



Acute Toxicity Test Of Some Potential Plants As Natural Contraceptive Materials Using The Bslt Method

Rahmi¹, Nindya Anggiani Sembiring², Syarifah Riska Mela Putri³

^{1,2,3} *Departement of Public Health, STIKes Widya Husada Medan, Jl. Williem Iskandar, Medan, Indonesia*

Email : rahmibhm@gmail.com

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Abstract

High birth rates will contribute to national and even global problems today. One of the government's efforts to overcome the relatively high birth rate in Indonesia is by emphasizing the Family Planning Program (KB). However, the use of synthetic contraceptives/chemicals can still cause various possible side effects. So it is necessary to do research to develop natural contraceptive ingredients with minimal side effects. The purpose of this study was to examine the toxicity of several

potential plant materials as natural contraceptives using the Brine Shrimp Lethality Test (BSLT) method and also to determine the mortality percentage of *Artemia salina* Leach larvae after administration of simplicia ethanol extract. The material for this study used the ethanol extract of the fruit plant *Zanthoxylum acanthopodium* DC, seeds and leaves of *Carica Papaya* L, seeds of *Momordica Charantia*, and leaves of *P. Guajava* L with concentrations of 0.100 µg/ml, 200 µg/ml, 500 µg/ml and 1000 µg/ml. This research is an experimental study with 5 treatment groups and 5 replications for each treatment group. The data from the toxicity test will be analyzed by Probit Analysis using SPSS 15.0 for windows to determine the value of LC 50. From the results of the study it was known that the higher the concentration of the compound given from each sample, the higher the mortality of *Artemia salina* lynch larvae. The results of the analysis obtained the LC50 value of each plant, namely Andaliman seeds of 3.85 µg/ml, papaya seeds of 3.93 µg/ml, bitter melon seeds of 3.74 µg/ml. guava leaves of 4.31 µg/ml and papaya leaves of 4 µg/ml.

Keywords : *Antifertility, Toxicity Test, BSLT, Artemia salina Leach*

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Introduction

Indonesia is ranked the 4th most populous in the world with a total of 264 million people (United State Cencus, 2019). The city with the highest population density is Medan City, which is 8,481 people per km². North Sumatra Province is recorded to have a population of 14,262,147 people consisting of 7,116,896 men and 7,145,251 women, with a sex ratio of 99.60 and an average population density of 196 people per km² (Provsu Health Office, 2018) .

Birth rates can be used to formulate new policy strategies in an effort to improve health status, especially the Family Planning Program (KB). The high birth rate will contribute to the current national and even global problem, namely the population explosion (over population). Dense population related to the need for land availability. However, with the lack of public knowledge about family planning (KB), this raises socio-economic problems, welfare, security, availability of land, clean water and food needs. The biggest impact is environmental damage (Christiani, et al., 2013). Family planning is an attempt to measure the number of children and the desired birth spacing. Therefore the government launched a program or method to prevent and delay pregnancy (Sulistyawati, 2013).

Based on the pattern in choosing the type of contraception, most of the Active Family Planning participants chose injections and pills as contraceptives and were even very dominant. The choice of contraception can be influenced by side effects experienced, ease of use, personal protection from sexually transmitted infections (STIs), and latex sensitivity. Other considerations include convenience, product accessibility, efficacy, and affordability (Colquitt & Martin, 2017). Side effects of injectable contraceptives and pills include nausea, bleeding in the form of spotting between menstrual periods, headaches and breast tenderness in the first month of use. Nor does it protect against sexually transmitted infections (STI) and Human Immune Deficiency Virus (HIV), Acquired Immuno Deficiency Syndrome (AIDS) (Irianto, 2014).

Currently, various studies have been carried out both hormonally and non-hormonally (NH). Herbal contraception is very promising as a future contraception because many natural ingredients have an antifertile effect (Cheng & Mrukk, 2011). There are natural contraceptive ingredients derived from plants that have potential as antifertility, including andaliman (*Zanthoxylum acanthopodium* DC.), papaya seeds (*Carica papaya*), papaya leaves (*Carica Papaya* L), bitter melon seeds (*Momordica Charantia*), and guava leaves (P , *Guajava* L.). However, it is necessary to carry out further research regarding the toxicity of some of the above plants to determine the safety level of using these plants as natural contraceptives.

