Identification of Sugar-Apple Seeds (Annona squamosa L.) Extract as A Candidate Against The Aedes aegypti L. Mosquito Vector Control DBD

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Abstract: Aedes aegypti L. mosquitoes are the disease vectors of dengue hemorrhagie fever (DHF). This diseases is caused by dengue virus which is transmitted by Ae. Aegypti mosquito. The effort to control Ae. aegypti vector have been done in so many times, including chemical, physical, and biological control method. Extraction by kinetic maceration have been done with sugar-apple seeds (Annona squamosa L.) with the solvent of 70% of ethanol. Subsequently, the obtained extract is tested phytochemical screening along with the powder and larvicidal activity against Ae. aegypti. The results of phytochemical screening of the powder and 70% ethanol extract of sugar apple seeds have obtained the compound of saponin, triterpenoid and coumarin. Based on the activity test against the larva of Ae. aegypti from ethanol 70% extract of of sugar-apple seeds, show LC50 values is 97,462 ppm. These are indicate that the compound which is found in sugar-apple seeds have a potential as a larvicides.

Keyword: Aedes aegypti L., Annona squamosa L., sugar-apple seeds, larvasida.

INTRODUCTION

The existence of a mosquito that is close to human life pose a serious health problem, because mosquitoes act as vectors of some diseases with high rates of morbidity and mortality caused. Ae. L. aegypti mosquito is getting attention because it is one of the vectors penyakit(1). Ae. aegypti is diurnal, or active during the morning and afternoon. This mosquito-colored striped black and white, would rather be in a protected area as home. Transmission of the disease carried by female mosquitoes, because only the female mosquito sucks blood. This was done to obtain the protein to produce eggs. The virus is transmitted by Ae. aegypti is the dengue virus, the virus that causes dengue hemorrhagic fever (DHF). Ae. aegypti mosquitoes carrying dengue virus obtained from infected individuals and multiply in the body and salivary glands of female mosquitoes.

Dengue disease not only in children but in all ages. DBD becoming known in Indonesia in 1968 in Surabaya and Jakarta, and then continue to expand as the spread of dengue endemic area. The number of cases of dengue and widely spread is increasing along with the increasing mobility and population density. There are 150,000 cases of dengue in 2007 and continued to increase until 2010. In addition, WHO reported more than 35% of the population living in urban areas affected by the disease. Until now there is no specific vaccine to treat dengue fever, and the only control vector disease control(2,3,4,5).

Controlling the mosquito vector can be done with the use of biological larvicides to control mosquito larvae stage. Biological larvicides are safer for humans and the environment and poses no resistance penyemaran target organisms. One of the plants that can be used as larvicides are sugar apple (Annona squamosa L.) of the family Annonaceae. The plant parts are potentially as larvicides are seed (semen)(6).

Previous research reported the main active compound of sugar apple seed is annonain and squamocin belonging asetogenin compound. Squamocin annonain compound of the family Annonaceae and is reported to have toxicity properties cukupefektif against Chrysomya bezziana fly larvae and insects of the order Diptera (Ae.aegypti L.) which are cytotoxic, and neurotoxic. Asetogenin...
compounds can inhibit the action of the enzyme NADH in the mitochondria, causing the death of larvae, as well as toxic contact and stomach poison to insects (7,8,9).

Based on these results, the authors want to identify the qualitative content of secondary metabolites and activity test seed extract sugar apple (Annona squamosa L.) against Ae. aegypti. Larvicidal activity test seed extract srikaya with some variation of the concentration method Efficacy Testing Standards Pestisida Household and vector control to determine the optimal concentration of sugar apple seed extract used 20 third instar larvae of Ae. aegypti. The data seen LC50 values were obtained by probit analysis.

The research objective is to identify chemical compounds / secondary metabolites qualitatively and larvicidal activity of seed extract sugar apple (Annona squamosa L.) against larvae of Aedes aegypti L. Results from this study are expected seed extract sugar apple (Annona squamosa L.) can be used as a biological larvicides dengue vector control with chemical compounds / secondary metabolites contained in sugar-apple seeds.

MATERIAL AND METHODS

MATERIAL. Sugar-Apple seeds (Annona squamosa L.) were obtained from Balitro (Research Institute for Spices and Medicinal Plants) Cimanggis, Bogor. Determination at Bogoriense Herbarium, Research Center, LIPI Cibinong, Bogor.

