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# Antioxidant activity of local Gompang Batu Plants (*Lobelia nummularia* Lam) *in vitro* DPPH Method

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**ABSTRACT**

*Free radicals are reactive atoms or molecules because they contain unpaired electrons that easily attack body structures, causing various diseases such as cancer, liver, heart and other degenerative diseases. Antioxidants are compounds or molecules that can reduce free radicals. Natural antioxidants are needed to reduce the effects of using synthetic antioxidants. The local plant gompang batu (Lobelia nummularia Lam) is found in Ruhut Bosi Village, Pangaribuan District and has been used by the community as a traditional medicine. This study aims to determine the content of secondary metabolites and antioxidant activity of ethanol extract of gompang batu in vitro using the DPPH (2,2 diphenyl-1-picrylhydrazyl) method. The results of the phytochemical screening test of the gompang batu plant contain secondary metabolites of flavonoids, alkaloids, steroids/terpenoids and saponins. The results of the antioxidant activity test of the ethanol extract of gompang batu obtained an $IC_{50}$ value of 135,095 ppm (moderate category) and an $IC_{50}$ value of vitamin C (as a positive control) of 39,390 ppm.*

Keywords: *Lobelia nummularia* Lam, Antioxidant, DPPH

## 1. INTRODUCTION

Free radicals are reactive atoms or molecules because they contain unpaired electrons so that they easily attack body structures which cause various diseases [1]. Excessive free radicals can cause diseases such as cancer, liver, heart and other degenerative diseases [2]. According to data from the World Health Organization (WHO), through the International Agency for Research on Cancer (IARC) cancer research institute, released data on new cancer cases in the world reaching 20 million cases, with a death toll of 9.7 million cases [3,4]. According to Global Cancer Statistics (Globocan) data released by WHO in 2022, in Indonesia there were 408,661 new cancer cases with 242,988 deaths caused by cancer[5]. Based on these data, it shows that free radicals have a very dangerous impact and need to be addressed, to neutralize the work of free radicals, antioxidants are needed. Antioxidants are compounds that inhibit oxidation reactions in the body by inhibiting



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the reactivity of free radicals by providing electrons or electron donors and through hydrogen atom donors [6]. Antioxidants based on their sources are of two types, namely natural antioxidants and synthetic (artificial) antioxidants. Synthetic antioxidants when used for a long period of time will cause negative impacts such as causing carcinogenic diseases. Natural antioxidants are safer than synthetic antioxidants because they come from natural ingredients that are easily obtained in the surrounding environment [7].

One of the plants that can be used as traditional medicine is the gompang batu plant found in Ruhut Bosi village, Pangaribuan District, North Tapanuli. Based on the results of the MEDA USU herbarium determination (No.1943/MEDA/2024) gompang batu has the species name *Lobelia nummularia* Lam, genus *Lobelia*, family *Campanulaceae*. The Lobelia genus has around 450 species included in the *Campanulaceae* family [8]. The results of phytochemical analysis in the *Lobelia* genus contained secondary metabolites of alkaloids as much as 46.05%, flavonoids 25%, terpenoids, 13%, polyacetylene 5.25%, coumarin 4%, fatty acids 4%, neolignan 1.35% and amide[9]. The pharmacological activity of the lobelia genus shows inhibitory activity against Mycobacterium tuberculosis, central nervous system disorders, anti-epileptic, anti-inflammatory, antidepressant, treating cuts, boils, gallbladder disorders, kidney stones and urinary tract infections, and antibacterial [8]. The results of the study of the antioxidant activity of the *Lobelia* genus in the ABTS test, have antioxidant activity of ethanol extract ($IC_{50}$ = 29.26 ± 0.49 µg / mL) and in the FARP test have antioxidant activity of ethanol extract with an $IC_{50}$ value = 329.03 ± 46.30 µmol / g [10].

The antioxidant activity testing method that can be used is the DPPH (*2,2 diphenyl-1-picrylhydrazyl*) method which is a stable purple free radical compound with one unpaired atom, inhibits the formation of free radicals and prevents cell damage, which is indicated by a decrease in the intensity of the purple color to yellow, depending on the antioxidant content [11]. In addition, this method also has the advantages of being simple, easy, fast and requiring few samples [12]. Based on the description above, a study was conducted on the antioxidant activity of the local gompang batu plant (*Lobelia nummularia* Lam) in vitro using the DPPH (*2,2 diphenyl-1-picrylhydrazyl*) method.