This research is in line with the RENSTRA of STIKes Widya Husada Medan. The research policy of the STIKes listed in the Widya Husada Medan STIKes Strategic Plan is primarily in the health sector. This proposed research is included in the field of drug raw material self-sufficiency technology as well as the development and strengthening of institutional systems, health policies, and community empowerment in supporting drug self-sufficiency. Furthermore , this proposal is part of a research theme which is one of Widya Husada's STIKes roadmap, namely developing the



potential of plants/plants as the development of local resource-based phytopharmaca and strengthening women's knowledge in the development of local knowledge-based phytopharmaca.

Methods

1. Time and Place of Research Implementation

This research was conducted from May to November 2022 at the STIKes Widya Husada Medan Microbiology Laboratory. This study was an experimental study using the *Post Test-Only Control Group Design approach* with the treatment of antifertility extracts from several plants against *Artemia salina Leach larvae*.

2. Research Materials

The materials used in this study were *Artemia salina Leach larvae*, ethanol, and aliman seeds, papaya seeds, bitter melon seeds, guava leaves and papaya leaves. The number of *Artemia salina Leach larvae* used was 10 larvae per treatment group with five treatment concentration groups and five replications for each treatment group. So, the total number of samples needed is 250 larvae for each plant sample.

3. Preparation of Test Materials

The extract was prepared by maceration with ethanol, followed by evaporation of the solvent using a rotary evaporator to obtain a thick extract.

4. Research Experiment Design

This research is an experimental study with 5 treatment groups. There were 5 replications for each treatment group. The treatment groups referred to are:

- negative control (K): shrimp larvae + simplicia ethanol extract 0 µg/ml.
- Treatment I (P1): shrimp larvae + simplicia ethanol extract 100 µg/ml.
- Treatment II (P2): shrimp larvae + simplicia ethanol extract 200 µg/ml.
- Treatment III (P3): shrimp larvae + simplicia ethanol extract 500 µg/ml.
- Treatment IV (P4): shrimp larvae + simplicia ethanol extract 1000 µg/ml.

5. Implementation of Toxicity Test

The simplicia extract was diluted by adding seawater to each test tube at a ratio of 1:2:4:8 to a volume of 5 ml. Then put 10 prawn larvae aged 48 hours each into the tube. Place the test tube under the light for 24 hours, then count the number of dead larvae.

6. Research Parameters

The parameters of this study are:

- a. Determine LC50 based on the concentration of each simplicia of several plants used



b. Measuring the percentage of mortality of *Artemia salina* Leach larvae after administration of simplicia ethanol extract

7. Data Analysis

Research data will be processed and presented in the form of tables and graphs. Data from the toxicity test will be analyzed by Probit Analysis using SPSS 15.0 for windows to determine the price of LC 50.

Results and Discussion

1. Toxicity Test Results of several plants have the potential as anti-fertility

The following are the results of the analysis of the toxicity test of the ethanol extract of andaliman seeds, bitter melon seeds, papaya seeds, guava leaves and papaya leaves using the (BSLT) method in the graph and table below. The percentage of larval mortality can be seen in Figure 1 below.

