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Survival Analysis Larvacide of Fractionation Plant Seeds Soursop (Annona muricata L) Against Larvae of Aedes Aegypti Vector Dengue Haemorrhagic Fever

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Abstract
Survival Analysis of a Natural Insecticide Seed Fractionation Soursop In larvae of Aedes aegypti vector of Dengue Haemorrhagic Fever in Manado City has done. This study aims to determine the most effective concentration of seed fractionation soursop (*A. muricata)* to kill mosquito larvae *A. aegypti*. The method used in extracting waste is soursop seeds by maceration with ethanol. Technical Data analysis was performed to find the concentration of deaths that affect the mortality of larvae of dengue mosquito *A. aegypti* instar III / IV with survival analysis methods. The test results show the MTTF or Mean Time To Failure (survival value) mosquito is the provision of 183 476 ppm concentration. Thus, the concentration of 183 476 ppm is a concentration of the most effective amount to kill mosquito larvae. According to toxicity criteria based *Australian Petroleum Energy Association*, 183 476 concentration of n-hexane extract fraction or soursop seeds (Mean (MTTF) = 183 476) at 24 hours of observation included in the criteria of Medium Toxicity (*Moderately Toxic*).
Keywords: Survival analysis, Natural Insecticides, Soursop Seeds, *Aedes aegypti*.

1. Introduction

Mosquitoes, including one of the types of insects that gained great attention in the human health, because it has potential as a vector in the transmission of a disease (Stocker, *et al*. 2005).

Dengue disease is an infectious disease caused by the dengue virus and transmitted by the mosquito *Aedes aegypti* and *Aedes albopictus* which is characterized by sudden fever 2 to 7 days with no obvious cause, weak or lethargic, anxiety, heartburn, accompanied by signs of bleeding in the skin a bleeding spots (petechie), hematoma (echymosis), or rash (purpura), occasional nosebleeds, dysentery, vomiting of blood, decreased consciousness or shock (shock) (Indrawan, 2001).

Until now still not found drugs and an effective vaccine for dengue fever. Mosquito nest eradication (PSN) is a vector control method as one of the efforts made to prevent the transmission of dengue disease PSN campaign has encouraged the government in this case the Ministry of Health with the motto 3M, which drain water regularly shelters, shelters closed water and bury the used goods that can become mosquito breeding (Departmen Kesehatan RI, 2008).

Manado city is endemic Dengue Hemorrhagic Fever (DHF) in the year 2010 where an increase in cases. Data dengue hemorrhagic fever (DHF) in Manado City in 2010 as many as 998 cases with 25 deaths (CFR = 2.5%). When compared with January of s / d April 2010, ie as many as 832 cases, the downward trend in the case of very sharp. However, the pattern of disease is subject to change and may have increased unexpectedly (Surat Edaran Walikota Manado, 2011).

The most rapid method of control to break the cycle of transmission is the use of larvicides and insecticides synthetic, but synthetic chemical compounds can cause resistant properties in mosquitoes. Some resistant cases were also reported in the world, such as mosquito resistance *A. aegypti* to organophosphat in Brazil (Araujo, *et al.,* 2006).

Control is a natural way to use the plant as biopesticides, as an environmentally friendly alternative to control, easy to apply and is not harmful to natural enemies and other beneficial insects. Insecticides of this plant is more selective and safer, as easily biodegradable (degraded) in nature so it does not leave a residue on the soil, water and air (Adebowale and Adedire, 2006).

One plant in the *Annonaceae* family who has studied the content of the active compound is *Annona muricata* Linn locally known as Soursop. Other studies mention the plant family *Annonaceae asetogenin* contains many compounds that are suspected of larvicides, and the ingredients are well *asetogenin* as insecticides, acaricides, antiparasitic and bactericidal (Alali, *et al.,* 1999).

Based on the above background, it is necessary to investigate the mean life (optimal concentration) or known as MTTF Mean Time To Failure (MTTF) or the expected value where mosquito larvae mortality rate will be obtained with the use of natural insecticides soursop seeds using larvae of dengue mosquito *Aedes aegypti* as a bio-indicator.

2. Method

2.1 Tools and Material

2.1.1 Tools

The tools used in this study are; blender, analytical balance, beaker glass, separating funnel, erlenmeyer, micro pipettes, vacuum rotary evaporator, desiccator, pipette, test tube.

