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Phytochemical screening and toxicological evaluation using Brine Shrimp Lethality Tes (BSLT) of ethanolic extract of *Morinda citrifolia* L.

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ABSTRACT

Introduction: *Noni*, which has the scientific name Morinda citrifolia L., is a plant often found in Indonesia and has many properties used for generations. However, this noni fruit has potential toxicity that needs to be studied further. This study aimed to determine the phytochemical compounds and determine the toxicity of the ethanol extract of noni fruit using the Brine Shrimp Lethality Test (BSLT) method.

Methods: The research was conducted at the Chemistry Laboratory, Universitas Nahdlatul Ulama Surabaya. This research includes experimental research. *Artemia salina* L. larvae aged 48 hours were put into a test tube containing a solution of noni fruit extract with concentrations of 10, 50, 100, 250, 500, 750, and 1000 ppm, then observed after 24 hours.

Results: In the phytochemical screening results, noni fruit ethanol extract contains tannins, saponins, flavonoids, and terpenoids. The test results obtained an LC50 value of 17.78 ppm, which indicates that the ethanol extract of the noni fruit is toxic.

Conclusion: The ethanol extract of the *noni* fruit contains tannins, saponins, flavonoids, and terpenoids and has toxicity to *Artemia salina* L. larvae. It can be concluded that the ethanolic extract of noni fruit has the potential as an anticancer.

Keywords: *Artemia salina* L., *Morinda citrifolia* L., phytochemical screening, LC50.
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INTRODUCTION

Noni which has the scientific name *Morinda citrifolia* L. is a plant that is often found in Indonesia and has various names in various regions in Sunda called *cangkudu*, "*pace*" in Java, "*pamarai*" Batak. In other countries, it is also known by various names, "*noni*" in mulberry, India, "*noni*" in Malaysia.[1] *Morinda citrifolia* L. or *Noni*, which comes from tropical Asia, is a valuable medicinal plant widely used in traditional medicine. Noni is usually cultivated for its roots, leaves, and fruit.[2] The bioactive components contained in noni fruit are effective in treating several diseases such as antioxidants, anti-inflammatory, analgesic, hypertension, anticancer, and immunomodulators.[3,4] Noni leaves contain flavonoid compounds, where these compounds are included in the class of natural phenolic compounds found in plants that have antimicrobial properties. Noni fruit contains an anticancer substance called damnacanthal, this substance is very effective against abnormal cells compared to anticancer substances found in other plants. Research by scientists from Germany showed that damnacanthal contained in noni leaves could inhibit the development of cancer cells.[5] Noni seeds contain alkaloid compounds, saponins, tannins, and cardiac glycosides.[6]

Drugs derived from natural ingredients that are considered safe by the community also need to be watched out for by the community. That is because each ingredient or substance has the potential to be toxic, depending on the dose. Given the diverse uses of noni fruit still, based on experience from generation to generation, it needs to be supported by scientific information regarding the efficacy, and side effects caused.[7]

Many studies have been carried out on aqueous and n-hexane extracts of noni fruit, but research on ethanolic extracts of noni fruit has not been widely studied. The toxicity test chosen in this study is the Brine Shrimp Lethality Test (BSLT) method because this method is the first step to testing the toxicity of an extract or compound.[8] According to Maryati et al. in their research, the n-hexane extract of noni fruit has cytotoxic activity against T47D cells in the medium range with an average IC50 value of 582.13 ± 61.64 g/mL.[9] This method uses test animals, namely shrimp larvae *Artemia salina* L. or at the nauplius stage chosen because it is identical to human cancer cells, namely HeLa cells.[10] BSLT can be determined by testing the Lethal Concentration 50% (LC50) to determine the potential bioactive compounds as anticancer. This study aimed to determine the secondary metabolite compounds and the toxicity activity of noni ethanol extract using the Brine Shrimp Lethality Test (BSLT) method.

METHODS

Study Design

The research was conducted at the Chemistry Laboratory, Universitas Nahdlatul Ulama Surabaya. This research includes experimental research. The ingredients used are noni fruit, distilled water, 0.1% FeCl3, chloroform, olive oil, dilute ammonia, concentrated sulfuric acid, and NaCl. The tools used are test tubes, tube racks, measuring cups, beaker glass, 10 ml measuring pipette, 1 ml measuring pipette, pump pipette, measuring flask, dropper, plastic wrap, analytical balance, rotary evaporator, spatula, glass funnel, and mortar.

