
7. Compressed using 1/4 inches flat-face, round punches and dried.

quefasciatus larvae. In addition, their toxicity to guppy fish was carried out.

Mosquitoes

The tested mosquito species were *Ae. aegypti* and *Cx. quinquefasciatus*. These mosquitoes were uninfected laboratory strains, reared for over 10 generations in the insectary of the Insecticide Research Unit at the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Thailand. The methods for mass rearing were slightly modified from the procedure in the manual "Rearing techniques for mosquitoes" (Limsuwan *et al*, 1987).

Late 3rd and early 4th instar larvae of *Ae. aegypti* mosquito were used to screen for the larvicidal activity of the prepared tablets.

Larvicidal tests

Standard methods for testing the larvicidal action of the prepared tablets were slightly modified from the WHO (1996). A stock solution was prepared by dissolving tablets containing 5% and 10% *R. nasutus* extract concentration in distilled water in a volumetric flask and stored in a refrigerator at 15ºC. After 20 healthy, late 3rd-early 4th instar larvae were introduced into each experiment cup [sterilized drinking plastic cup (150 ml capacity)], which contained 100 ml of dechlorinated water, an appropriate volume of stock solution was added to obtain the desired concentration. Experiments were carried out in a series of five concentrations in 5 replicates with the final total number of 100 larvae for each concentration. Each replicate set contained one control, which was 100 ml of dechlorinated water alone, and the other control was 100 ml of water containing a maximum volume of lactose solvent in the test medium. Since very few larvae succumbed within 24 hours of exposure to the tested solutions, the mortality was recorded at 48 hours of exposure, during which no food was offered to the larvae. The mortalities of mosquito larvae were recorded according to the following criteria: moribund larvae, which were incapable of rising to the surface or did not show the characteristic diving reaction when the water was disturbed, had discoloration, an unnatural position or rigor. The LC_{50} and LC_{90} values were determined by the Probit analysis program (Finney, 1971). The control mortality rate was accounted for by Abbott's formula (Abbott, 1925).

Toxicity test on fish

P. reticulata, with a total length of 1.5-2.5 cm, were purchased from the Sunday (Jatujak) market, Bangkok. They were acclimatized in the laboratory for 2 weeks in dechlorinated tap water at a room temperature of 28-30ºC. The most active and healthy fish were selected for the bioassay.

Toxicity experiments were performed using

Table 2
Physical properties of two tablet formulations from the crude methanolic extract of *R. nasutus* and the control.

Physical properties	Formulations		
	5% extract	10% extract	0% extract (placebo)
Shape	Round	Round	Round
Color	Milky pink	Dark pink	White
Weight variation (mg)	102.4 ± 4.04	104.6 ± 2.26	103.7 ± 2.62
Thickness (mm)	2.7 ± 0.15	2.7 ± 0.04	2.6 ± 0.07
Diameter (mm)	5.9 ± 0.02	5.9 ± 0.01	5.9 ± 0.03
Hardness (N)	56.4 ± 8.39	62.5 ± 6.14	66.7 ± 11.77
Friability (%)	0.07	0.08	0.08
Disintegration time (minute)			
- Used equipment	14.6 ± 1.06	14.4 ± 0.65	16.3 ± 0.95
- Without equipment	143.3 ± 15.96	158.1 ± 1.86	114.8 ± 3.60

Figures in parentheses represent mean ± SD

the methods of Wangsomnuk (1997) and the standard procedures for laboratory screening of the WHO (1971). In order to determine the approximate lethal limits of the prepared tablet, 10 *P. reticulata* were tested in 5 liters of the various desired concentrations of tested solution. Dechlorinated tap water was used as a control. The concentration at which all *P. reticulata* survived and the concentration at which all *P. reticulata* died within 48 hours were determined. Three replications of 5 liters between the two concentrations from the consecutive 7 concentration series and dechlorinated tap water control were made in 7-liter glass jars, and 10 *P. reticulata* released in each concentration. Observations of the mortality of the fish were made at intervals of 6, 12, 24, 32, 40 and 48 hours. During each observation, the fish were prodded gently to see whether there was any response. Dead fish were removed from the test media to avoid fouling, and the mortality was recorded at 24 and 48 hours. All bioassay tests were conducted without aeration or renewal of water at a room temperature of 28-30ºC. The LC_{50} and LC_{90} values were determinated by the Probit analysis program (Finney, 1971). Control mortality was accounted for by the Abbott's formula (Abbott, 1925). The lethal concentrations were defined as the concentrations at which 50% and 90% of the experimental animals died during the exposure time.

RESULTS

The physical property

The MeOH extract of *R. nacantus* was prepared as a tablet. No problems emerged in the compression process. The physical properties of the three tablet formulations are shown in Table 2. All three formulas of the prepared tablets were similar, with a smooth, shiny surface, round in shape, 5.9 mm in diameter and 2.7 mm in thickness. The MeOH extracted was red in color, so the 10% concentration was darker pink than the 5% concentration, while the tablet containing only lactose was white in color. These prepared tablets had a weight variation of 102.4-104.6 mg, a hardness of 56.4-62.5 N and a percentage friability of 0.07-0.8%. Using a disintegration test apparatus their disintegration time was 14.6-14.4 minutes, whereas without the apparatus the time was 143.3 -158.1 minutes. All the physical properties met the requirements of the USP XX standard (Lachman, 1976). However, these tablet forms easily absorbed humidity because lactose was used as a filler.

Table 3
Biological properties of three formulations of the crude methanolic extract of *R. nasutus* against the larvae of *Ae. aegypti* and *Cx. quinquefasciatus* and both female and male *P. reticulata* after 48-hour exposure.