Extraction. Sugar-Appleseeds (Annona muricata L.) which have been dried in the sun was directly crushed and blended into a fine powder. Powdered crude drug was extracted by maceration kinetic in stages using different solvent polarity is n-hexane, ethyl acetate, and ethanol 70% at room temperature until the extracted perfectly, then filtered with cotton and proceed with filter paper, pulp, and each extract n-hexan, ethyl acetate, and ethanol is 70% separated. Each extract was concentrated by vacuum rotary evaporator at a temperature of 45°C to obtain a viscous extract n-hexane, ethyl acetate and ethanol 70%.

Identification with phytochemical screening. Phytochemical screening performed on pollen and seed extract of soursop with Farnsworth method in Biological and phytochemical screening of Plant seed sirsakdilakukan to identify the qualitative content of secondary metabolites in seed soursop.

Flavonoids. 2 grams of powder simpisia or 0.15g of extract ethanol 70% boil with 100 ml of hot water for 5 minutes, then filtered with filter paper, 5 mL filtrate of extract solution coupled with a bit of powdered zinc or magnesium and 1 mL of 2 N HCl and 5 mL amyl alcohol. Flavonoids compounds would pose orange to red (12).

Saponins. Entering 10 ml sample into a test tube and shake for 30 seconds and observe what happens. If the foam is formed solid (not lost for 30 seconds) the identification showed the presence of saponin (12).

Coumarin. 2.12 grams of powder simpisia or 0.15g of extract ethanol 70% included in the test tube and add 10 mL of chloroform, heated 20 minutes on waterbath is then cooled. After it is filtered with filter paper, the filtrate waterbath until dry. The residue was added 10 mL of hot water, then cooled and put into a test tube, add 0.5 mL of 10 % ammonia solution and then observed under UV light at a wavelength of 365 nm (blue or green fluorescence showed the presence of cumarin (12).

Volatile oil. 2 of powder simplicia and 0.67 g extract put into a test tube, then added 10 mL of petroleum ether, at the mouth of the tube fitted with a mouthpiece that has given cotton that has been moistened with water, then heated above waterbath10 minutes after the cold water and filtered with a filter paper. The Obtained filtrate is evaporated in the vaporizer cup, the residue is dissolved in 5 mL ethanol and then filtered with filter paper. If residues smelling aromatic indicate a of compounds volatile oils (12).

Kuinon. 5 ml of solution experiments inserted into a test tube, add a few drops of 1 N sodium hydroxide solution. Occurs in red indicate a compounds of quinine (12).

Steroids/Triterpenes. 1.10 grams of powder or sugar-apple seed extract: 0.67 g of ethanol extract 70% extract, macerated with 20 mL ether for 2 hours, then filtrated the solution, and A total of 5 mL of the extract solution evaporated to dryness, then added with a reagent Lieberman- Burchard. green - red color arising indicates compounds terpenoids or steroids (12).

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**Tannin.** 2 grams of powder simplisia or 0.15g of extract ethanol 70% added 100 mL of water, boil for 15 Minutes, cooled and filtered. divided to each 5 mL filtrate (reaction tubes): Added a few drops of solution of iron (III) chloride 1 %, Changes blue or blackish green and Added a few drops of 1 % solution of gelatin to form white precipitate indicates the compounds of tannins . To 5 mL Second filtrate was added 15 mL reagent Stiasny (formaldehyde 30% - hydrochloric acid  = 2 : 1), the precipitate formed pink color indicates the presence of tannins katekuat . Subsequently the precipitate is filtered, the filtrate who saturated with sodium acetate powder, add a few drops of solution of iron (III) chloride 1 %, occurred in blue ink Showed the presence of tannins galat (12).

**Alkaloids.** 2.12 grams of powder simplisia or 0.15g of extract ethanol 70% is inserted in a porcelain bowl and then add 5 mL of ammonia 30% crushed and then added 20 mL chloroform and crushed again, then filtered. The filtrate obtained was added HCl 1 N as much as 5 ml and then separated into 2 sections namely A and B. The filtrate A coupled with Mayer reagent, filtrate B coupled with Dragendorff reagent. With reagent Meyer gives a white precipitate, and Dragendorff reagent give an red brick precipitate (12).