Preparation of Noni Fruit Simplicia

Noni extract was carried out by washing the noni fruit with distilled water. The fruit was cut and dried until it was dry enough in an oven at 40°C for 24 hours. After the dried fruit is then mashed, the noni fruit simplicia is ready to be used.

Noni Fruit Ethanol Extract Preparation

Next, extraction was carried out by the maceration method by weighing 1 gram of noni simplicia powder and putting it in a glass vessel and then extracting with ethanol solution. This process was carried out for 24 hours for three days. The macerate obtained was then evaporated with a rotary evaporator to obtain the ethanol extract of the noni fruit.

Phytochemical Compound Test

Tannin Test

Samples of noni ethanol extract have added as much as 2-3 drops and 3-5 drops of 0.1% FeCl3, then homogenized, and the color changed to greenish-yellow or greenish-brown, which was declared positive (+) for tannin compounds.

Saponin Test

Samples of noni fruit ethanol extract were taken 10 ml, added 5 ml of distilled water, then shaken until foam appeared, then 3-5 drops of olive oil were added and shaken again, if there was still foam and it did not disappear, then it was declared positive (+) saponin compounds.

Flavonoid Test

Next, 5 ml of noni fruit ethanol extract and 5 ml of dilute ammonia were added and homogenized. So, there will be a color change to slightly yellow then it is declared positive (+) flavonoid compounds.

Terpenoid Test

Samples of 50-100 mg were placed on a drip plate, and acetic acid was added until all samples were submerged, left for 15 minutes then 6 drops of the solution were transferred into a test tube and 2-3 drops of concentrated sulfuric acid were added. We observed the color changing and the intensity of the resulting color was used as a relative measure of the triterpenoid and steroid content in the sample. The presence of triterpenoids is indicated by the occurrence of a red-orange or purple color.

Toxicity Test with Brine Shrimp Lethality Test (BSLT) Method

Noni ethanol extract toxicity test used BSLT (Brine Shrimp Lethality Test) with *Artemia Salina* L. larvae or brine shrimp. The breeding of shrimp larvae was carried out by weighing 20gr of NaCl and dissolved in 1L of distilled water, then filtered, and 0.5 grams of shrimp larvae cysts were added and left for 2×24 hours until the larvae hatched. Breeding is done with sufficient lighting and the help of an aerator because larval growth needs much air to keep the larvae alive.

Furthermore, after 2×24 hours of larval breeding, the *noni* fruit ethanol extract test solution was made at several concentrations, namely 10, 50, 100, 250, 500, 750, and 1000 ppm, and 1% DMSO was homogenized, added as much as 5 ml to the cup test and 5 ml of seawater. Add 10 *Artemia salina* L. larvae, then cover with plastic wrap and give the air holes. Furthermore, it was observed after 24 hours. Lethal concentration for 50% mortality after 24 hours of exposure, LC50 was determined by the probit method where the LC50 value was greater than 1000 ppm for inactive plant extracts. The LC50 value was calculated by linear regression obtained by the percent mortality on the probit scale.

RESULTS

Phytochemical Test

The results of the phytochemical screening of the ethanol extract of noni fruit contained tannins, saponins, flavonoids, and terpenoids. The results of identifying phytochemical compounds in the ethanol extract of *noni* fruit can be seen in Table 1.

The results of the phytochemical screening test showed that the tannin test showed a brownish color change, the saponin test showed a stable foam, the flavonoid test showed a yellowish color change, and the terpenoid test showed an orange color change. This indicates that the ethanol extract of the noni fruit contains tannins, saponins, flavonoids, and terpenoids.

Toxicity Assay

Based on the table 2, the percentage value of mortality obtained from the analysis of the probit method to obtain a linear regression equation is y=0.746x+4.068, and it is known that the LC50 value is 17.78 ppm so it can be categorized as very toxic due to testing of the ethanol extract of the noni fruit (*Morinda citrifolia* L.) indicates the value of LC50 < 1000 g/ml or ppm. The range of LC50 toxicity values is said to be very toxic 0-250 g/ml, toxic 200-250 g/ml, moderate 500-750 g/ml, and non-toxic 750-1000 g/ml.