## 2. EXPERIMENTAL

### *2.1. Chemicals, Equipment and Instrumentation*

The materials used in this study were samples of gompang batu plants from Ruhut Bosi Village, Pangaribuan District, North Sumatra, DPPH powder (Sigma Aldrich), n-Hexane ($C_6H_{14}$), ethyl acetate ($C_4H_8O_2$), 96% ethanol, methanol p.a ($CH_3OH$) (Merck), ascorbic acid ($C_6H_8O_6$), distilled water ($H_2O$), hydrochloric acid HCL 2 M, Whatman filter paper No. 1, concentrated hydrochloric acid (HCL) (Merck), Narium Hydroxide (NaOH) 10%, Dragendorff's reagent, Wagner's reagent, anhydrous acetic acid ($C_4H_6O_3$), sulfuric acid ($H_2SO_4$), magnesium powder (Mg) and iron (III) chloride ($FeCl_3$) 1%. The tools used in this study were: beaker glass, measuring cup, test tube, measuring flask (pyrex), analytical balance (Shimadzu ATX224 A), dropper pipette, blender, glass funnel, stirring rod, spatula, buchner funnel, rotary evaporator (Heidolph), hot plate (Thermo), distillation apparatus, dropper pipette, micro pipette (Dragon Lab), test tube rack, 80 mesh sieve, vortex (VORTEX MVOR-01, SBS tube vibrating agitator) and UV-Vis device (BMG LABTECH), incubator (Memert) and microplate.

### *2.2 Research Procedure*

**Preparation and Extraction of Gompang Batu (*Lobelia nummularia* Lam) Samples**

Samples of gompang batu (*Lobelia nummularia* Lam) plants were taken from Ruhut Bosi Village, Pangaribuan District, North Sumatra, cleaned and dried without exposure to sunlight and ground to produce 80 mesh gompang batu simplicia powder. Gompang batu simplicia powder was macerated using graded solvents starting from non-polar solvents (*n*-hexane), semi-polar (ethyl acetate) and polar (ethanol) each for



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3x24 hours. The filtrate results were then evaporated using a rotary evaporator to obtain thick extracts of *n*-hexane, ethyl acetate and ethanol extracts of gompang batu.

**Phytochemical Screening Test**

1. Flavonoid Test

   A total of 0.5 g of gompang batu extract was dissolved with ethanol solvent, then pipetted 1 mL and added Magnesium powder and then dripped with 1 mL of HCL (p) solution. The color changes of red, yellow, orange that occurred indicated positive flavonoid content [13].
2. Alkaloid Test

   As much as 0.5 g of ethanol extract of gompang batu was dripped with 2 ml of 2 M HCL, then the filtrate was divided into two parts. In the first tube, 3 drops of Wagner reagent were added, if the brown precipitate formed indicated a positive alkaloid. In the second tube, 3 drops of Dragendorff reagent were added, if the orange precipitate formed indicated a positive alkaloid [14].
3. Steroid/Terpenoid Test

   As much as 0.5 g of ethanol extract of gampang batu was dissolved with ethanol solvent, then added anhydrous acetic acid reagent, and concentrated sulfuric acid. The blue or green color change formed indicates the presence of steroids, while orange or purple indicates the presence of terpenoids [15].
4. Tannin Test

   As much as 0.5 g of ethanol extract of gompang batu was dissolved in ethanol solvent, then 3 drops of $FeCl_3$ were added. If a dark blue or blackish green color was formed, it was said to be positive for containing tannin [16].
5. Saponin Test

   As much as 0.1 grams of ethanol extract of gompang batu was dissolved with ethanol solvent, added 1 mL of warm water and then shaken. After that, the foam that emerged was observed. Left for 5 minutes and if the foam did not disappear, 2 M HCL was added, it was said to be positive if there was still constant foam [16].

