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Toxicity of Gompang Batu Plant Extract (*Lobelia nummularia* Lam) with BSLT (*Brine Shrimp Lethality Test*) Method as Raw Material for Medicine

Agnes Normawati Silaban[1], Murniaty Simorangkir[2]*

Program Studi Kimia, Fakultas Matematika dan Ilmu Pengetahuan Alam, Universitas Negeri Medan, Medan 20221, Sumatera Utara Indonesia

**Corresponding author : murniatysimorangkir@unimed.ac.id*

ABSTRACT

Gompang Batu is a traditional medicinal plant found in Pangaribuan, North Tapanuli, Sumatra, Indonesia. Identification confirmed that gompang batu is Lobelia nummularia Lam from the Campanulaceae family. This study aims to determine the toxicity and phytochemical content of three types of gompang batu extracts. The Brine Shrimp Lethality Test (BSLT) method.was used for toxicity testing. Extraction was conducted through maceration using n-hexane, ethyl acetate, and ethanol solvents. LC_{50} values were 18.05 µg/mL for ethanol extract (very toxic), 27.59 µg/mL for ethyl acetate extract (very toxic), and 51.34 µg/mL for n-hexane extract (toxic). Phytochemical analysis showed that the ethanol extract contains abundant alkaloids, flavonoids, saponins, terpenoids and tannins. Ethyl acetate extract contains alkaloids, steroids, flavonoids, saponins, and tannins moderate tannins. N-hexane extract contains steroids and a small amount tannins. L. nummularia extract is classified as toxic and show potential as a medicinal raw material.

Keywords: *Lobelia nummularia* Lam, BSLT toxicity, phytochemical screening.

1. INTRODUCTION

The use of medicinal plants as alternative raw materials for drugs is increasingly popular due to their abundant availability, relatively low prices, and fewer side effects compared to synthetic drugs. One of the plants traditionally used by the community in Pangaribuan District, North Tapanuli Regency, North Sumatra is gompang batu, which is known to be effective in treating boils. Based on the determination results, gompang batu is *Lobelia nummularia* Lam, belongs to the Campanulaceae family (MEDAN USU NO.1943/MEDAN/2024). Previous studies have stated that the Lobelia genus contains secondary metabolites

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of alkaloids, flavonoids, and terpenoids which have antibacterial, anti-inflammatory, and antidepressant activities.[13] Vadeo (2023) reported that *L. nummularia* is traditionally used as a wound medicine, and Zheng et al. (2021) identified the content of lobeline compounds in this genus which have the potential as pharmacological active ingredients.[13, 15] However, until now there have not been many scientific studies that have specifically tested the toxicity and bioactive compound content of this plant, so further research is needed to support its use as a raw material for herbal medicine.

Several previous studies have used the *Brine Shrimp Lethality Test* (BSLT) method to assess the toxicity of various plant extracts. This method is considered simple, economical, and can provide an initial overview of the cytotoxic potential of natural compounds.[3, 12] Simorangkir et al. in their study of *Clerodendrum fragans* extract showed variations in toxicity levels between ethanol, ethyl acetate, and n-hexane extracts.[11] In addition, Fikayuniar et al. (2022) emphasized that the BSLT method is very suitable for initial toxicity testing of traditional medicinal plants that have not been widely studied.[6] The level of toxicity in the BSLT test can be determined based on the number of shrimp larvae (*Artemia salina* Leach) that die due to exposure to extracts or compounds from natural materials.[8]

Based on the description above, a study was carried out on the toxicity of gompang batu plant extract (*Lobelia nummularia* Lam) using the BSLT method as a raw material for medicine. Extraction was carried out using the maceration method using solvents with varying polarity. Phytochemical screening was carried out to determine the secondary metabolite content of *L. nummularia* extract.

2. EXPERIMENTAL

2.1. Chemicals, Equipment and Instrumentation

The main chemicals used in this study include *n*-hexane (C_6H_{14}) (Merck), ethyl acetate ($C_4H_8O_2$) (Merck), ethanol (C_2H_5OH) 96% (Brataco), Dragendorff's reagent (Merck), hydrochloric acid (HCl) 2N (Merck), iron (III) chloride ($FeCl_3$) 5% and 1% (Merck), anhydrous acetic acid (CH_3COOH) (Merck), sodium chloride (NaCl) (technical), distilled water (H_2O), Artemia salina Leach eggs (Ocean Star International), yeast extract (technical grade), and dimethyl sulfoxide (DMSO) 1% ($(CH_3)2SO$) (Merck). The glassware used included Erlenmeyer flasks, beakers, test tubes, measuring flasks, Buchner funnels, and separating funnels, rotary evaporators (DLAB RE100-Pro), analytical balances (Kern ADB 200-4), hotplate stirrers (Onilab M57-H550-S), vacuum pumps (B-One DVP-3-73), filter paper (Whattman), electric blenders (Goldfruit Electric Grinder), 60 mesh sieves, desiccators, and 20 watt incandescent lamps (TL).