Table 1. Phytochemical Compound of Ethanol Extract of Noni Fruit.

Phytochemical Compound	Ethanol Extract of Noni Fruit
Tanin	+
Saponin	+
Flavonoid	+
Terpenoid	+

Note : (+) contain compound, (-) contain no compound

Table 2. **Mortality of *Artemia salina* L. Larva after administration of Ethanol Extract of Noni Fruit (*Morinda citrifolia L.*).**

Sample	Concentration (ppm/µg/ml)	Log 10 (Concentration)	% Mortality	Probit
Ethanol Extract of Noni Fruit	10	1	50%	5
	50	1.698970004	60%	5.25
	100	2	60%	5.25
	250	2.397940009	80%	5.84
	500	2.698970004	90%	6.28
	750	2.875061263	90%	6.28
	1000	3	90%	6.28

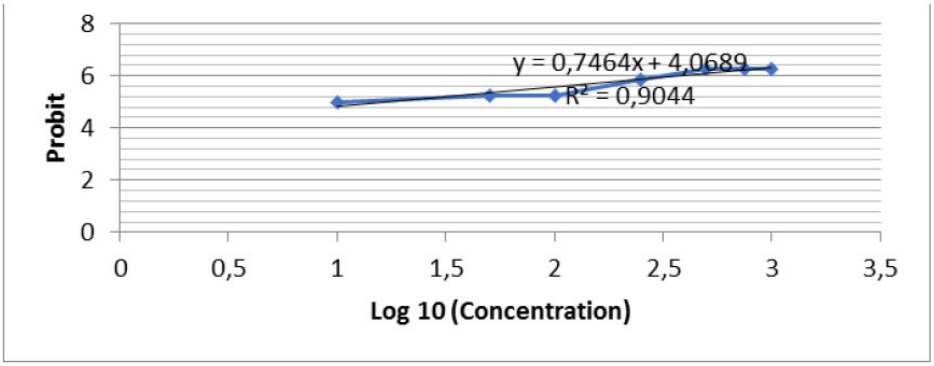

Figure 1. Mortality Curve of *Artemia salina* L. Larva on Ethanol Extract of Noni Fruit.

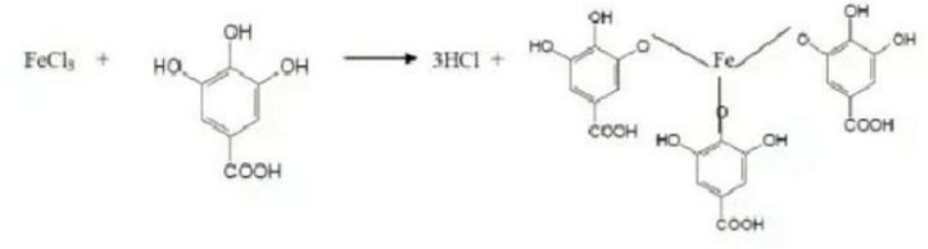

Figure 2. Chemical reactions of tannin compounds.[11]

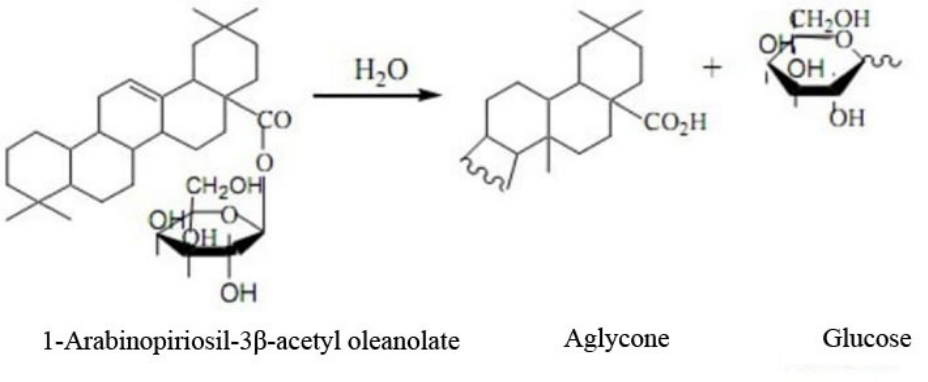

Figure 3. Chemical reactions of saponins.[11]

DISCUSSION

Phytochemical Screening

Based on the results obtained in the phytochemical screening test of noni fruit ethanol extract, positive results were obtained containing tannins, saponins, flavonoids, and terpenoids. A greenish-brown color change indicates the result of a positive reaction for tannin compounds (Figure 2). The function of adding 0.1% FeCl3 aims to form complex compounds when added with tannin compounds.