2.1.2 Material

The chemicals used in this study is ethanol, butanol, ethyl acetate, n-hexane, soursop seeds, mosquito larvae *A. aegypti.*

2.2 Work Procedures

This research was conducted in the Laboratory of the Department of Biology and Department of Chemistry, Faculty of Mathematics and Natural Sciences, State University of Manado. Materials used in this study is the fruit seeds are old soursop (*A. muricata)* waste collected from restaurants and eating houses side product soursop fruit juice in the city of Manado. Preparation of a study conducted among plant determination, collection materials, cleaning, drying material is dried by - aired (not in direct sun) and grinding to a powder using a blender.

Endosperm samples soursop 1.0 Kg seed powder was extracted by maceration using 10 liters of 70% ethanol for 24 technical hours, over and over until all components are extracted out. Ethanol extracts were then evaporated with a rotary vacuum evaporator to produce ethanol extract thick. Instar stage III / IV larvae of A. aegypti used in the study consideration at this stage of the instar mosquito organs are complete (the feathers) and the larvae is relatively stable against external influences (Dep. Kes. RI, 1987).

Subsequently condensed ethanol extract 50 g was taken and dissolved with 200 ml of ethanol-water with a ratio of 7: 3. Ethanol extract water and then partitioned with 200 ml n-hexane for 3 times until the whole thing apart to obtain the results of the ethanol-water and n-hexane fraction.

Ethanol-water extract was evaporated with a rotary vacuum evaporator to evaporate ethanol. Then extract the remaining water in the same manner successively partitioned with ethyl acetate and n-butanol. Each extract obtained by the fraction of n-hexane, ethyl acetate fraction and n-butanol fractions and tested its biological activity against mosquito larvae A. Aegypti.

Media mosquito larvae *A. aegypti* is made by filling plastic containers with water and lined the inner walls of the filter paper. Filter paper serves as a mosquito A. aegypti females attach their eggs. The eggs are attached to the filter paper and then dried at room temperature and stored in a sealed container. For hatching eggs, filter paper dipped in a plastic tray containing water and after 24 hours the eggs will hatch and grow into larvae instar I.

Instar larval stage of development I will have a larval instar II, III (4 days) and fourth instar (2 days). Every 2 days larvae fed pellet fish as 1-2 grams. Larval culture medium every 2 days once the water is replaced. Larvae will grow into pupae for 8 days. Larval instar III / IV were used in testing.

Ten vials prepared for testing, where for each sample takes nine vials and one vial as control. Extract as viscous weighed 0.04 g dissolved in 4 mL of ethanol. Solution pipetted as many as 10; 100; 500 and 1000 mL. Respectively inserted into the vial, the solvent was evaporated in a desiccator for 24 hours.

Prepared vial filled with 5 mL of water, 100 mL dimethylsulfoxide, 25 larvae mosquito A. aegypti. then add water until the extract solution volume to 10 mL in a concentration of 10; 100; 500: 1000 ppm. For controls, into a small bottle of water added 5 mL, 100 mL dimethylsulfoxide, 25 larvae mosquito A. aegypti then add water until the volume is 10 mL. Observations were made after 24 hours of the death of mosquito larvae (Suirta, *et al.,* 2007).

Data analysis was performed to determine the extent of the pattern (shape) of the mortality rates of *Aedes aegypti* larvae by administering increasing concentrations, performed using Survival Analysis.

3. Results and Discussion

3.1 Mortality patterns of Aedes aegypti Mosquito Larvae In Data Concentration and Fraction

The data in Table 1 presents the number of deaths and the mortality rate of larvae mosquito *A. aegepty* at four concentration levels ie 1000 ppm, 500 ppm, 100 ppm, and 10 ppm with a combination of three types of fractions, namely fractions of n-hexane, ethyl-acetate, and butanol. The data used is the death rate (mortality) in the form of a percentage score from 0% to 100%. States of the 0% 25 mosquitoes, no one died, while the 100% of the stated overall 25 dead mosquitoes.

