Larvicidal Activity of *Aedes Aegypti* from a Simple Preparation of Cashew (*Anacardium occidentale* L.) Nut Shell Extract for Community Level Use

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Abstract

Dengue fever is a major risk to public health due to the recent worldwide spread. Chemical vector control are usually considered, therefore, negative consequences are commonly occurred due to chemical resistance and environmental effects. Volume of cashew nut shell is become increasing as by-product after nut processing. This study was aimed to evaluate larvicial activity and toxicity of cashew shell extract with simple preparation for community use. Fresh cashew nut shell was dried and grinded in powder form. Material was immersed in water tank for 72 h and ratio of flavor and material and water ratio was 100 g/L (10% W/V). The supernatant of stock solution was diluted to 1:5, 1:10, 1:20, 1:50 and 1:100 of larvicidal solution for phenolic content measurement, analysis of alkyl phenols by gas chromatography-mass spectrometry (GC/MS) and larvicial activity test. Larvicidal activity was effective at LC_{50} = 3.8 mg of GAE/ml; LC_{90} = 11.5 mg of gallic acid equivalent (GAE) /ml. The solution was contained five alkyl phenols, which included saturated cardol, monounsaturated cardol, anacardic acid, diunsaturated cardanol and monounsaturated cardanol. Anacardic acid was major active compound, which was correlated to mass spectra. This larvicidal solution preparation was simple and cost effectiveness, which may useful in community level especially in cashew cultivating area. Field application and public training on this study will be conduct on controlling of mosquito-borne diseases.

***Keywords**: Aedes aegypti, Anacardium occidentale, cashew, lavicidal activity, arthropod-borne disease, vector control,*

Introduction

Dengue fever is a major risk to public health due to the recent spread of the virus worldwide, which caused by dengue virus [1, 2]. The most important vector-borne disease transmitted by *Aedes* spp. (Diptera: Culicidae). Southeast Asia, including Thailand, the primary vector for dengue virus is *Aedes aegypti* and the secondary vector is *A. albopictus* [3]. *Aedes* spp. is also vectors of chikungunya virus [4] and Zika virus [5]. The vector control is one of most health policy for prevention of mosquitoes borne diseases, including larva and adult, which may controlled by include elimination of breeding sites, use of chemical control, genetic and biological control [4]. Chemical control are usually considered as the first method for mosquito control, therefore, negative

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consequences are commonly occurred due to chemical resistance and environmental effects [6, 7]. Plant origin is currently attention larvicides and consider because of harmless and biodegrade characteristics. larvicides of plant origin are also currently receiving considerable attention due to relatively harmless biodegradable properties [8]. Interactions between plants and insects are revealed the potential use of plants for fundamental pest control programs [9]. The various forms of natural products in folk medicinal treatments are more attend and consider for vector control [10, 11]. Green larvicides are now being considered because most plants are said to be nontoxic and biodegradable. Unlike the conventional insecticide which is based on single active ingredient, green insecticides comprise of bioactive chemical variations which have behavioral and physiological activities [11, 12]. Cashew (*Anacardium occidentale* L.) is well-known specie belongs to the *Anacardiaceae* family, which is a perennial-flowering plant and originate from Brazil. The pseudo fruit (cashew apple) can be used to make juice, jelly, jam, wine and syrup [13, 14], and its waste has been proposed as a feedstock for protein-enriched animal feed or as a high fructose source [15]. Different parts of the cashew tree have traditionally been used across the world to treat various diseases. Leaves or nut shell extracts from cashew, commonly known as the cashew tree, have long been used to treat inflammation and other conditions, including asthma, ulcers, and cancer [16]. In addition, cashew tree bark and leaves have been used in ancient medication for toothache and malaria [17]. Currently, Brazil, India, Vietnam, Tanzania and also Thailand are the main producers of cashew nuts. The nut is considered as more important in international market due to its widespread acceptance and demand [13]. Cashew nut shell is produces a heavy vesicant liquid called cashew nut shell liquid (CNSL) and contains high concentration of unsaturated long chain phenols, such as anacardic acid, cardanol, cardol and their derivatives [18, 19]. Recently, insecticidal action of cashew nut shell liquid (CNSL) on larvae of *A. aegypti* which is safe to mammals [20]. The remarkable toxicity effects exhibited by the solvent fractions of the ethanol extract of cashew shell wastes against the third and fourth instars larvae of *A. aegypti* [21]. Southern Thailand is major region of cashew nut production, which located in tropical zone and characterized by high humidity and rain throughout the year [22]. In addition, cashew nut is one major of agriculture product in Ranong province and after nut processing; the volume of cashew nut shell is become increasing as by-product. Hence, this study was aimed to evaluate larvicial activity and toxicity of cashew shell extract with simple preparation for community use.