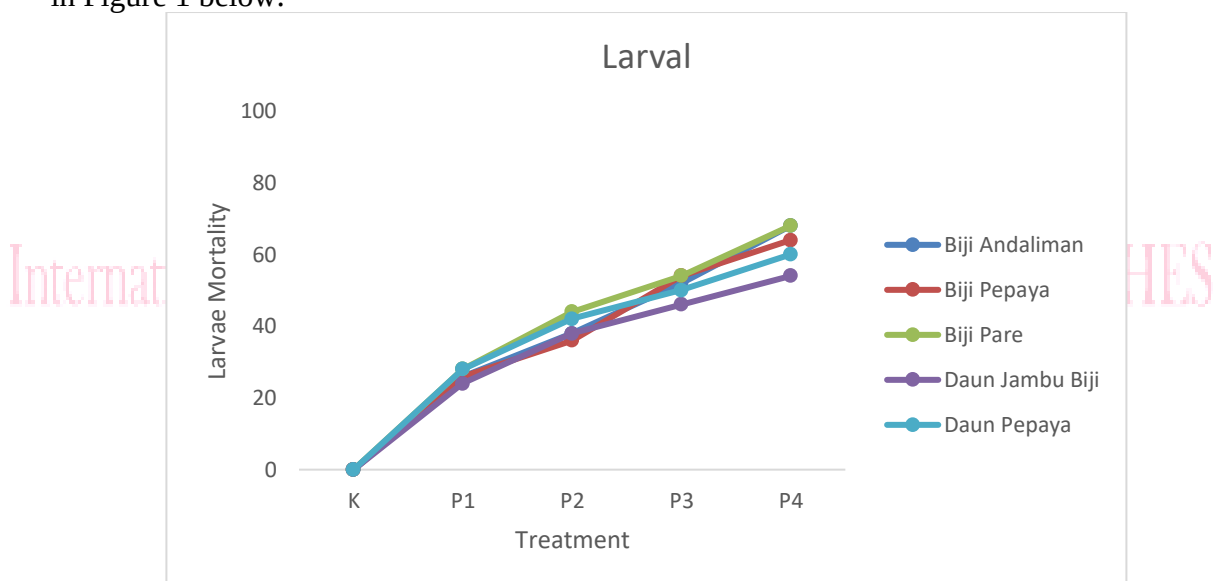


Figure 1. Percentage of larvae death by ethanol extract of some plants as potential antifertility by BSLT method. Description: K (Control/ 0 µg/ml), P1 (100 µg/ml), P2 (200 µg/ml), P3 (500 µg/ml), P4 (1000 µg/ml).



The results of the probit analysis to obtain the LC50 value for each sample can be seen in table 1 below.

SAMPLE	Percentage of Larva Mortality					LC 50 (µg/ml)
	K	P1	P2	P3	P4	
Andaliman seeds	0	26	38	52	68	3,854
Papaya Seeds	0	26	36	54	64	3,929
Bitter gourd seeds	0	28	44	54	68	3,738
Guava Leaves	0	24	38	46	54	4,311
Papaya leaf	0	28	42	50	60	4,001

Table 1. LC50 values and percentage of larval death by ethanol extracts of some plants as potential antifertility using the BSLT method. Description: K (Control/ 0 µg/ml), P1 (100 µg/ml), P2 (20 0 µg/ml), P3 (500 µg/ml), P4 (1000 µg/ml).

The results of the toxicity test of the ethanol extract of andaliman on shrimp larvae in the graph show the lowest concentration mortality of 26% while the highest concentration mortality is 68%. Based on the results of the probit analysis, the LC50 value of the extract was 3.854 µg/ml . The LC50 value in the test results shows the concentration where the extract can kill as much as 50% of the tested animals or larvae. So it can be stated that andaliman has an LC value of 50 which is lower than 1000 ppm.

From the results of the study it was found that the content contained in andaliman was toxic to shrimp larvae. The content contained in the Andaliman extract contains essential oils in the form of *Flavonoids* potency acute toxicity and can cause death of *Artemia salina* Leach larvae. Mechanism of death larvae associated with the function of secondary metabolic compounds such as triterpenoids, saponins and flavonoids in andaliman which can inhibit the eating power of larvae (*antifedant*) (Budiono, et all., 2021).

Procedure these compounds are by acting as *stomach poisoning* . The mechanism of action in inhibiting the growth of larvae is thought to be by inhibiting signal transduction to the nucleus through protein kinase inhibition thereby inhibiting proliferation and cell growth. Tyrosine kinase receptors play a role in increasing cell growth. The content of saponins works by inducing *cell cycle arrest* and cell apoptosis. If these compounds enter the body of the larvae, the digestive system will be disrupted. In addition, this compound inhibits taste receptors in the mouth area of the larvae, resulting in larvae fail to get a taste stimulus so they are unable to recognize their food and the larvae die hunger (Supriningrum et. all., 2016).

The results of the toxicity test of papaya seed ethanol extract on the larvae shown in the graph show the lowest concentration mortality of 26% while the highest concentration mortality is 64% so that the LC50 value of the extract is 3.929 µg/ml . The LC50 value in the test results shows the concentration where the extract can kill as much as 50% of the test animals or larvae. So it can be stated that papaya seeds have an LC value of 50 which is lower than 1000 ppm.