Table 1
Description Average Value and Variance of Each Concentration

Extract	Concentration	Average	Variance
n-hexane	10 ppm	28.000	4.000
	100 ppm	66.67	6.11
	500 ppm	97.33	2.31
	1000 ppm	100.00	0.00
Ethyl-acetate	10 ppm	0.0	0.0
	100 ppm	16.0	4.0
	500 ppm	78.7	14.0
	1000 ppm	100.00	0.00
Butanol	10 ppm	0.00	0.00
	100 ppm	0.00	0.00
	500 ppm	9.33	6.11
	1000 ppm	94.67	4.62

Graphically presented as follows:

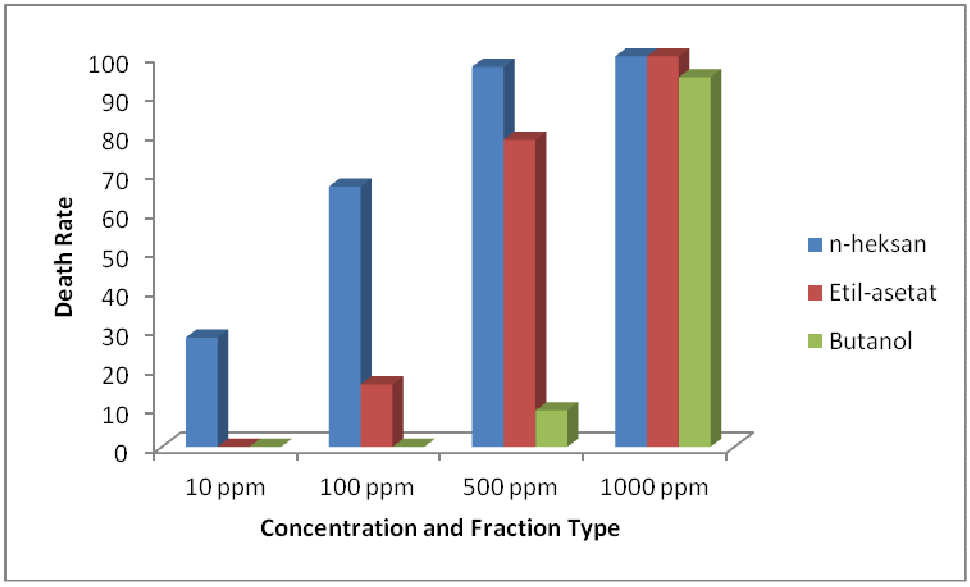

Figure 1
Description Average Value and Variance Each Concentration

From the table and figure above appears there are differences in the mortality rate of mosquitoes at different concentration levels from 10 ppm to 1000 ppm, and various types of fractions. Graphically seen that among the three types of fractions at a concentration of 1000 ppm have mosquito larvae mortality rate almost as highly, seen also in the n-hexane fraction had a better mortality rate at a concentration of 10 ppm, 100 ppm, and 500 ppm compared to the two other factions namely Ethyl-acetate and butanol. The data used was obtained from the overall 25 mosquitoes in each repetition (there are 3 replicates) to obtain 75 as a whole mosquitoes.

Here is presented a survival analysis model parameter estimation:

Butanol Fraction

Table 5.
Survival Analysis Model Parameter Estimation

Parameter Estimates Standard 95.0% Normal CI				
Parameter	Estimate	Error	Lower	Upper
Shape	8.00142	3.51436	3.38302	18.9247
Scale	1015.56	23.6229	970.295	1062.93

Ethyl Acetate Fraction

Table 6.
Survival Analysis Model Parameter Estimation

Parameter Estimates Standard 95.0% Normal CI				
Parameter	Estimate	Error	Lower	Upper
Shape	1.98465	0.185205	1.65292	2.38296
Scale	595.789	36.3559	528.629	671.482

n-Hexane Fraction

Table 7.
Survival Analysis Model Parameter Estimation

Parameter Estimates Standard 95.0% Normal CI				
Parameter	Estimate	Error	Lower	Upper
Shape	0.886843	0.0704492	0.758977	1.03625
Scale	172.954	24.2031	131.466	227.534

Density function of opportunities weibull distribution is;

$$f(y) = \frac{\lambda y^{\lambda-1}}{\theta^{\lambda}} \exp\left\{-\left(\frac{y}{\theta}\right)^{\lambda}\right\}$$

With y is the concentration (in ppm) which causes mosquito larvae mortality rate, λ is the parameter form (shape) and θ is the distribution scale parameter (scale).
Thus obtained survival analysis models for mosquito larvae mortality rate data as follows:

For Butanol Fraction, $$f(y) = \frac{8.01 y^{8.01-1}}{1015.56^{8.01}} \exp\left\{-\left(\frac{y}{1015.56}\right)^{8.01}\right\}$$

For Ethyl-asetate Fraction, $$f(y) = \frac{1.98 y^{1.98-1}}{595.78^{1.98}} \exp\left\{-\left(\frac{y}{595.78}\right)^{1.98}\right\}$$

For n-Hexane Fraction, $$f(y) = \frac{0.88 y^{0.88-1}}{172.95^{0.88}} \exp\left\{-\left(\frac{y}{172.95}\right)^{0.88}\right\}$$

In the graph, the curve survival analysis are presented as follows:

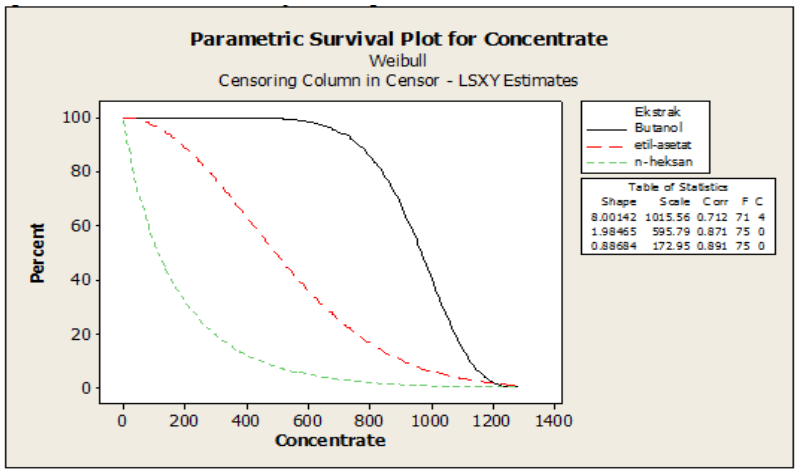

Figure 2
Curves Survival Analysis

Graphically, the curve shape if done numeracy as presented in the following table:

Tabel.8. Table of Percentiles

	Percentile		
Percent	**n-heksan**	**Etil-asetat**	**Butanol**
0.1	0.07	18.35	457.12
1	0.97	58.68	593.60
2	2.12	83.42	642.44
3	3.37	102.59	673.02
4	4.69	118.90	695.71
5	6.07	133.39	713.93
6	7.50	146.62	729.26
7	8.98	158.88	742.55
8	10.50	170.39	754.32
9	12.07	181.30	764.91
10	13.67	191.71	774.57
20	31.87	279.81	843.27
30	54.08	354.40	889.27
40	81.09	424.72	926.19
50	114.41	495.32	958.76
60	156.72	570.12	989.55
70	213.22	654.20	1020.63
80	295.78	757.23	1054.73
90	442.96	906.99	1098.39
91	465.88	927.67	1103.97
92	491.65	950.26	1109.96
93	521.06	975.25	1116.45
94	555.24	1003.34	1123.60
95	595.98	1035.59	1131.62
96	646.27	1073.76	1140.86
97	711.76	1121.09	1151.97
98	805.23	1184.63	1166.34
99	967.84	1286.12	1188.08
99.9	1528.84	1577.64	1243.91

From the table above shows that the concentration level (second column) 0:07 ppm n-hexane granting, or granting 18:37 ppm Ethyl-acetate, or 457.12 ppm Butanol administration, will result in mosquito larval mortality rate was 0.1%. Then the concentration level of 0.97 ppm n-hexane fraction gift, or 58.68 ppm granting Ethyl-acetate, or 593.60 ppm, would result in mosquito larval mortality rate was 1.0%. With the same interpretation, to the provision of 1528.84 ppm concentration for the provision of n-hexane, 1577.64 ppm for ethyl-acetate administration, and 1243.91 ppm for granting Butanol, will result in mosquito larval mortality rate was 99.9%. 1528.84 ppm concentration point for the provision of n-hexane, 1577.64 ppm for ethyl-acetate administration, and 1243.91 ppm for granting Butanol is the highest concentration level of provision that would reach the conclusion that the mosquito larvae will die as a whole (approaching 100%).