Materials and Methods

Sample collection and preparation of larvicidal solution

Cashew nut shell waste was collected on March, 2019 from local agricultural group nearby Suan Sunandha Rajabhat University, Ranong academic campus, Thailand. Cashew tree were botanically identified as Phayam variety, which most common cultivated in this area. Fresh cashew nut shell (5 kg) was sundried and then dried material (~3 kg) was grinded in powder form. Grinded material was immersed in water tank for 72 h and ratio of flavor and material and water ratio was 100 g/L (10% W/V). The supernatant of stock solution was diluted to 1:5, 1:10, 1:20, 1:50 and 1:100 of larvicidal solution for phenolic content measurement, analysis of alkyl phenols by gas chromatography-mass spectrometry (GC/MS) and larvicial activity test.

Total phenolic content

In this step, each diluted larvicidal solution (0.1 ml) was added to 4.6 ml of distilled water and 1 ml of Folin-Ciocalteu reagent, and mixed by vortex mixer. After that, mixture was left in room temperature for 3 minutes. Next, 3 ml of 2% Na_2CO_3 (w/v) was filled into the tube, and shaken with the speed of 150 RpM for 2 hours. Then, the extract was measured to find out the light absorbance at 760 nm by comparing with the gallic acid at the intensity of 1, 0.875, 0.75, 0.625, 0.5, 0.375, 0.25 and 0.125 mg/ml. The total phenolic content was calculated into mg of gallic acid per g of the extract [23].

Analysis alkyl phenols in larvicidal solution

Extraction of alkyl phenols in larvicidal solution was modified according from previous study, which was extracted active of constituents in cashew nut liquid shell (CNLS) [24]. GC/MS was performed on a Hewlett-Packard 5971 instrument with the following components and conditions: dimethylpolysiloxane DB-1 coat-fused silica capillary column (30 m x 0.25 mm); He carrier gas (1 mL/min); injector temperature and detector temperature were 250 and 200 °C, respectively. Programming of column temperature was 35-180 °C, at 4 °C/min; and 180-250 °C, at 10 °C/min. Mass spectrometer operating conditions were 70 eV of ionization energy. Mass spectra were recorded from 40 to 450 m/z. Percentage of peak area was obtained electronically from GC/MS response without the use of an internal standard or correction factors; and *m/z* of individual compounds were evaluate based on previous study [25].

Larvae rearing and larvicidal assay

A. aegypti eggs were reared in Laboratory of Faculty of Public Health, Western University, Kanchanaburi, Thailand. The egg rafts were kept in the basin containing tap water that served as the culture medium at 28±2 °C and a photoperiod of 12-h light followed by 12-h dark (12L:12D). Appropriate amount of biscuit powder were added to enhance the growth of the larvae. The third and fourth instar larvae were used in the study. The late third or early fourth instar larvae were used for bioassay test following the WHO standard larvicidal bioassay method. The procedures was followed briefly, 25 larvae in 249 ml of declorinated-tap water with 1 ml of each concentration of larvicidal solution was tested and repeated at least 4 times. Mortality counts were made after 24 hours. The results were analysed to obtain the LC_{50} by probity analysis using a computer program. 1% (w/v) Temephos (Abate, Cyanamid, USA) was used for positive control [26, 27].

Results and Discussion

The concentration of stock and diluted larvicidal solutions were represent as total phenolic content (mg of gallic acid equivalent/ml); and larva mortality of each solution was shown in Table 1. Chromatographic analysis of solution was contained five alkyl phenols, which included saturated cardol, monounsaturated cardol, anacardic acid, diunsaturated cardanol and monounsaturated cardanol (Fig. 1). Anacardic acid was major active compound, which was correlated to mass spectra (Fig. 2). This finding was corresponded to previous studies, which were applied on mosquito control with safe to mammals [20, 21]. Our result was less effective than previous studies; however, active alkyl phenols were still remained in appropriate amount. This larvicidal solution was simpler to prepare and cost effectiveness was considered by use of water as solvent and container, which may useful in community level especially in cashew cultivating area. Field application and public training on this study will be conduct on controlling of mosquito-borne diseases.

Table 1: Total phenolic content and activity from each dilution of larvicidal solution*

Larvicidal solution	Stock (10% W/V)	1:5	1:10	1:20	1:50	1:100
Total phenolic content (mg of GAE/ml)	180.1 ± 9.5	35.6 ± 0.5	15.1 ± 1.2	7.2 ± 2.0	4.0 ± 1.7	3.1 ± 0.4
Larva mortality (%)	100	100	92	65	51	30

* LC_{50} = 3.8 mg of GAE/ml; LC_{90} = 11.5 mg of GAE /ml; GAE = gallic acid equivalent; positive control = 1% temephos