From the results of the study it was found that the content contained in papaya seeds is toxic to shrimp larvae. The chemical constituents contained in papaya seeds are phenol, triterpenoid, flavonoid, alkaloid, and saponin groups (Rajab, et. all., 2015). Due to the presence of these active compounds, papaya seeds have antifertility properties and can be used as male contraceptives (Basha, et. all., 2013). Papaya seeds can affect the arrangement of the seminiferous tubules, namely damage to the germinal epithelium, degeneration of spermatids and spermatids.

In this study it was found that papaya seeds have the potential for acute toxicity. This is related to the four compounds contained in papaya seeds based on test results namely alkaloids, flavonoids, steroids and triterpenoids, at certain levels these compounds have potential toxicity and can cause death in *Artemia salina* Leach larvae. The process of killing the larvae by the compounds found in papaya seeds can inhibit the eating power of the larvae. The way these compounds work is by acting as *stomach poisoning* or stomach poison. Therefore, if this compound enters the body of the larvae, the digestive roof of the larvae will be disrupted. In addition, this compound inhibits taste receptors in the mouth area of the larvae. This results in the larvae failing to get a taste stimulus so they are unable to recognize their food and the larvae die from hunger (Muflihah, 2015).

The results of the toxicity test of the ethanol extract of bitter melon seeds on the larvae shown in the graph show the lowest concentration mortality of 28% while the highest concentration mortality is 68% so that the LC₅₀ value of the extract is 3.738 µg/ml. The LC₅₀ value in the test results shows the concentration where the extract can kill as much as 50% of the test animals or larvae. So it can be stated that bitter melon seeds have an LC 50 value which is lower than 1000 ppm.

From the results of the study it was found that the content contained in bitter melon seeds is toxic to shrimp larvae. The bitter taste of bitter melon seeds is caused by the content of kurbitasin. The results of examining the chemical elements of bitter melon seeds showed the presence of iron, potassium, calcium and magnesium. The phytochemical screening identified the presence of alkaloids, saponins, flavonoids, and steroids/triterpenoids. In the ethyl acetate, n-butanol and ether fractions resulting from liquid extraction, the presence of flavonoids was detected in the ethanol extract. which can inhibit cell growth and development. Steroids can act as an obstacle to spermatogenesis and are reversible (Nurhadijah, et. all., 2018). The way these compounds work is by acting as *stomach poisoning* or stomach poison. Therefore, if this compound enters the body of the larvae, the digestive organs of the larvae will be disrupted.

The results of the toxicity test of the guava leaf ethanol extract on the larvae shown in the graph show the lowest concentration mortality of 24% while the highest concentration mortality is 54% so that the LC₅₀ value of the extract is 4.311 µg/ml. The LC₅₀ value in the test results shows the concentration where the extract can kill as much as 50% of the test animals or larvae. So it can be stated that guava leaves have an LC value of 50 which is lower than 1000 ppm.

From the results of the study it was found that the content contained in and guava is toxic to shrimp larvae. The chemical constituents of guava leaves include psidiolic acid, ursolic acid,



categonic acid, oleanolic acid, guajavolic acid, krategolic acid, guajaverin, isoquercetin, hyperin, flavonol compounds, tannins, casuarinin and quercetin. One of the chemical constituents of guava leaves, namely quercetin, is a flavonoid compound which has the function of inhibiting gamete membrane fusion during fertilization. Quercetin also inhibits hyaluronidase activity so that spermatozoa cannot penetrate the cumulus prior to fertilization (Husni, et. all., 2016).

The mechanism of larval death is related to the function of these compounds in guava leaves which can inhibit the eating power of the larvae (*antifedant*). The workings of these compounds act as stomach poisoning so that they will interfere with the digestive system. In addition, taste receptors in the mouth area of the larvae will also be inhibited, causing the failure of the taste stimulus to the larvae to be unable to recognize their food (Novita, et. all., 2020).