2.2. Research Procedure

2.2.1. Plant Sampel Preparation

Fresh *Lobelia nummularia* Lam plant samples were taken from Rahut Bosi village, Pangaribuan, North Tapanuli as much as 4.0 Kg, then ground using a blender, until 300 grams of fine powder of 60 mesh size simplicia were obtained.

2.2.2. Extract Preparation

300 grams of *L nummularia* simplicia powder were extracted gradually by the maceration method using solvents with varying polarity. First, the powder was soaked in 900 mL of *n*-hexane for 3 x 24 hours, then

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filtered and the filtrate was concentrated using a rotary evaporator. The remaining residue was then re-extracted with 900 mL of ethyl acetate, filtered and concentrated using a rotary evaporator. Finally, the residue was re-extracted using 900 mL of ethanol, filtered, and concentrated using a rotary evaporator. All thick extracts were stored in the refrigerator.

2.2.3. Phytochemical Screening

2.2.3.1. Alkaloid Test

The 0.5 grams of extract was added to 1 mL of 2N HCl and 9 mL of distilled water, then stirred until homogeneous. Then 5 drops of Dragendorff's reagent were added to the solution. The formation of a brick-red precipitate indicates the presence of alkaloids in the exctract.[10]

2.2.3.2. Flavonoid Test

Five drops drops of extract in a test tube, then 2 drops of 5% iron (III) chloride ($FeCl_3$) solution were added. The appearance of a blackish green or blackish blue color indicates the presence of flavonoid compounds.[10]

2.2.3.3. Saponin Test

One mL of extract was added to 10 mL of distilled water, then the solution was shaken for 10 minutes. If stable foam is formed, wait for at least 10 minutes. Then a few drops of 2N hydrochloric acid (HCl) were added. If the foam does not disappear, it indicates the presence of saponin compounds.[10]

2.2.3.4. Steroid and Terpenoid Test

The 0.5 grams of extract was added with 10 drops of acetic anhydride ($(CH_3CO)_2O$) and 2 drops of concentrated sulfuric acid (H_2SO_4). Color changes to green and blue indicate the presence of steroids, while red and purple indicate the presence of terpenoids.[10]

2.2.3.5. Tannin Test

The 0.5 grams of extract was added with 10 mL of distilled water, then 3 drops of 1% $FeCl_3$ solution were added. The blackish green color that appears indicates the presence of tannins.[10]

2.2.4. Phytochemical Screening

2.2.4.1. Artemia salina Larvae Culture

A total of 0.5 grams of *Artemia salina* eggs were placed in a hatching container containing seawater that had gone through a filtering process. After aeration and irradiation using a 20 watt TL lamp for 24 hours, the eggs hatched into nauplii which were then ready to be used as test organisms.[11]

2.2.4.2. Sample Preparation

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Each extracted sample of 40.0 mg was dissolved in seawater to reach a volume of 20.0 mL to obtain a solution with a concentration of 2000 µg/mL. Because *n*-hexane and ethyl acetate extracts have low solubility in seawater, 1% dimethyl sulfoxide (DMSO) of 1.0 µL was addes as an additional solvent. Furthermore, a dilution series was made to a concentration of 1000, 500, 100, 50, and 25 µg/mL.[11]

2.2.4.3. Toxicity Test

The 5.0 mL of test sample from each concentration was put into a container containing 10 two-day-old *Artemia salina* shrimp larvae. Next, seawater was added to the container until it reached a final volume of 10 mL, each concentration was made in three replications (triplicate). All incubation containers were placed in a place with sufficient lighting for 24 hours.[11]

2.2.4.4. Data Analysis

After 24 hours of incubation, observations were made on the number of *Artemia salina* larvae that died at each sample concentration. The percentage of mortality was calculated by comparing the number of dead larvae to the total number of larvae used in the test. The LC_{50} value was obtained through regression analysis, namely by entering the log concentration value and probit value into the regression line equation. A compound is categorized as toxic or active if its LC_{50} value is <1000 µg/Ml.[11]

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening Results

Phytochemical screening test aims to identify the types of compound groups contained in an extract.[7] The results of phytochemical screening of n-hexane extract of *Lobelia nummularia* Lam are presented in Table 1.

