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SAM (Saponin Anti Mosquitoes) - Biolarvicide activity of Rambutan (*Nepheliumlappaceum*) towards Mosquito Larvae

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ABSTRACT

SAM is an innovation of the saponin as a larvicide bioactive from rambutan (Nepheliumlappaceum) seeds and rinds against the larvae of mosquito, the transmitters of several public health problems. SAM contains active ingredient saponin to control mosquito larvae in aquatic habitat. The seeds and the rinds of rambutan, which are normally discarded, were found to contain saponin. The purpose of this project was to optimize the utilization of natural saponin from rambutan seeds and rinds in mosquito control to create biolarvicide as an alternative to the use of Temephos (trade name: Abate) as mosquito larvicides. Natural saponin offer an advantage over synthetic larvicide as this is less prone to development of resistance and easily biodegradable. Besides, this project aims to provide a cheap and efficient alternative of mosquito control system to the mass produced by chemical substances used today. Experiments to extract saponin from the seeds and rinds of rambutan as well as to test the effect of saponin extract on mosquito larvae were conducted to produce the best larvicide product. The best concentration of saponin on larvae biological activity was also determined. The contributions of this project are considerably great as many people out there are suffering from dengue. SAM is relatively low-cost and affordable to wide range of poor communities affected by these diseases. Hence, the use of saponin as a biolarvicide will definitely benefit all of mankind due to its efficiency, while still maintaining its eco-friendly status.

Keywords:SAM, Saponin, rambutan, larvicide, Nepheliumlappaceum

INTRODUCTION

The Saponin Anti Mosquitoes (SAM) is an innovation of the saponin as a larvicide bioactive from rambutan *(Nepheliumlappaceum*) seeds and rinds against the larvae of mosquito. Mosquito is the principal vector for transmitting several tropical fevers such as dengue fever, malaria, filariasis and Japanese encephalitis. This study was focused mainly on *Aedes aegypti* that transfers dengue virus. The most widely used mosquito control method is the chemical-based control. The reason for this selection is the prompt results of this control. However, chemical control using synthetic larvicides actually causes adverse side effects, such as the mosquitoes should become resistant, human and livestock poisoning, as well as environmental pollution (Ray Sahelian, 2005). The development of new larvicides that are more environment friendly and do not pose hazard needs to be done. The use of biolarvicide looks promising. Therefore, our research focuses on insect control using saponins which is naturally derived from plants. SAM is a biological larvicide which is derived from plant material containing chemical (bioactive) that are toxic to insects but are easily biodegradable in nature. So, it will not pollute the environment and relatively safe for human (Manafet. *al*., 2013). Saponins have historically been understood to be plant-derived, but they have also been isolated from marine organisms. Saponins are indeed found in many plants. Saponins are high molecular weight glycosylated plant secondary metabolites, consisting of a sugar moiety linked to a triterpene or steroid aglycone (Chai *et. al*., 2018). Detergent properties are the typical characteristics of saponins. They produce stable foam when dissolved in water, which is why some saponin-containing plants have been used as soaps for hundreds of years (Palanisamyet. *al*., 2008). In Africa, plant extracts containing a high percentage of saponin are commonly used to treat water supplies and wells contaminated with disease vectors. After

treatment, the water is safe for human drinking. This proves that saponin is really a good biolarvicide to fight mosquitoes. It is soluble in both organic soluble and water. Furthermore, it doesn't cause any pollution and it's less toxic than any other chemical method of vector control (Solís-Fuentes *et. al.*, 2010).Our product, SAM is a good option to replace current synthetic larvicide. This is simply because rambutan is easily found in a tropical country like Malaysia. The aim of this study was to determine Biolarvicide activity of rambutan towards Mosquito Larvae. The seeds and the rinds of rambutan are normally discarded. Therefore, the purpose of this project was to optimize the utilization of natural saponin from rambutan seeds and rinds in mosquito control to create biolarvicide as an alternative to the use of Temephos as mosquito larvicides. The qualities of SAM which creating a healthier living environment, non-polluting, environment friendly and cheap, proves that it is able to be commercialized. Therefore, in the near future, we hope that by the production and commercialization of this project on a larger scale, this eco-friendly mosquito larvicide will achieve great success in the international market.