The results of the toxicity test of the papaya leaf ethanol extract on the larvae shown in the graph show the lowest concentration mortality of 28% while the highest concentration mortality is 6% so that the LC50 value of the extract is 4.001 $\mu\text{g/ml}$. The LC50 value in the test results shows the concentration where the extract can kill as much as 50% of the tested animals or larvae. So it can be stated that papaya leaves have an LC value of 50 which is lower than 1000 ppm.

Papaya leaves contain the alkaloids karpain, karpain, pseudokarpain, choline and carposid. Papaya leaves contain a glucosinolate called benzyl isothiocyanate. Papaya leaves also contain minerals such as potassium, calcium, magnesium, copper, iron, zinc and manganese which are known to have active substances such as tannins and flavonoids. The mechanism of larval death is related to the function of these compounds in papaya leaves which can inhibit the eating power of the larvae (*antifedant*). The workings of these compounds act as stomach poisons or *stomach poisoning* so that it will interfere with the digestive system (Alfath, 2018).

Conclusion and Recommendation

From the results of the study it was known that the higher the concentration of the compound given from each sample, the higher the mortality of *Artemia salina* lynch larvae. The results of the analysis obtained the LC50 value of each plant, namely Andaliman seeds of 3.85 $\mu\text{g/ml}$, papaya seeds of 3.93 $\mu\text{g/ml}$, bitter melon seeds of 3.74 $\mu\text{g/ml}$. guava leaves of 4.31 $\mu\text{g/ml}$ and papaya leaves of 4 $\mu\text{g/ml}$. Further research is needed to determine the types of plants that have the most potential to be used as natural contraceptives with the least effect.

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References

- Budiono Putri, Rizki ,WH Nugrahaningsih, Nur KusumaDewi. (2021). Uji Toksisitas Ekstrak Daun Cassava Terhadap Larva Artemia salina Leach dengan Metode Brine Shrimp Lethality Test. Indones. J. Math. Nat. Sci. 44(2) 2021. Doi: <http://journal.unnes.ac.id/nju/index.php/JM>
- Supriningrum, R., Sapri, & Pranamala, V.A. (2016). Uji toksisitas akut ekstrak etanol akar kb (Coptosapelta tomentosa Valetton ex K.Heyne) dengan metode brine shrimp lethality test (BST). Jurnal Ilmiah Manuntung, 2(2), 161-165.
- Rajab, et al, 2015. Uji Antifertilitas Kombinasi Fraksi Kloroform Biji Pepaya dengan Fraksi Etanol Biji Saga (Abrus precatorius Linn.) terhadap Spermatogenesis Tikus Jantan Galur Wistar. e-Jurnal
- Basha H, Lalithamma A, Govardhan Naik A, Changamma C. Evaluation of antifertility efficacy of Carica papaya seed extract through histological indices in male albino rats. International Journal of Chemical and Pharmaceutical Sciences. 2013 Sept; 4(3) Pustaka Kesehatan, vol. 3 (no. 2), Mei 2015
- Muflihah, 2015. Analisis Variasi Konsentrasi Terhadap Uji Toksisitas Akut Golongan senyawa Mmetabolit Sekunder Dari Ekstrak Biji epaya (Carica papaya L) Pada Larva Udang (Artemia Salina Leach)
- Nurhadijah, lilis, alfa A Perdana, dkk. 2018. Aktivitas Formulas Biji Jarak Pagar dan Pae terhadap Spermatogenesis pada Tikus Wistar. Jurnal Jamu Indonesia (2018) 3(1):26-31.
- Husni, Ervi & Sukes. 2016. Efek Zat Aktif Ekstrak Daun Jambu Biji Merah (Psidium Guajava.L) Terhadap Spermatogenesis Pada Tikus Putih Jantan (Rattus Norvegicus). Jurnal Ners Vol. 11 No. 2 Oktober 2016: 269- 276.
- Nofita, Ade Maria Ulfa, Miera Delima. 2020. Uji Toksisitas Ekstrak Etanol Daun Jambu Biji Australia (Psidium guajava L) Dengan Metode BSLT (Brine Shrimp Lethality Test). Jurnal Farmasi Lampung. Vol. 9 No, Juni 2020.
- Alfath, tita. 2018. Uji Toksisitas Ekstrak Daun Pepaya Terhadap Larva Artemia Salina Leach. Dengan Brine Shrimp Lethality Test (BSLT). Semarang