**Larvical activity test. Larvae Maintenance.** Mosquito eggs incubated in a plastic container (tray) measuring 20 x 15 x 10 cm³ yang containing distilled water. The eggs will hatch within 24 hours of becoming the first instar larvae, then the 2nd day will have become instar II stage of development, at this stage larvae fed chicken liver, then after 1-2 days will be changed again to the third instar.

![Figure 1. Larvae rearing.](image)

**Implementation of Experimental Test Larvicidal Activity.** Larvicidal activity test was conducted using “Pesticide Efficacy Testing Standards Household and Vector Control”. Carefully weigh approximately 100 mg extract and then dissolved in 100 mL of of solvent. This solution is a mother liquor (1000 ppm). The mother liquor 18.750 ml pipette; 12.500 mL; 6.250 mL; 3.125 mL; 1.250 mL respectively inserted into plastic cups that have ditara 25 mL to obtain a concentration of 750 ppm, 500 ppm, 250 ppm, 125 ppm, 50 ppm, then evaporated completely. Each concentration was made in 3 plastic cups (triplo), then into individual plastic cups partially added to 25 mL of distilled water homogenkan, and included 20 third instar larvae of *Ae. aegypti*. Observations were made after
24 hours of exposure to the test solution and counted the number of larvae were dead and stated in the presentation of death.

Negative controls only solvent without the extract, in the same way. Positive controls carried out on Temephos 1 ppm.

**Data Processing Methods.** Test data processing is done systematically using probit analysis method. Probit analysis is used to determine the percentage of larval mortality LC50 of Ae. aegypti L. uses Epa Probit Analysis Program Used For Calculating LC/EC Values Version 1.5. In Epa Probit Analysis Program Used For Calculating LC/EC Values Version 1.5, the data entered is the relationship with the concentration of the value of the average percentage mortality of larvae of Ae. aegypti.

**RESULT AND DISCUSSION**

**Making the Ethanol 70% Extract.** Sugar apple seed is extracted by cold by maceration kinetic (use maserator). The solvent used for extraction is 70% ethanol. Extracts were obtained in the form of extracts viscous blackish brown. 36.9 g of 506.2 g of crude drug powder sugar apple seeds with 7.29% yield.

**Phytochemical screening.** The phytochemical screening via Farnsworth method was conducted using powder of simplicia and Soursop seeds and sugar-apple seeds extract. In powder and extract having metabolite compound such as saponin, triterpenoid, and cumarin. The result of phytochemical test is shown in Table 1.

<table>
<thead>
<tr>
<th>No</th>
<th>Secondary Metabolites</th>
<th>Simplicia powder</th>
<th>Ethanol 70% Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Kuinon</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Tannin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Steroids / triterpenoids</td>
<td>- / +</td>
<td>- / +</td>
</tr>
<tr>
<td>7</td>
<td>Volatile oil</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Coumarin</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Notes: + = giving positive reaction  
− = giving negative reaction

In Table 1 it can be seen that the results of the qualitative identification of secondary metabolite content of the seed powder soursop (Annona muricata L.) by means of screening phytochemical compounds derived class of saponins, triterpenoids, and coumarin. While the 70% ethanol extract derived class compound saponin, triterpenoids, and coumarin.

**Larvicidal Activity Test.** Larvicidal activity test 70% ethanol extract sugar apple seeds is done by the method of Pesticide Efficacy Testing Standards Household and Vector Control for mosquito larvae Ae.aegypti L. Larvae used test is the third instar larvae of mosquitoes Ae.aegypti because it has a fairly good resistance against external environment and durability stronger mechanically when the transfer of the larvae, and have a long time to turn into adult mosquitoes. Test solution at a concentration of 50, 125, 250, 500 and 750 ppm generated triplo, then put 20 third instar larvae of Ae. aegypti L. and counted the number of larvae mortality after 24 hours of observation. Negative controls only the solvent used and Temephos (larvicidal commonly used) as a positive control.
Table 2. The average percentage mortality of larvae of *Ae.aegypti* L., after exposure to 70% ethanol extract sugar-apple seeds in the 24-hour observation.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Sugar-apple Seeds Ethanol 70% Extract</th>
<th>% Kematian Negative (Solvent)</th>
<th>Control Positive (Temephos 1 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>750</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>500</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>250</td>
<td>98,35</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>125</td>
<td>48,35</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>18,35</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>LC50 (ppm)</td>
<td><strong>97,462</strong></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Linear regression</td>
<td>a = -103,6709</td>
<td>b = 75,0693</td>
<td>r = 0,9372</td>
</tr>
</tbody>
</table>