**Antioxidant Testing with DPPH Method**

The antioxidant activity of gompang batu plants was tested using the DPPH (*2,2 diphenyl-1-picrylhydrazyl*) method, with a UV-Vis spectrophotometer at a wavelength of 513 nm. The ethanol extract sample solution of gompang batu was provided with concentrations of 5, 10, 25, 50, and 100 ppm. The concentration of the DPPH solution was 0.4 mM in methanol p.a. As a positive control, vitamin C solution with concentrations of 5, 10, 25, 50 and 100 ppm in methanol p.a. The percentage of sample inhibition was calculated based on the difference in absorbance of the DPPH solution with the absorbance of the sample solution divided by the absorbance of the DPPH solution multiplied by 100%. The concentration of antioxidants that can inhibit free radicals by 50% is called the $IC_{50}$ value. The $IC_{50}$ value can be calculated using a linear regression equation, where Y equals 50 and X indicates the $IC_{50}$ value of test sample [17].

## 3. RESULTS AND DISCUSSION

### 3.1. Preparation and Extraction of Gompang Batu Plant (*Lobelia nummularia* Lam)

This study used gompang batu plants from Ruhut Bosi Village, Pangaribuan District, North Sumatra. The weight of gompang batu simplicia was 325 grams. The obtained simplicia powder was then extracted. The extraction separation process uses the principle of solubility, namely like dissolves like. Simply put, polar



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solvents will dissolve polar molecules while nonpolar solvents will dissolve nonpolar compounds. Types of solvents with different levels of polarity are used to obtain optimal extracts from compounds of unknown types[18]. The extraction process is carried out to separate compounds or substances found in natural materials. The extraction process is influenced by several factors, including the type of solvent, extraction time, solvent concentration, and extraction method. Maceration is one of the chemical separation techniques used in this study, namely by soaking organic solvents at room temperature[19]. This study used solvents with graded polarity consisting of non-polar solvents (*n*-hexane), semi-polar solvents (ethyl acetate), and polar solvents (ethanol) for 3x24 hours in a closed container. This method can be used on materials that are not heat resistant and compounds that are not resistant to high temperatures. The filtrate obtained was concentrated using a rotary evaporator to produce a thick extract of gompang batu obtained in greenish black with an extract weight of *n*-Hexane 10.50 grams (yield percentage 3.25%), ethyl acetate extract 9.602 grams (yield percentage 2.95%) and ethanol extract 15.421 grams (yield percentage 4.74%).

3.2 Results of Phytochemical Screening Test of Gompang Batu Plant Extract (*L.nummularia* Lam)

Phytochemical screening is the initial step in qualitatively identifying chemical compounds contained in the sample's simplicia. This test is carried out by taking a small amount of thick extract of the sample from the evaporation results. Reagents that match the compounds to be identified are used in phytochemical screening. This study conducted phytochemical tests such as alkaloids, flavonoids, terpenoids/steroids, and saponins. The results of phytochemical screening of gompang batu ethanol extract are presented in Table 1.

**Table 1.** Phytochemical Screening Results of Ethanol Extract of Gompang Batu Plant (*L. nummularia* Lam)

| Phytochemical Test | Reagent | Test Results |
|---|---|---|
| Flavonoid | Mg + $HCL_{(p)}$ | +++ |
| Alkaloid | Wagner | +++ |
| | Dragendorff | +++ |
| Steroid | $CH_3COOH$ Anhidrat + $H_2SO_{4(p)}$ | - |
| Terpenoid | $CH_3COOH$ Anhidrat + $H_2SO_{4(p)}$ | +++ |
| Uji Tannin | $FeCl_3$ 1 % | +++ |
| Uji Saponin | Air + HCL 2 M | +++ |

**Information :**

(-) = Negative (++) = Clear
(+) = Quite Clear (+++) = Very clear

Table 1. shows secondary metabolites in the ethanol extract of gompang batu (*Lobelia nummularia* Lam) plants that positively contain terpenoids, flavonoids, alkaloids, tannins, and saponins.

Identification of flavonoids with Mg powder and HCLp shows a color change from green to orange. The oxidation reaction of flavonoid compounds, $Mg^{2+}$ will oxidize flavonoid compounds and form complexes with magnesium ions. The formation of yellow indicates good flavonoid content. In ethanol solution, magnesium metal will reduce polyhydroxy will be reduced by magnesium metal in HCL into benzo pyrylium salts which are red, yellow, or flavilium salts [20].


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Identification of alkaloids shows the presence of secondary metabolite content which is marked by the formation of orange precipitate, this is due to the presence of free electron pairs of alkaloids on the nitrogen atom which will bind to $K^+$ ions in the alkaloid reagent. Because bismuth salts are easily hydrolyzed to form bismuth ions ($BiO^+$), bismuth nitrate ions are dissolved in HCl to prevent hydrolysis reactions. Furthermore, $Bi^{3+}$ ions from bismuth nitrate react with potassium iodide to form a black precipitate of bismuth (III) iodide, which then dissolves in excess potassium iodide to form potassium tetraiodobismuthate alkaloid [21].