Table 1. Phytochemical Screening Results of *Lobelia nummularia* Lam

Compound grups	Fraction		
	***n*-hexane**	**Ethyl acetate**	**Ethanol**
Alkaloid	-	++	+++
Flavonoid	-	++	+++
Saponin	-	++	+++
Steroid	+++	+++	-
Terpenoid	-	-	+++
Tannin	+	++	+++

Description: (-) not detected, (+) small amount, (++) moderate amount, (+++) strong amount

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The ethanol extract of *L. nummularia* contained many alkaloids, flavonoids, saponins, terpenoids, and tannins. This is because ethanol is a polar solvent that can dissolve various types of secondary metabolite compounds, both polar and semi-polar. Based on research by Meray et al. (2024) stated that ethanol can extract bioactive compounds such as alkaloids, flavonoids, and tannins because it has the ability to form hydrogen bonds with the polar groups of these compounds.[7] The ethyl acetate extract of *L. nummularia* contains many steroids and alkaloids, flavonoids, saponins, and tannins in moderate amounts. This is because ethyl acetate is known to be effective in extracting compounds with medium polarity, such as steroids and certain phenolic compounds.[9] The n-hexane extract of *L. nummularia* contains many steroids and few tannins. This is because n-hexane is a non-polar solvent that selectively extracts non-polar compounds such as steroids and triterpenoids, and tannins.[9] This extraction pattern is in line with the principle of "like dissolves like", where compounds with a certain polarity will be more easily dissolved in solvents that have similar polarity.

3.2. Toxicity Test

The toxicity test of *Lobelia nummularia* Lam extract (gompang batu) was conducted using the Brine Shrimp Lethality Test (BSLT) method. Mortality data of larvae at various concentrations and types of extracts are presented in Table 2.

Table 2. Results of Toxicity Test of *Lobelia nummularia* Lam Extract with the BSLT Method

Sample Test	Extract Concentration (µg/mL)	Number of Dead Larvae			Total Larva Mati	Mortality (%)	Probit value	LC_{50} (µg/mL)
		Pengulangan						
		I	II	III				
Ekstract of *n*-hexane	**Control**	0	0	0	0	0		
	25	4	4	3	11	36.66	4.4	
	50	4	5	6	15	50.00	5.0	
	100	5	7	6	18	60.00	5.25	51.34
	500	10	9	9	28	93.33	6.48	
	1000	10	10	10	30	100.00	7.37	
Ekstract of ethyl acetate	**Control**	0	0	0	0	0		
	25	5	4	6	15	50.00	5.00	
	50	7	6	6	19	63.33	5.3	
	100	7	9	8	24	80.00	5.84	27.59
	500	10	10	10	30	100.00	7.37	
	1000	10	10	10	30	100.00	7.37	

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Ekstract of ethanol	**Control**	0	0	0	0	0		
	25	6	7	6	19	63.33	5.33	
	50	7	6	8	21	70.00	5.52	
	100	8	8	9	25	83.33	5.95	18.05
	500	10	10	10	30	100.00	7.37	
	1000	10	10	10	30	100.00	7.37	

Description: a). Average with three repetitions per concentration

b). % Larval Mortality = (Number of larval deaths)/(Number of larvae tested) x 100%

c). LC50 is obtained from the equation Y = aX + C, where Yi is the Probit value and X is the concentration.[12]

Based on the research that has been done, the results of the toxicity test showed that ethanol, ethyl acetate and n-hexane extracts caused the highest death rate of test animals, namely 100%, where the LC_{50} value in the ethanol extract was 18.05 µg/mL, ethyl acetate extract 27.59 µg/mL and n-hexane extract 51.24 µg/mL.

According to Meyer et al. (1982), extracts with LC_{50} values <30 µg/mL are categorized as very toxic, while between 30–100 µg/mL are categorized as toxic and LC50> 1000 µg/mL are non-toxic which have the potential as anticancer agents.[8] Therefore, the ethanol and ethyl acetate extracts of *Lobelia nummularia* Lam are included in the very toxic category, while the n-hexane extract is included in the toxic category. These results are in accordance with phytochemical tests which show that the ethanol extract contains a combination of alkaloid, saponin, and terpenoid compounds which are known to have cytotoxic effects.[11]

Compounds such as alkaloids, steroids, flavonoids, saponins, and tannins have toxic properties that work by disrupting the digestive process in larvae. The mechanism of action includes inhibiting the activity of digestive enzymes such as protease, lipase, amylase, and invertase, thereby disrupting the ability of larvae to digest and absorb nutrients from their food.[5] The mechanism of action of these compounds as stomach poisoning that inhibits taste receptors in the mouth area of the larvae, so that the larvae cannot respond to taste stimuli, have difficulty recognizing their food which ultimately causes death in the larvae.[4,5,11] So that the results of this study indicate that the *L. nummularia* plant has the potential as a raw material for natural medicine.

4. CONCLUSION

Toxicity test using BSLT method showed that ethanol extract has toxicity with LC_{50} value of 18.05 µg/mL (very toxic), then ethyl acetate extract 27.59 µg/mL (very toxic) and n-hexane extract 51.34 µg/mL. Gompang batu plant (*Lobelia nummularia* Lam) contains secondary metabolite compounds such as alkaloids, flavonoids, saponins, steroids, terpenoids, and tannins. These results indicate that *L. nummularia* plant has

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the potential as a raw material for drugs that can be further developed for pharmaceutical purposes, especially as a natural-based antimicrobial or anticancer agent.

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