The following table for the Mean Time To Failure Butanol Fraction:
Table 9. Mean Time To Failure (MTTF) Butanol Fraction

Characteristics of Distribution Standard 95.0% Normal CI				
Mean(MTTF)	Estimate	Error	Lower	Upper
	956.400	16.0146	925.521	988.309

The following table for the Mean Time To Failure Ethyl Acetate Fraction:
Table 10. Mean Time To Failure (MTTF) Ethyl Acetate Fraction

Characteristics of Distribution Standard 95.0% Normal CI				
Mean(MTTF)	Estimate	Error	Lower	Upper
	528.083	31.8807	469.153	594.415

The following table for the Mean Time To Failure n-Hexane Fraction:
Table 11. Mean Time To Failure (MTTF) n-Hexane Fraction

Characteristics of Distribution Standard 95.0% Normal CI				
Mean(MTTF)	Estimate	Error	Lower	Upper
	183.476	22.5943	144.131	233.562

The above table presents the mean life (optimal concentration) or known as MTTF Mean Time To Failure (MTTF) or the expected value where mosquito larvae mortality rate will be obtained. The test results show the value of the mosquito life expectancy is the provision of 183 476 ppm concentration for n-hexane, 528 083 ppm for ethyl-acetate, and 956 400 ppm for Butanol. Thus, the number concentration of 183 476 ppm for n-hexane, 528 083 ppm for ethyl-acetate, and 956 400 ppm for a massive concentration of butanol is the most effective way to kill mosquito larvae.
To test whether there are differences in survival (survival rate) mosquito larvae in the three extracts tested with the Chi-Square Test as follows:
Table 12. Test for Equal Shape Parameters

Chi-Square	DF	P
60.8279	2	0.000

Test results in the table above shows that the value of Chi-Squarehitung of 60 827, and Sig Chi Square of 0000. Statistics from the table-Chi Chi-Square with squaretabel obtained at 5,991. Because the value of Chi-squarehitung> Chi-squaretabel and Sig Chi-square <0.05 indicates that there are differences in survival (survival rate) mosquito larvae in the third fraction real (significant). Further up Bonferonni test.
Table 12. Bonferroni Comparison Test Next Three Types Faction

Jenis Fraksi	**Daya Tahan**	**Notasi**
Butanol	956.400	A
Etil-asetat	528.083	B
n-Heksan	183.476	C

In the table above shows that the provision of Butanol fraction type, will provide survival (survival rate) the highest mosquito larvae. It means the mosquito larvae will die on Butanol concentrations reaching 956 400. By administering ethyl-acetate fraction type, will provide survival (survival rate) mosquito larvae better (different notation) when compared with Butanol fraction type, mosquito larvae will die in the concentration of ethyl acetate at 528.08. While the provision of n-hexane fraction, will provide survival (survival rate) mosquito larvae are the lowest when compared to the fraction of type-butanol and ethyl acetate, the mosquito larvae can only survive on 183 476 ppm concentration alone. It can be concluded that the provision of n-hexane fraction type will provide survival (survival rate) most low mosquito larvae. According to toxicity criteria based Australian Petroleum Energy Association (1994) in Ratningsih (2008) 183 476 ppm concentration of fractions of rice grains soursop on 24 hours of observation included in the criteria Toxicity Medium (*Moderately Toxic*).

4. Conclusion

1. Giving butanol fraction, will provide survival (survival rate) the highest mosquito larvae. It means the mosquito larvae will die at concentrations that reach 956.400.ppm Butanol.

2. Provision of ethyl-acetate fraction, will provide survival (survival rate) mosquito larvae better (different notation) when compared with the butanol fraction, mosquito larvae will die in ethyl-acetate concentration of 528.08 ppm.

3. Giving n-hexane fraction, will provide survival (survival rate) mosquito larvae are the lowest when compared to type-butanol and ethyl acetate, the mosquito larvae can only survive at a concentration 183 476 ppm.

4. The test results MTTF or Mean Time To Failure (survival value) mosquitoes by giving 183 476 ppm concentration was the most effective concentration to kill larvae of the mosquito *Aedes aegypti.* According to toxicity criteria based Australian Petroleum Energy Association 183.476 ppm concentration of n-hexane fraction soursop seeds in 24 hours of observation included in the criteria of Toxicity Medium (*Moderately Toxic*).

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