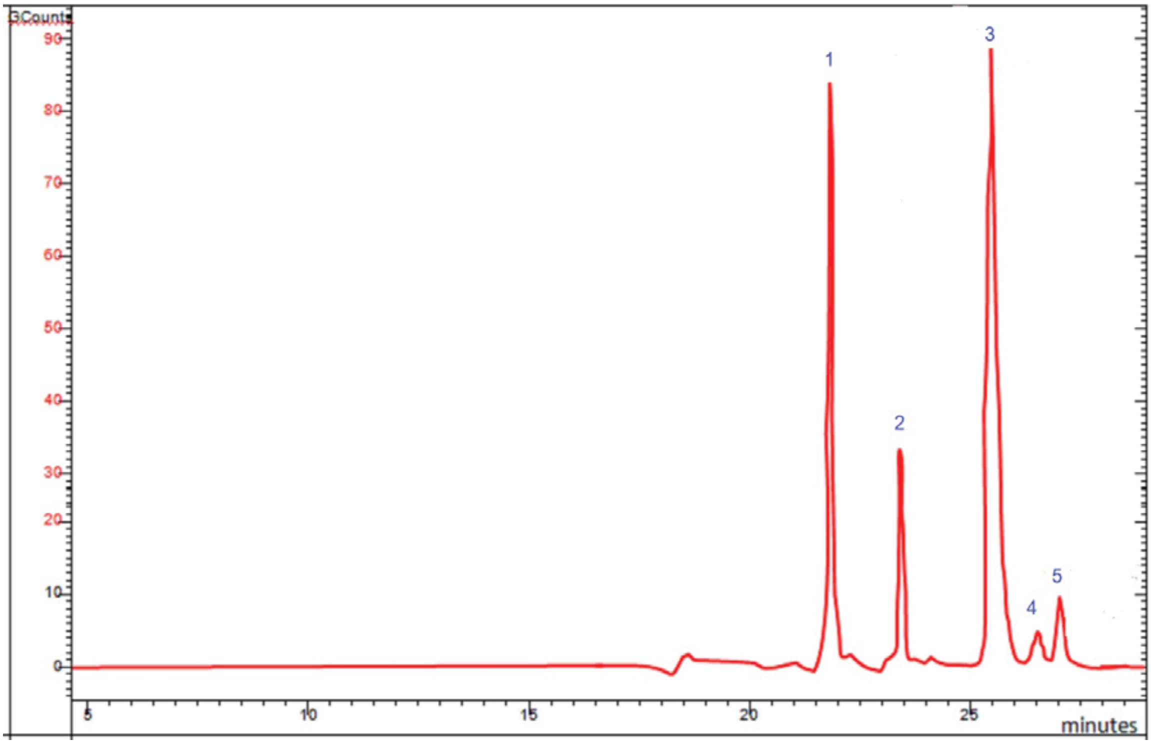

Figure 1: Chromatographic separation: (1) saturated cardol; monounsaturated cardol (2); anacardic acid (3); diunsaturated cardanol (4); monounsaturated cardanol (5)

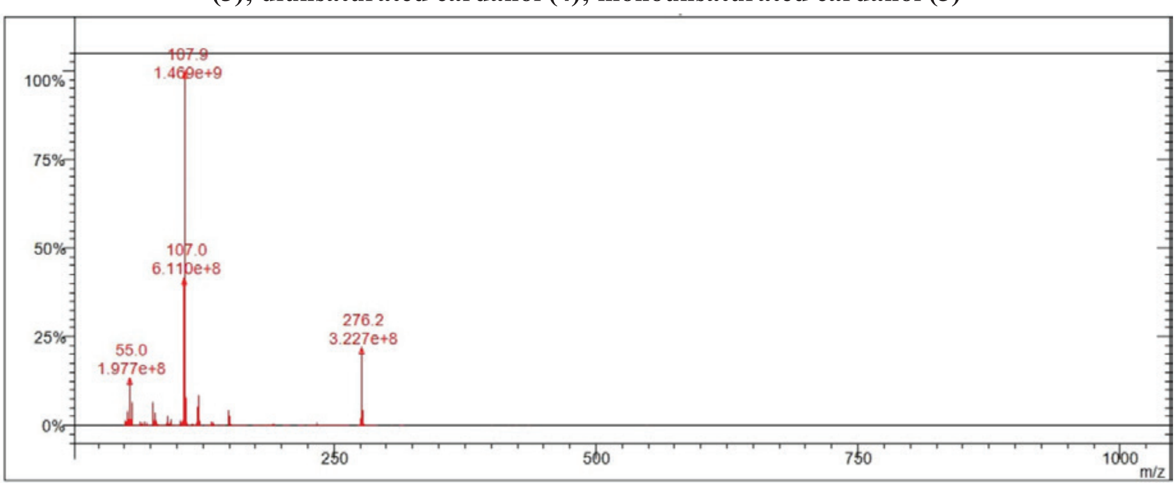

Figure 2: Mass spectra of anacardic acid as main constituent in larvicidal solution

Conclusion

Simple cashew nut shell preparation of larvicidal solution may useful on controlling mosquito-borne diseases, such as, dengue fever. Anacardic acid was active alkyl phenol, which was remained in preferable concentration.

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Conflicts of Interest: The authors confirm that there are no conflicts of interest.

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