The test results larvicidal activity against sugar-apple seed LC50 values obtained from the ethanol 70% extract is 97.462 ppm. From the LC50 values of 97.462 ppm can be concluded that the sugar apple seed extract has larvicidal activity against *Ae. aegypti*. This can be caused by chemical compounds / secondary metabolites contained in the sugar-apple seed sapinon, coumarin suspected triterpenoid and potentially as larvicidal. Saponins allegedly able to diffuse into the cuticle layer of larvae that can damage cell membranes and toxic compounds can be entered and off the larvae. Saponins have a bitter taste and sharp and can cause irritation of the stomach. Larvae digestive tract, particularly the midgut (midgut) is the major site of absorption of nutrients and digestive enzymes seksresi. Saponin absorption into the intestine larvae can inhibit the action of digestive enzymes and cause damage to the cells in the channel pencernaan. Saponin also thought to be as antifeedant (antimakan) on the larvae so that the larvae loss of appetite, this led to the loss of energy and development of larvae will be hampered even can cause death 24. In addition, coumarin is also reported as larvicides because potentially able to change the ability of detoxification with reversible and irreversible inhibits the enzyme cytochrome P450. Dari third ability of secondary metabolites in seed srikaya concluded that sugar apple seeds potentially sebagail larvicides against mosquito larvae *Ae. aegypti* L.

Mortality of larvae on seed extract sugar apple (*Annonasquamosa* L.) allegedly also because of the effects of the component compounds acetogenindansquamosin toxic contact. Where after the larvae exposed to the extract, the compound into the body of *Ae. aegypti* through physical contact and the case of death of the larva. Prijono (1994) in Wardhana *et al.* (2005) states that the absorption of toxic insecticides contact occurs largely in the cuticle. Active compounds will penetrate into the insect's body through the part that is covered by a thin cuticle, such as membrane between segments. Stomach poison ability of the compound to absorb seyawaasetogenin work on sugar apple seed extract into the wall fosfolirasi larvae and able to inhibit oxidative chain so that the cell respiration is inhibited activity of *Ae.aegypti* because of breathing stopped. *Squamosin* compounds in seeds srikaya allegedly able to diffuse from the thin cuticle layer to spread throughout the body *Ae. aegypti* through hemolimfa flow.

Mortaitas larvae of *Ae.aegypti* showed signs as follows: larvae do not move when touched, bodies pale white larvae, elongated body shape or rigid. The color can be seen more clearly with the aid of a stereo microscope and optilab. Differences larvae of *Ae.aegypti* normal and who have died can be seen in Figure 2.
Figure 2. Third instar larvae of *Ae. aegypti* normal (A); and third instar larvae of *Ae. aegypti* die (B).

Figure 3. Graph average percentage mortality soursop seed extract on a 24 hour observation (x-axis: % average mortality of larvae and the y-axis: concentration sugar apple seeds extract (ppm)).

From Figure 3 shows that the higher the concentration of sugar apple seed extract, the higher the death rate of *Ae. aegypti* L. Ethanol 70% and distilled water as a negative control test the same activity against larvae of *Ae. aegypti*, and the results obtained all the larvae do not occur death. This indicates that the solvent does not affect the mortality of larvae. *Temephos* as a positive control, in which the larvicidal activity at a concentration of 1 ppm trials have demonstrated 100% mortality against larvae of *Ae. aegypti* L.

**CONCLUSION**

Based on the results of phytochemical screening of the seeds sugar apple (*Annonasquamosa* L.) obtained secondary metabolites content of saponins, triterpenoids, and coumarins. Test results with the larvicidal activity and data analysis has been done, it can be concluded that the 70% ethanol extract of the seeds have activity sugar apple seeds against larvae of *Ae. aegypti* L. with LC$_{50}$ values of 97, 462 ppm.

Suggestion. Ethanol 70% extract of the seeds sugar apple (*Annonasquamosa* L.) has a good chance to be used as biological insecticides to control mosquito larvae that are environmentally friendly.

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