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LARVICIDAL ACTIVITY OF THE MIXTURE OF CASHEW NUT SHELL LIQUID (CNSL) AND AQUEOUS EXTRACT OF *Sapindus rarak* DC AGAINST LARVAE OF *Culex quinquefasciatus*

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ABSTRACT

The aim of this study was to evaluate the larvicidal activity of **C**ashew **N**ut **S**hell **L**iquid (CNSL) against the *Culex quinque fasciatus* in larval stage. The CNSL was diluted in water by addition of aqueous extract of *Sapindus rarak* DC to increase its solubility. Larvae were exposed to varying concentrations of that mixture. The larvae mortality was observed after 24 h exposure. LC_{50} and LC_{90} value by extrapolation were 20,52 ppm and 55,41 ppm respectively. CNSL were specified by characterizing its physico-chemical properties and anacardic acid as marker compound by High Performance Chromatography (HPLC). The results were the mixture of Cashew Nut Shell Liquid (CNSL) and Aquous extract of S*apindus rarak* DC **had** larvicidal activity against *Cx. Quinque-fasciatus* and further investigations were needed to identify the fatty acid derivative as active compound of CNSL which responsible for larvicidal activity.

Key word: larvicidal, larvae, *Culex quinquefasciatus, Cashew Nut Shell Liquid, Sapindus rarak*

INTRODUCTION

Culex quinquefasciatus is the most common mosquito species found in Indonesia. There are the main cause of the mosquito bites in the evenings and night[1]. The main factor supporting this species is probably due to poor sanitation caused by human migration to urban areas. Interest in the control of *Cx.quinquefasciatus* lies in the fact that it acts as a vector of filarial disease as a serious public health problem in Indonesia and many tropical developing countries. Filariasis is an endemic, disabling, and disfiguring disease. The filarial worm, *Wuchereria bancrofti* responsible for human filariasis is carried by *Cx.quinquefasciatus* which is a tropical pest and probably the most abundant and ubiquitous house mosquito in towns and cities, in the tropical countries[1].

One of the strategies of WHO in combating tropical disease is to destroy their vectors or intermediate hosts. Since no effective vaccine is available for filariasis, the only effective approach of minimizing the incidence of this disease is to eradicate and control mosquito vectors mainly by application of organic insecticides to larval habitats. Control of mosquito at this stage is efficient because during the immature stages, mosquitoes are immobile[2].

The use of natural products for controlling of insect pests offers an economically viable and eco-friendly approach, besides being harmless to beneficial insects when adopted on a larger scale. Chemical pesticides have been used for several decades in controlling pests and vectors of various human diseases as they have a quick knock down effect. However, their indiscriminate use resulted in several problems such as resistance and resurgence of pests, elimination of natural enemies, toxic residues in food, water, air, and soil which affect human health and disrupt the ecosystem, leading to the threat that their continued use may further harm the environ-ment[3].

Anacardium occidentale L. (*Anacardiaceae)*, a fruit tree grown widely in tropical and sub-tropical areas is cultivated in Indonesia for its cashew nuts. Tyman & Morris[7] in 1989 described the composition of CNSL which found between the seed coat (pericarp) and the nuts. It is not a triglyceride and contains a high portion of phenolic compound, mainly are anacardic acid, cardol, and cardanol. Recently, Lomonaco[5] and Oliveira[6] noted insecticidal action of CNSL on larvae of *Aedes aegypti* (Diptera: Culicidae). The three CNSL compo-nents demonstrated good larvicidal activity against *Ae. aegypti.*

Despite the potential to be developed as larvicides, phenolic compounds in the CNSL was toxic because it induced dermatitis[8]. In addition, application of CNSL as larvicides in the medium of water is also cumbersome, so it is necessary to add other materials intended to dilute it. In this study, aqueous extract of *Sapindus rarak* DC was added to increase the solubility of CNSL in water.