Identification of steroid in ethanol extracts showed negative results that formed an orange color, this color change reaction was triggered by acetic anhydride which is an acetylation reaction of the -OH group. The terpenoid test showed positive results containing terpenoid compounds that turned orange. Showing that the addition of acetic anhydride and sulfuric acid causes a bond with terpenoid or steroid compounds, which causes a color change reaction [15].

Identification of saponins in ethanol extracts showed positive results in the formation of stable foam because glycosides have the ability to obtain foam in water and hydrolyze it into glucose and other compounds, stable foam can be formed because saponins have active polar and nonpolar groups on their surface [22]. The results of tannin identification showed positive results with a color change to blackish green due to the presence of OH groups, tannin compounds are polar. As a result, after the addition of $FeCl_3$, tannins react with iron ions to form complex compounds (tannins and $Fe^{3+}$ ions) [21].

### 3.3 DPPH Method Antioxidant Test Results

The test results in this study on ethanol extract of gompang batu and on vitamin C (as a positive control) at concentrations of 5, 10, 25, 50 and 100 ppm obtained the results of absorbance measurements at a wavelength of 513 nm. In testing with DPPH was carried out in conditions of minimal light which can cause unstable results. The $IC_{50}$ value indicating the concentration of extract capable of inhibiting 50% of free radicals is shown in Table 2. The $IC_{50}$ value can be calculated from the linear regression equation ($y = ax + b$) obtained from the sample concentration and free radical activity (% inhibition) on the x and y axes respectively.



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***Table 2.*** *Antioxidant Test Results of Ethanol Extract of Gompang Batu Plant and Vitamin C*

| Extract Type | Concentration (ppm) | Absorbance Value Results | | | Average Absorbance | % Inhibition | $IC_{50}$ (ppm) |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | | |
| Vitamin C | 5 | 0,344 | 0,345 | 0,340 | 0,343 | 17,150 | 39,390 |
| | 10 | 0,305 | 0,307 | 0,304 | 0,305 | 26,248 | |
| | 25 | 0,245 | 0,242 | 0,244 | 0,244 | 41,143 | |
| | 50 | 0,134 | 0,130 | 0,132 | 0,132 | 68,116 | |
| | 100 | 0,035 | 0,031 | 0,034 | 0,033 | 91,948 | |
| Ethanol Extract | 5 | 0,400 | 0,392 | 0,399 | 0,397 | 4,106 | 135,095 |
| | 10 | 0,380 | 0,378 | 0,379 | 0,379 | 8,454 | |
| | 25 | 0,343 | 0,341 | 0,34 | 0,341 | 17,552 | |
| | 50 | 0,310 | 0,312 | 0,315 | 0,312 | 24,557 | |
| | 100 | 0,264 | 0,263 | 0,262 | 0,263 | 36,473 | |
| DPPH | 0,4 mM | 0,413 | 0,415 | 0,414 | 0,414 | | |

Based on the results of antioxidant test measurements on ethanol extract and vitamin C can be seen in Table 2 which shows the $IC_{50}$ value in ethanol extract of 135,095 ppm indicating moderate antioxidant activity which is marked by a color change from purple to yellow indicating a decrease in absorbance from free radicals can be seen in Figure 1. The linear equation between % inhibition and the concentration obtained can be seen in Figure 2, with the equation y = 0,3243x + 5,9046; $R^2$ = 0,949. While the results of the antioxidant test on the ascorbic acid (Vitamin C) comparison solution obtained an $IC_{50}$ value of 39,390 ppm indicating very strong antioxidant activity with a linear regression equation y = 0,7765x + 19,413; $R^2$ = 0,9559.