Biological properties	Formulations		
	5% extract	10% extract	0% extract (placebo)
Ae. aegypti			
LC_{50} value (mg/l)	13.6 (12.65-14.66)	14.2 (13.00-15.58)	0
LC_{90} value (mg/l)	23.3 (21.59-25.50)	24.3 (22.16-27.29)	0
Cx. quinquefasciatus			
LC_{50} value (mg/l)	18.7 (7.42-20.08)	17.3 (13.08-18.68)	0
LC_{90} value (mg/l)	36.9 (30.16-40.32)	31.1 (28.80-33.94)	0
P. reticulata, female			
LC_{50} value (mg/l)	105.2 (90.72-123.78)	110.8 (80.65-132.46)	0
LC_{90} value (mg/l)	233.8 (219.95-273.80)	238.6 (196.48-286.53)	0
P. reticulata, male			
LC_{50} value (mg/l)	99.1 (88.85-230.68)	103.4 (200.46-256.50)	0
LC_{90} value (mg/l)	222.9 (192.43-263.46)	242.6 (224.35-272.68)	0

LC_{50} = median lethal concentration; LC_{90} = 90% lethal concentration; Figures in parentheses represent 95% confidence interval; Confidence interval is asymmetric because of logarithmic transformation.

The biological property

Larvicidal activity. The results of the mosquito larvicidal activity of the prepared tablets containing the 5% and 10% *R. nasutus* extract and only lactose are shown in Table 3. The activity against *Ae. aegypti* of the tablets containing 5% and 10% extract were not significantly different from each other ($p>0.05$) with 48-hour LC_{50} values of 13.6 and 14.2 mg/l, respectively. Their activity against *Cx. quinquefasciatus* was also similar ($p>0.05$) with LC_{50} values of 18.7 and 17.3, respectively. The larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* were not different ($p>0.05$). No larval mortality was observed in the two control groups, the lactose solution and the dechlorinated water.

Toxicity to fish. No fish died in the two control groups, the lactose solution and the dechlorinated water. Behavioral reactions in the order of their appearance were swimming to the surface for respirations, a decrease in activity, sinking to the bottom and death. The 48-hour LC_{50} and LC_{90} values for the tablets containing 5% and 10% extract are shown in Table 3. The toxicity of the tablets containing 5% and 10% extracts were not significant different for the *P. reticulata* females, with LC_{50} values of 105.2 and 110.8 mg/l, respectively, and for the *P. reticulata* males, with LC_{50} values of 99.1 and 103.4 mg/l, respectively. The female and male *P. reticulata* were sensitive to the same dose of the extract.

DISCUSSION

There have been many reports of promising crude plant extracts with LC_{50} values less than 15 mg/l, which were reported to have eco-

nomical effectiveness, as these can be purified to get more active ingredients (Kalyanasundaram and Das, 1985; Sujatha *et al*, 1988). However, large-scale chemical synthesis of these insecticides is precluded by the extremely high cost of synthesizing the complex structure of the active ingredients (Cragg *et al*, 1993; Mulla and Su, 1999). It is only practical to extract bioactive agents from the renewable parts of a tree to manufacture various insecticide formulations. The commercial formulations of products containing Neem, *Azadirachta indica*, are a good example. The experimental formulation of azadirachta (AZ), such as wettable powder (WP); Azad WP10, and emulsifiable concentration (EC); Neemix EC0.25 and Neemazad EC4.5 provided 80% cumulative mortality (larvae, pupae and adults) after treating 4th instar larvae at 0.1-1 mg/l. A significant level of antifeeding activity was indicated at 5 and 10 mg/l AZ for all the test formulations against *Cx. tarsalis* and *Cx. quinquefasciatus* (Su and Mulla, 1998; Mulla and Su, 1999). Neem was formulated to be wettable powder AzadTM WP10 and an emulsifiable concentrate AzadTM EC4.5 which have been reported to have effective mosquito ovicidal activity and good persistence (Su and Mulla, 1998).

From our initial study, *R. nasutus* root extract appears to have promising insecticidal activity against *Ae. aegypti*, *Cx. quinquefasciatua*, *An. dirus* and *Ma. uniformis*. This present study also showed that the crude extract of *R. nasutus* can be formulated into a tablet containing 5% and 10% extract, with their physical properties following the USP XX standard. These prepared tablets easily absorbed humidity because they contained lactose as a filler, therefore they should be kept in suitable containers. For disintegration without equipment, which was not listed as a USP XX standard test, we conducted this investigation due to the condition of mosquito breeding places. Results without equipment show the disintegration times of tablets containing 5% and 10% extract averaged 143.3 and 158.1 minutes, respectively. There are no reports similar to this type.

The small tablet size of 10 mm in diameter containing a high concentration of 5% or 10% extract as an active ingredient, can be suitably applied to artificial containers, which are breeding places for *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus*. Until now, no detailed studies of the toxicity of *R. nasutus* root extract on large animals have been carried out, so avoidance of application of these prepared tablets to containers of drinking water should be kept in mind. Acute toxicity tests, sub-chronic tests, and mutagenic tests to mammals should be carried out on rats to determine the side effects. This plant is medicinally important; it has been reported to provide effective treatment for rheumatism, skin diseases and other ailments (Chopra *et al*, 1956; Smitinand, 1980).

Our experiment studying acutetoxicity in fish showed that 48-hour LC_{50} values for the tablets containing 5% and 10% extracts ranged from 99.1-110.8 mg/l, which is 5 to 10-fold higher than the LC_{50} values of *R. nasutus* against mosquito larvae. These tablets can be used to control mosquitoes and be introduced into a mosquito control program.

Further research is needed to develop effective controls mosquito larvae, especially concerning breeding places for each mosquito vector species.

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