MATERIALS AND METHODS

Plant materials

Cashew nuts were obtained from cashew trees (*A.occidentale*) at Wonogiri, Central Java, Indonesia. The fruits of *Sapindus rarak* DC were collected from the areas of "Perhutani" located in Situbondo, East Java, Indonesia. The plant material was identified by Mr.Joko Santosa and Voucher specimen was deposit at Deprtment of Pharmaceutical Biology Faculty of Pharmacy, UGM

Oil Extraction of CNSL

The dried cashew nut shell (1,3 kg) were pressed by *hydraulic pressor* to obtain *Cashew Nut Shell Liquid* (CNSL).

Preparation of aquous extracts of S.rarak

The dried pericarp from *S.rarak* (10 g) were cut into small pieces then extracted with 1000 mL. of distilled water by reflux process at 50°C for 30 minutes. The concentration of aqueous extract of *S.rarak* fruits was 1% $^{b}/_{v.}$

Reagents

N-Toluene (E.Merck), Ethyl acetate (E.Merck), Acetic acid glacial (E.Merck), Chloroform (Brataco), Methanol (Brataco). Iodium P, Anisaldehyde-sulphuric acid LP, Vanilin-sulphuric acid LP. ethanol 96% (Brataco), n-hexane (Brataco), Hydrochloric acid (E.Merck). All other chemicals used were analytical grade.

Larvicidal assay

The activity of the test materials were evaluated by the World Health Organization recommended guidelines [4].

The larvicidal assay consisted of two steps. First was the orientation to determine a certain concentration of aqueous extract of *S. rarak* DC. (It was the biggest concentration which is not found any died larvae). The chosen concentration then was used for making the mixture of CNSL and aquous extract of *S. rarak* DC. The next step was dissolving CNSL in various amount so that it was obtained 6 concentration series of the mixture for used in the testing, which were 0; 8; 8,56; 9,16; 9,8; and 10,49 ppm.

Characterization of CNSL's physico-chemical properties

Physico-chemical properties of cashew nut shell oil was characterized through the iodine number, esther number, and acid number. Those properties were characterized by Pusat Antar Universitas (PAU) - UGM.

HPLC analysis of CNSL constituents

The identity of anacardic acid was confirmed by HPLC. The analysis was carried out using a Shimadzu SPD-10VP chromatograph, UV-VIS detector, which utilized a Lichochart RD-C18 analytical column. The mobile phase consisted of methanol-acetic acid 4% (9:1v/v), which was run in the isocratic mode phase (1 mL/min) at a UV wavelength of 280 nm.

Determination of Foaming Index of S.rarak *source*

5 g of dried *S.rarak* fruits was dissolved in distilled water and then heated 50^{o}C for 5 minutes. 10 mL filtrate was used for foaming test. Saponin test was performed by agitation in 10 mL of the filtrate in a closed tube for 10 minutes. Incidence foam up interval of 10 minutes (stable foam) showed a saponin.