Materials And Methods

Extraction of Saponin

Firstly, the rambutan seeds and rinds were air dried and cut into smaller pieces. Dried rambutan seeds and rinds were ground with blender to make 1 kg of powdered rambutan seeds and rinds. Then the samples were put in plastic container and were soaked it in distilled water for 24 hours. After 24 hours, the soaked samples were transferred into 2000 ml beaker. The samples were soaked in 2L of 96% methanol and stirred well and were left for 48 hours. After 48 hours, the samples were rinsed for 2 times with ethanol again. Then, it was filtered and the filtrate was collected. All the collected liquid material (ethanol + extract) were put in a capped bottle (can even use the ethanol bottle).

Collecting the extract

The wet sample were put in rotary evaporator. This is the cold extraction method. Methanol was used if it sticks to the wall of flask of rotary evaporator. Extract was collected in vial. The weight of the extract was measured.

The best concentration of saponin on larvicidal activity of mosquito

Total of 8 cups were used and were labelled with each different concentration respectively. (4 cups for sample, 4 cups for control). Each cup was filled with 200 ml of distilled water. The extract was added to labeled cup with different concentrations accordingly: a) 1%, 2.5 ml sample b) 0.5%, 1.25 ml sample, c) 0.25%, 0.63 ml sample, d) 0.125%, 0.32 ml sample.For control, methanol was added accordingly to the labeled cup with different concentrations. The volume was the same like sample. Cups were left in the laboratory for 15-30 minutes under laboratory conditions at 25-30^0 C and 80-90% relative humidity. Twenty-five *Aedes aegypti* mosquito larva were added into each cups. The cups were filled with distilled water until it reaches 250 ml. The number of death mosquito larvae was observed and recorded after 15 minutes, 30 minutes, 1 hour, 2 hours and 24 hours' days. Steps 1-8 were repeated for 2 times to get the best result.

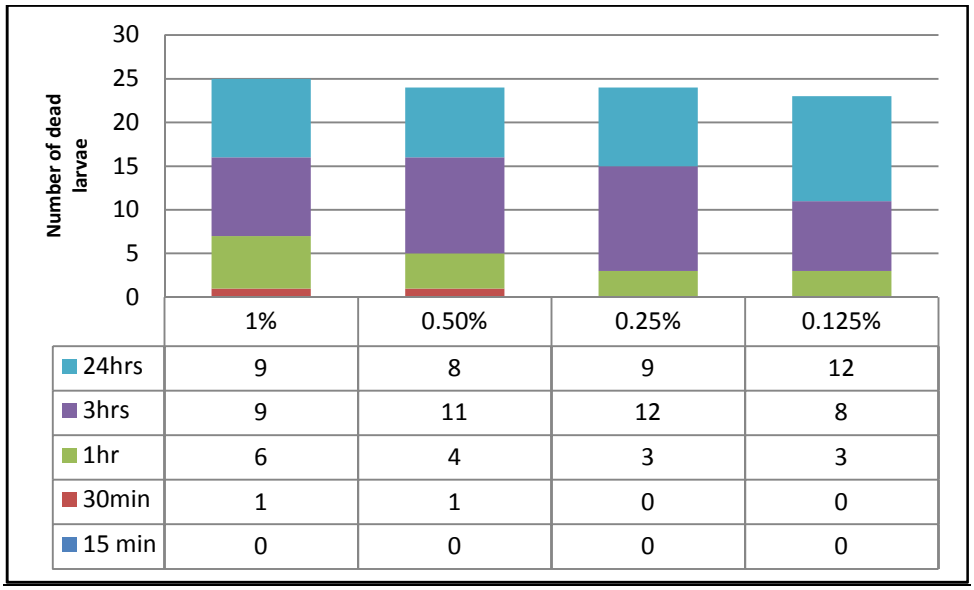
Results and Discussion

Tables 1: Viability of larva in the samples.