The positive reaction of the saponin compound test was indicated by the presence of foam and did not disappear (Figure 3). In the saponin compound test, olive oil was added as a source of cholesterol, which caused the formation of complex compounds that were insoluble in water. Saponins can be efficacious in reducing surface tension to inhibit fungal growth.[12]

The positive reaction for the flavonoid compound test is marked by a change in color to slightly yellow. This is because flavonoids are phenolic compounds and when phenol is added to a base, a color change will occur due to the conjugation of aromatic groups (Figure 4). Flavonoids are phenol-derived compounds that are efficacious in lowering cholesterol and lipids because they are antibacterial.[13] Flavonoid compounds also have potential as antioxidants because their structure contains hydroxyl groups that can donate hydrogen atoms to free radicals.[14]

The content of terpenoids/steroids in plants was tested using the Liebermann-Buchard method (acetic acid), which will give a red-orange or purple color for terpenoids. With the addition of the Liebermann-Buchard reagent, molecules of acetic anhydride and sulfuric acid will bind to molecules of terpenoid/steroid compounds, resulting in a reaction that appears in the color change (Figure 5). From the results of the terpenoid screening, it turned out that the sample contained terpenoid compounds.[11]

Brine Shrimp Lethality Test (BSLT)

The Brine Shrimp Lethality Test (BSLT) is a preliminary test that aims to determine the mortality rate directly proportional to the concentration of the extract. This test used *Artemia salina* L. larvae aged 48

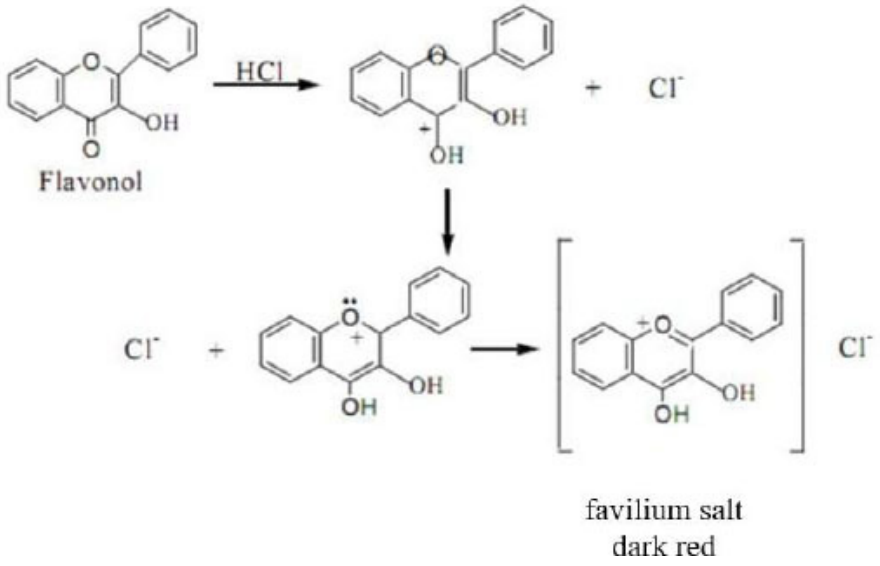

Figure 4. Chemical reactions of flavonoid compounds.[11]