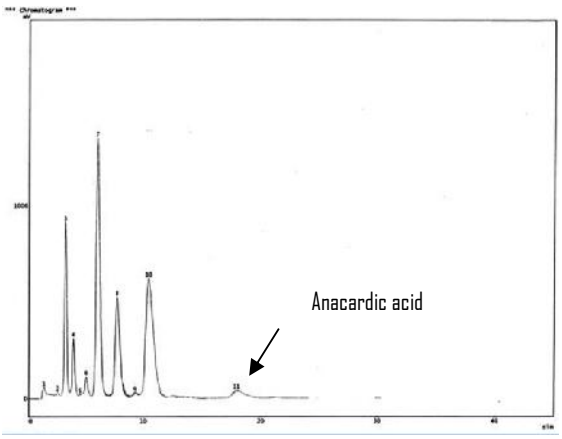

Fig.1. Chromatogram of CSNL

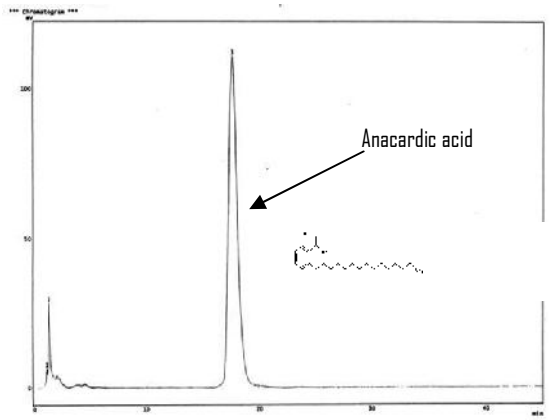

Fig.2. Chromatogram of Anacardic acid

RESULTS AND DISCUSSION

One of the marker constituent of CNSL, anacardic acid, was detected in the sample of CNSL in relative concentration of 2,58%. The HPLC chromatograms were shown in fig.1 and fig.2.

Due to its bad solubility, CNSL was diluted in water with *S.rarak*'s pericarp extract as a co-solvent, in concentration which was determined by the orientation test. Concentration of 400 ppm was selected as the optimum concentration to dillute CNSL in water but it had no effect to cause larval mortality. The mixture of CNSL and *S.rarak* extract

used in this study was visually clear. There were also not detected visible oil particles or any breaking phase.

Table 1. Physico-chemical properties of CNSL used in this study

Physico-chemical properties of CNSL	Value
Iodium number (g iod/10 g CNSL)	6,3
Acid number (mg NaOH/g CNSL)	104,1
Saponification number	127,1
Esther number	45,9
Density (g/mL)	0,8232

It had no published data available of effect of CNSL on immature stages of Culex mosquitoes. Effect of different concentrations of CNSL solution on aquatic stages of *Cx.quinquefasciatus* larvae in the laboratory conditions was shown in Tables 2.

There was significant decreasing mortality percentage of the larvae by increasing CNSL concentration(table 2). As shown in that Table, the biggest death rate was noticed in exposure to 10,49 ppm concentration.

LC line equation obtained from probit analysis was y = 3.01 x + 1.05, so that the calculation could be known that LC_{50} and LC_{90} value were 20.52 ppm and 55.41 ppm, respectively. This value was the result of extrapolation and therefore could not guarantee that these results show the actual value of LC_{50} and LC_{90}.

Essential oils or plant extracts with LC_{50} values below 100 ppm can be considered potentially have larvicidal activity[9]. Our present observation reveals that *Cx.quenquefasciatus* larvae were susceptible to 8 ppm to 10,49 ppm CNSL.

In Indonesia, population of *Cx.quinquefasciatus* is controlled by using of organophosphate insecticides, such as temephos and malathion. Temephos, which is also known as "Abate" has been used in areas of water where the *Cx.quinquefasciatus* mosquito breeds in order to reduce the population of this disease-carrying insect. Resistance to temephos by *Cx.quinquefasciatus* has been reported in Brazilian[10] and Malaysia[11]. Temephos was very lethal toward *Cx.quinquefasciatus* larvae with a LC_{50}=0,00088 ppm[11]. CNSL consti-tuents were less active than temephos, but could be important models for the further development of new larvicides. It is necessary to investigate the effectiveness of these mixture compounds in a field setting and the toxicity toward other organisms, for example by doing brine shrimp lethality test.

CONCLUSIONS

In the present work the mixture of CNSL in aquaeous extract of *S.rarak* DC could be used as larvicide against

Table 2. Larvicidal activity of CNSL and *S.rarak* extract mixture against *Cx.quinque fasciatus* larvae

CNSL (ppm)	Number of larva died (3 replications)			Total larvae	Mortality (%)
	1	2	3		
0	1	1	0	75	2.67
8	2	4	4	75	13.33
8,56	5	3	3	75	14.67
9,16	7	3	3	75	17.33
9,8	6	9	0	75	20
10,49	8	5	3	75	21.33

Cx.quinquefasciatus. Given the continual search for renewable and biodegradable sources of new medicinal products, CNSL from cashew nut processing industries in Indonesian agribusiness represents an opportunity to increase the value of this by-product by developing green larvicidal compounds to be used in filariasis control, which is cheap, non-toxic, and biodegradable.

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