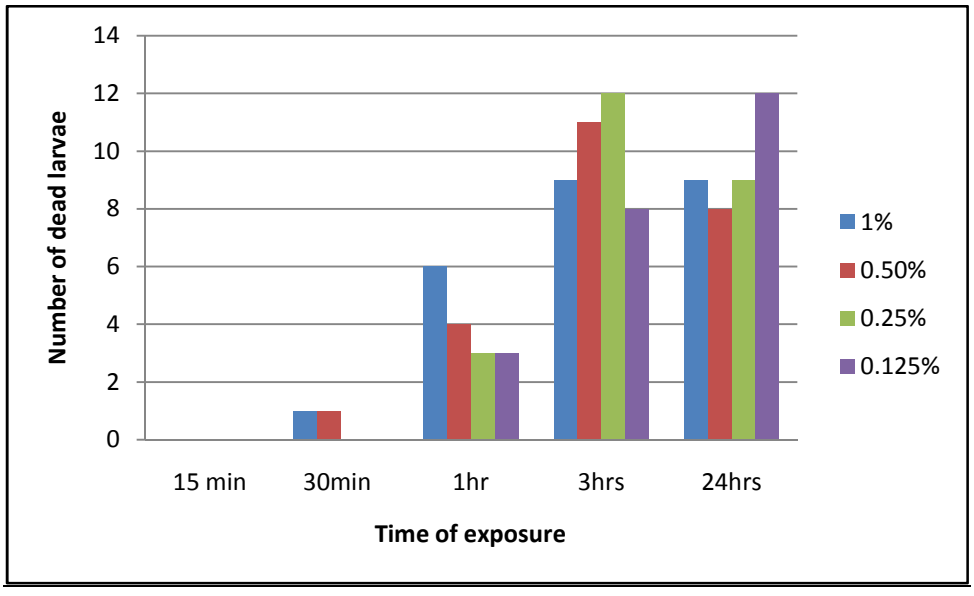
Amount of extract	Sample (mean number of larvae dead after time, t)					Total larvae alive in sample after 24 hours
	15 min	30 min	1 hour	3 hours	24 hours	
1% (2.5 ml)	0	1	6	9	9	0
0.5% (1.25 ml)	0	1	4	11	8	1
0.25% (0.63 ml)	0	0	3	12	9	1
0.125% (0.32 ml)	0	0	3	8	12	2

Tables 2: Viability of larva in the control.

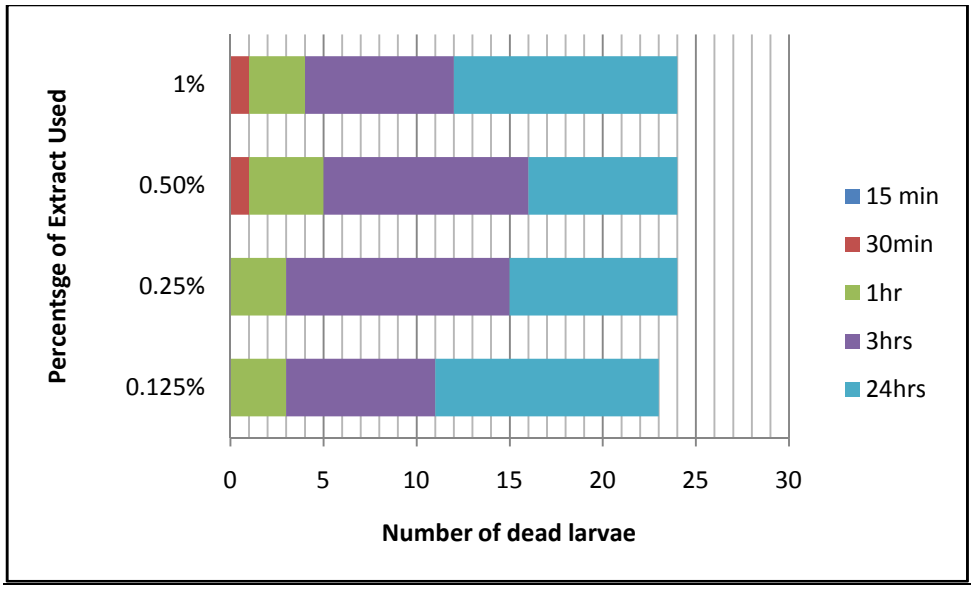
Amount of extract	Control (mean number of larvae dead after time, t)					Total larvae alive in sample after 24 hours
	15 min	30 min	1 hour	3 hours	24 hours	
1% (2.5 ml)	0	0	0	0	0	25
0.5% (1.25 ml)	0	0	0	0	0	25
0.25% (0.63 ml)	0	0	0	0	0	25
0.125% (0.32 ml)	0	0	0	0	1	24



Graph 1: Number of larvae dead versus the percentage of Saponin extract used



Graph 2: Number of larvae dead versus time exposure to different percentage of Saponin extract used



Graph 3: Number of larvae dead versus the percentage of Saponin extract used

Interpretation of Data.

Concentration 1% (25ml)

All the larvae died at the end of 24 hours

Concentration 0.5% (1.25ml)

73 out of 75 of the larvae dead after 24 hours and this shows positive effect of saponin even at half of the concentration.For the control only 1 larva is dead.