(a) (b) (c)

**Picture 1.** Color Changes in Antioxidant Activity Test (a = DPPH Solution, b = Vitamin C + DPPH Solution, c = Ethanol Extract Solution + DPPH)



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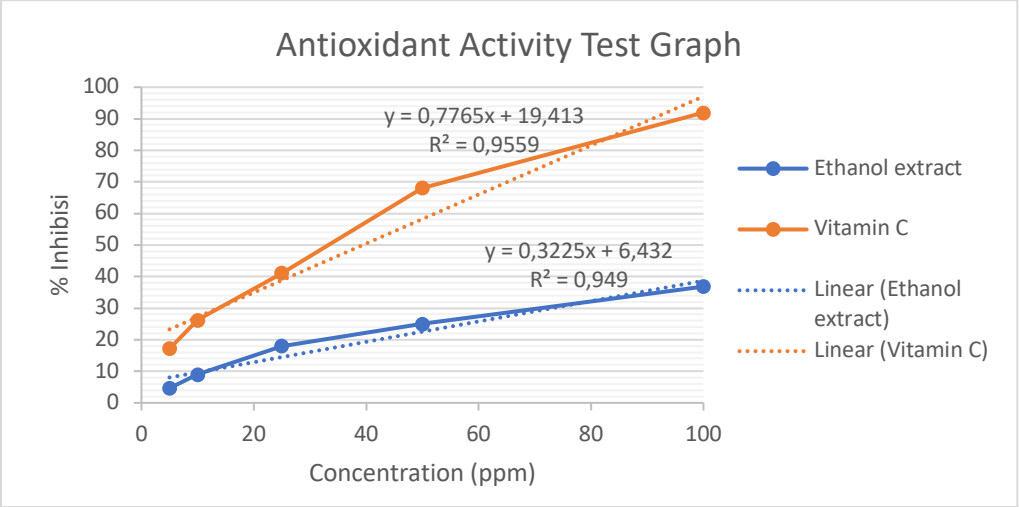
Antioxidant testing in Table 2 shows that the higher the sample concentration, the lower the absorbance value and the higher the % inhibition value. This is due to the concentration that has the potential to inhibit DPPH free radicals which increases, resulting in a decrease in the residual DPPH absorbance value. The results of antioxidant testing on gompang batu plant samples (*Lobelia nummularia* Lam) from the results of measuring the residual DPPH absorbance in the ethanol extract obtained an $IC_{50}$ value of 135,095 ppm. The $IC_{50}$ value is in the range of 100-150 ppm which indicates antioxidant activity in the moderate category. Antioxidant activity has a correlation of $R^2 = 0,949$. This shows that there is a relationship between the concentration of the solution and the absorption value and the standard curve obtained linearly.

The electron transfer reaction that occurs in the DPPH method is marked by a color change from purple to yellow, this is caused by the presence of secondary metabolites such as flavonoids, alkaloids, sterols or terpenoids, tannins, and saponins, which have the ability to function as antioxidants. The antioxidant mechanism of flavonoids is to capture ROS (Reactive Oxygen Species) directly. Flavonoids are oxidized by radicals, which produce more stable and non-reactive radicals [23]. Alkaloid content has the potential as an antioxidant by providing H atoms to free radicals through a reaction mechanism. This mechanism shows how alkaloids function as primary antioxidants. Terpenoids or steroids, perform primary antioxidant functions by stopping the formation of new free radicals by breaking the chain reaction and converting them into more stable products. Saponins consist of sapogenins, which are free parts of glycosides called aglycones. The antioxidant properties of this compound are indicated by the formation of hydroperoxides as secondary antioxidants, which inhibit the formation of lipid peroxides [24].




**Picture 2.** Antioxidant Activity Test Graph

The results of antioxidant activity in ascorbic acid (vitamin C) as a positive control have an $IC_{50}$ value of 39,390 ppm indicating very strong antioxidant activity, this is due to the fact that vitamin C is an effective antioxidant substance that has the ability to function as an antioxidant and prevent the negative effects of free radicals, by donating its electrons, this substance functions as an antioxidant. Comparison between the $IC_{50}$ value of gompang batu ethanol extract with vitamin C, the $IC_{50}$ value of vitamin C is greater than the ethanol extract, because vitamin C is a pure/single compound that has very strong antioxidant activity, while the ethanol extract still hangs complex compounds or a combination of other compound components.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## 4. CONCLUSION

Secondary metabolites contained in the ethanol extract of gompang batu (*Lobelia nummularia* Lam) are flavonoids, alkaloids, steroids, tannins and saponins. Ethanol extract of gompang batu has antioxidant activity with an $IC_{50}$ value of 135,095 ppm. The $IC_{50}$ value of vitamin C (positive control) was obtained at 39,390 ppm.s

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