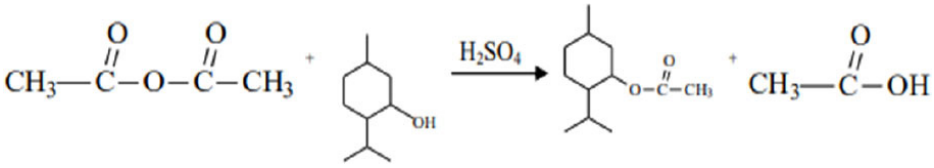

Figure 5. Chemical reactions of terpenoid compounds.[11]

hours and active. The test results obtained an LC50 value of 17.78 ppm. The value of LC50 is the value of the concentration of substances that can cause death 50% obtained by using a linear regression equation. Thus, the LC50 value can be calculated using the linear regression equation y=ax+b, where y is the probit value while x is the concentration value used in the test. Death in shrimp larvae was caused by drastic changes in the concentration gradient between inside and outside the cell, causing toxic compounds to spread well to the body of shrimp larvae. The effects of metabolic damage that occur quickly can be detected within 24 hours, causing 50% of shrimp larvae mortality.[15]

The mechanism of larval death is related to the function of flavonoid compounds that inhibit larval feeding power. Apart from that, according to Woo et al., the mechanism of flavonoids as an anticancer has several theories. Flavonoids as antioxidants are through the mechanism of activating the apoptotic pathway of cancer cells. The mechanism of cell apoptosis in this theory is due to DNA fragmentation. This fragmentation begins with releasing the proximal DNA chain by reactive oxygen compounds such as hydroxyl radicals.[16] Another effect is flavonoids as an inhibitor of tumor/cancer proliferation, one of which inhibits protein kinase activity by inhibiting signal transduction pathways from membranes to nuclear cells. Flavonoids inhibit receptor tyrosine kinase activity because increased receptor tyrosine kinase activity plays a role in the growth of cancer cell malignancies. Flavonoids also function to reduce tumor resistance to chemotherapeutic agents.[16]

According to the American National Cancer Institute (NCI), the standard for the effectiveness of bioactive components against cancer cells is 30 g/mL.[17] Based on previous research, if a plant extract is toxic according to the estimated LC50 with the BSLT method, the plant can be developed as an anticancer drug.[18]

CONCLUSION

Based on the research that has been done, it can be concluded that the ethanol extract of the noni fruit on phytochemical screening contains tannins, saponins, flavonoids, and terpenoids. In the Brine Shrimp Lethality Test (BSLT), the ethanol extract of the noni fruit obtained an LC50 value of 17.78 ppm, indicating toxicity to *Artemia salina* L. larvae with an LC50 value of <1000 g/ml. Also, further research with different study designs and larger samples needs to be done to identify the factors that influence the phytochemical and toxicity of the ethanol extract of the noni fruit.

AUTHOR CONTRIBUTION

All authors contributed to this study's conception and design, experiment, data analysis and interpretation, article drafting, critical revision, final approval of the article, and data collection.

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CONFLICT OF INTEREST

There is no conflict of interest for this manuscript.

ETHICAL CONSIDERATION

This research was approved by the Health Research Ethics Committee of the Faculty of Health, Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia.

REFERENCES

1. Piaru SP, Mahmud R, Abdul Majid AMS, Mahmoud Nassar ZD. Antioxidant and antiangiogenic activities of the essential oils of Myristica fragrans and *Morinda citrifolia*. Asian Pac J Trop Med. 2012;5(4):294–8. Available from: http://dx.doi.org/10.1016/S1995-7645(12)60042-X
2. Krishnaiah D, Bono A, Sarbatly R, Anisuzzaman SM. Antioxidant activity and total phenolic content of an isolated *Morinda citrifolia* L. methanolic extract from Poly-ethersulphone (PES) membrane separator. J King Saud Univ-Eng Sci. 2015;27(1):63–7. Available from: http://dx.doi.org/10.1016/j.jksues.2013.01.002
3. Kaur H, Gurjar N, Gill R. The noni fruit (*Morinda citrifolia* L.): A systematic review on anticancer potential and other health beneficial pharmacological activ ities. 2018;86–93.
4. Almeida-Souza F, De Oliveira AER, Abreu-Silva AL, Da Silva Calabrese K. In vitro activity of *Morinda citrifolia* Linn. fruit juice against the axenic amastigote form of Leishmania