Concentration 0.25% (0.63ml)

For this concentration only 1 larva is noted alive after 24 hours.The control only shows 1 dead larva same as the first and second concentrations.

Concentration 0.125% (0.32ml)

At the lowest concentration for the sample there is increase in the number of alive larva compared to other concentrations. Thus there is correlation that low concentration of the saponin will decrease the effectiveness of larviciding effect.There are one dead larvae of the control and it still does not exceed 10% of the number of larvae. So, the result is still valid. The larvae may be dead because of the handling.From the graphs (Graph 1, 2 and 3) we can see that, saponin is effective and has larvicidal activity against *Aedes aegypti* mosquito larvae.

Discussion

Rambutan is known to contain Saponin and is easily found in Malaysia. Saponin are mostly derived from plant source. Plant that are being used can either be prepared fresh or dried. In this experiment, we prepare it dried since the measurement of weight will not include the watery content of the seeds and rinds of rambutan. The cold extraction method is utilised in which the dry sample being soaked in the methanol before evaporation process. To improve the extraction method, other solvents can also be used like ethanol, chloroform, hexane, acetone etc. (Edenbotanicals, 2014). This is because different solvents will attract different molecules from the sample based on polarity. Aqueous solvent is not preferable due to high chance of contamination. (H. LumaKhairy*et. al*., 2018). In this experiment, a very basic extraction method of just using one type of solvent was used and the content of extract that we get may not be all pure saponin. There will be presence of other bioactive chemical constituents that are also being attracted by the solvent like phenols, tannins, flavonoids, terpenoids, and glycosides. There are quite a lot of phytochemical analysis for screening and identification of each bioactive component (Busakorn et. al., 2017). The one that we use for testing the presence of saponin is foam test in which positivity is indicated by formation of emulsion. Their ability to foam is caused by the combination of the non-polar sapogenin and the water soluble side chain. Another test for saponin is froth test. If the honeycomb froth is greater than 2 cm, height from the surface of the liquid persists after 10 minutes, the sample is considered positive for saponin. For other bioactive chemical constituents, they have their own specific tests like Salkowski's test for terpenoids and Shinoda test for flavonoids. Phenols and tannins are tested with chloride while glycosides with the same reagent as well as glacial acetic acid and sulphuric acid. If further experiments are done, we can improve it by trying to extract only saponin to ensure that larvicidal activity is contributed by saponin only and not by other components. This can be done by knowing what classes saponin belongs to and what solvents are appropriate for extraction process. If not possible, we can also try to get the percentage of larvicidal activity contributed by saponin as well as other bioactive compounds. For evaporation process, the methanol is

removed from the extract by using the rotary evaporator. The extract can be either in solid, jelly-like or liquid form. The most preferable is solid, jelly-like as to ensure there is no methanol (or any solvent used) being left in the extract. To use this extract as to prepare for stock solution used in the next experiment, we can use two different approach. If it is in solid form, use weight / volume. If it is in solution form, use volume / volume. This is for preparing the different concentrations of sample by applying serial dilution method. There are few precautions needed to be taken during the experiment, like protection from sunlight which can affect the bioactive chemical constituents and while using the rotary evaporator, the extract should be collected properly as to avoid any methanol left in the extract. To correct this, cover the sample container with aluminium foil to avoid sunlight. For the rotary evaporator, use some methanol to collect the stuck extract at the flask of rotary evaporator. When using the vial, cover the vial opening with aluminium foil and make some holes on it. Place the vial in fume chamber to let the remaining methanol evaporate. The calculation of weight and volume of extract must be done properly for preparation of the next experiment.From this study, it is determined that the best concentration for larvicidal activity of *Aedes aegypti* mosquitoes are 1% since all larvae died at the end of 24 hours. When the concentrations of sample used decreased, the effectiveness of larvicidal activity also decreased. While comparing the different concentrations, 1% concentration of Saponin showed better result than the lower concentrations with dead larvae seen as early as at the end of 30 minutes. However, for commercial use, using 1% of concentration would be costly even though it is faster in giving larvicidal effect. The best concentration for commercial purpose would be 0.5% as it showed same result as in 1% of concentration.

Conclusion

The extraction experiment was successful and the foam test confirmed the presence of Saponin. The presence of saponin extract will cause death of mosquito larvae. The best concentration of saponin for larvicidal activity of mosquito is 1%.

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