amazonensis and its hydrogen peroxide induction capacity in BALB/c peritoneal macrophages. BMC Res Notes. 2018;11(1):1–7. Available from: https://doi.org/10.1186/s13104-018-3555-7
5. Nirawati C. Uji Daya Hambat Ekstrak Daun dan Buah Mengkudu (*Morinda citrifolia* L.) Terhadap Pertumbuhan Bakteri Escherichia coli Sebagai Penunjang Praktikum Mata Kuliah Mikrobiologi. Universitas Islam Negeri AR. RANIRY Darussalam; 2016.
6. Ariyanto W, Sadimin S, Sariyem S. Daya Hambat Ekstrak Biji Mengkudu Terhadap Pertumbuhan Bakteri Streptococcus Mutans. J Kesehat Gigi. 2016;3(1):34–40.
7. Fikri A, Mugiyanto E. Uji Toksisitas Ekstrak Etanol Daun, Daging Buah Dan Biji Mengkudu (*Morinda citrifolia* Linn.) Dengan Metode BSLT (Brine Shrimp Lethality Test). Jurnal Farmasi dan Kesehatan. Uji Toksisitas Ekstrak Etanol Daun, Daging Buah Dan Biji Mengkudu (*Morinda citrifolia* Linn) Dengan Metod BSLT (Brine Shrimp Lethality Test) J Farm dan Kesehatan. 2018;1(2):1–8.
8. Antonius P, Eko M. Uji Toksisitas Daun Ketepeng (Cassia Alata L.), Kulit Buah Pisang Ambon (Musa Paradisiaca L. Var Sapientum) dan Kulit Rimpang Kencur (Kaempferia Galanga Linn.) dengan Metode Brine Shrimp Lethality Test (BSLT). In: Proceeding of The URECOL. 2018. p. 189–94.
9. Maryati A, Khairudin M. Aktivitas Sitotoksik Ekstrak Heksan, Ekstrak Etil Asetat, Ekstrak Etanol Buah Mengkudu (*Morinda citrifolia* L) Terhadap Sel T47D. J Farm Indones. 2020;17(1):1–10.
10. Juhaeriah E. Uji Toksisitas Akut Ekstrak Etanol Daun Paku Pedang (Nepholepis Falcata) Terhadap Larva Artemia salina Leach Dengan Metode BSLT. Universitas Islam Negeri Syarif Hidayatullah.; 2013.
11. Sangi MS, Momuat LI, Kumaunang M. Uji Toksisitas Dan Skrining Fitokimia Tepung Gabah Pelepah Aren (Arenga Pinnata). J Ilm Sains. 2012;12(2):127.
12. Khotimah K. Skrining Fitokimia dan Identifikasi Metabolit Sekunder Senyawa Karpain Pada Ekstrak Metanol Daun Carica pubescens Lenne dan K. Koch Dengan LC/MS. Uin Maulana Malik Ibrohim Malang. 2016.
13. Rustina. Uji Aktivitas Antioksidan dan Antibakteri Ekstrak Etil Asetat Labu Kuning (Cucuma moschata Duch.Poir). Universitas Muhammadiyah Yogyakarta; 2016.
14. Supomo S, Warnida H, Said BM. Uji Aktivitas Antioksidan Ekstrak Etanol Umbi Bawang Rambut (Allium chinense G.Don) Dengan Penagkal Radikal DPPH (1,1- Difenil-2-Pikrilhidrazil). J Ris Kefarmasian Indones. 2019;1(1):30–40.
15. Dwijayanti E, Alimuddin AH, Wibowo MA. Skrining fitokimia dan uji aktivitas sitotoksik pada kulit batang tampoi (Baccaurea macrocarpa) terhadap Artemia Salina Leach dengan Metode BSLT. J Kim Khatulistiwa. 2014;3(4):6–10.
16. Woo HD, Kim J. Dietary flavonoid intake and risk of stomach and colorectal cancer. World J Gastroenterol. 2013;19(7):1011–9.
17. Albuntana A, Yasman, Wardhana W. Toksisitas Ekstrak Empat Jenis Teripang Suku Holothuridae Dari Pulau Penjaliran Timur, Kepulauan Seribu, Jakarta, Menggunakan Brine Shrimp Lethality Test. J Ilmu dan Teknol Kelaut Trop. 2011;3(1):65–725.
18. Kramy P, Triawanti A. Uji Sitotoksik Ekstrak Etanol Caun Tumbuhan Jinjit (Desmodium Heterocarpon) terhadap Larva Artemia Salina Leach dengan Metode Brine Shrimp Lethality Test (BSLT). J Kedokt Univ Palangka Raya. 2017;2(1):1.