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Secondary Metabolites Compounds and Bioactivity Extract of Telang Flowers

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ABSTRACT

Indonesia has many medicinal plants that are good for health. Telang flower (*Clitoria ternatea* L.) is used by the community as a traditional medicine. The purpose of this study is to ascertain what secondary metabolite compounds, antioxidant activity, and toxicity of telang flower extract. Extraction of telang flower was carried out using the maceration method with n-hexane, ethyl acetate, ethanol, and methanol solvents. Antioxidant activity test used 2,2-diphenyl-1-picrylhydrazyl (DPPH), while toxicity to shrimp larvae (*Artemia salina*) was tested using the Brine Shrimp Lethality Test (BSLT) method. Based on the results of phytochemical tests, telang flowers are understood to include secondary metabolites, such as alkaloids, flavonoids, steroids, triterpenoids, saponins, and phenolics. The antioxidant activity of ethanol extract of telang flower was classified as very strong compared to methanol, ethyl acetate, and n-hexane extracts, with IC_{50} values of 49.442 ppm, 50.648 ppm, 54.950 ppm, and 59.935 ppm, respectively. Meanwhile, methanol extract showed higher toxicity to *Artemia salina* than ethanol, ethyl acetate, and n-hexane extracts, with LC_{50} values of 76.995 ppm, 97.073 ppm, 266.162 ppm, and 297.934 ppm, respectively. Based on these results, ethanol extract of telang flower has antioxidant activity with a very strong category, while methanol, ethyl acetate, and n-hexane extracts are included in the strong category. On the other hand, the toxicity of methanol extracts and n-hexane extracts was not significant. In contrast, the toxicity of methanol and ethanol extracts to *Artemia salina* was categorized as strong, while the toxicity of ethyl acetate and n-hexane extracts was classified as weak. Telang flowers have potential as an alternative anticancer drug, but further research is needed.

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I. INTRODUCTION

The nation of Indonesia is a tropical country rich in various types of plants, so that various types of plants that are beneficial for health can be found. Research on medicinal plants continues to be carried out, one example used by the community as a traditional medicine is the butterfly pea flower (*Clitoria ternatea* L.). This plant is believed to be able to treat various diseases such as skin disorders, red and tired eyes, urinary problems, throat, vaginal discharge, purulent wounds, and is also used as an antidote (Sofiah et al., 2022). Telang flowers contain delphinidin (the main anthocyanin) which is dark blue or purple. Anthocyanin is one of the flavonoid groups. One of the naturally occurring substances in plants with a variety of pharmacological and biological effects is flavonoids. Flavonoids can boost antioxidant capacity and lessen damage from dangerous elements like UV rays and free radicals, according to several studies (Nair et al., 2015; Dacullo & Bitacura, 2022; Yang et al., 2024). According to (Juswardi et al., 2024; Kaushik et al., 2021) asserted that flavonoids, tannins, alkaloids, and saponins-

secondary metabolites present in Telang flowers have a variety of biological characteristics, such as anti-inflammatory, anti-cancer, anti-microbial, and antioxidant qualities. Other studies have shown that the pharmacological activity of several secondary metabolite compounds has been employed to treat a number of illnesses. Telang flowers from Tenggarong Seberang, East Kalimantan. Potentially found bioactive compounds that have antioxidant properties and are toxic to Artemia salina. Furthermore, telang flower can be further developed as herbal medicine and pharmaceutical drug preparations based on natural components.

II. MATERIALS AND METHODS

A. Materialst

The tools used in this study include vials, aluminum voil, analytical scales, volume pipettes, test tubes, aerators, dropper plates, cuvettes, UV-Vis spectrophotometers, rotary evaporators, and study lamps. The materials used in this study include butterfly pea flower samples from the species *Clitoria ternatea* L., methanol solvent, ethanol solvent, n-hexane solvent, ethyl acetate solvent, H_2SO_4, Mayer's reagent, Dragendorff's reagent, Wagner's reagent, Pb acetate 10%, HCl, $FeCl_3$ 3%, DMSO and Artemia salina shrimp larvae.

B. Methods

Preparation of Sample

The sample of this study was telang flowers taken from Tenggarong Seberang District, Kutai Kartanegara, East Kalimantan. Then, it was wet sorted using running water and then dried by airing it without direct sunlight. It was refined using a blender, sieved Telang Flowers powder. The weight of the Telang flower powder was measured at 500 g. Solvents (ethyl acetate, n-hexane, ethanol, and methanol) were used to extract the material for 3 × 24 hours. After filtering it via filter paper, a vacuum rotary evaporator was used to evaporate the solvent until a thick extract was produced. The thick extract obtained from this process was stored in a desiccator at a temperature of approximately 4ºC (Anggraini & Kusuma, 2019).

Phytochemical Test

Phytochemical testing is a qualitative testing method used to identify and de termine the compound content in plants by observing color changes (Maheshwaran et al., 2024).

Alkaloid Test

The alkaloid test was carried out by dissolving the concentrated extract using distilled water, then put into three test tubes. After adding a few drops of H_2SO_4 to each test tube, homogenize. Mayer reagent was introduced to the first test tube, followed by Dragendorff reagent in the second and Wagner reagent in the third, in amounts of three to five drops. Alkaloid test is considered positive if there is a precipitate or turbidity of at least two reactions of the three test tubes (Cahyaningsih et al., 2019).

Flavonoid Test

Flavanoid test is done by dissolving the concentrated extract using distilled water, then put into a test tube. Then, 1 mL of 10% Pb acetate was added to the test tube and homogenized. Positive results for flavanoid compounds are indicated by the formation of a yellowish brown color (Pramushinta & Ajiningrum, 2022).

Steroid / Terpenoid Test

Steroid and terpenoid tests were carried out by dissolving the concentrated extract using distilled water, then put into a test tube. Then, glacial acetic acid and $H_2SO_{4(p)}$ were added slowly through the test tube wall. Triterpenoids are indicated by the formation of a purple or orange hue, whereas steroids are indicated by the generation of a green or blue tint (Muhammad Ezzudin & Rabeta, 2018).

Saponin Test

Steroid and terpenoid tests were carried out by dissolving the concentrated extract using distilled water, then put into a test tube. Then, glacial acetic acid and $H_2SO_{4(p)}$ were added slowly through the test tube wall. Triterpenoids are indicated by the formation of a purple or orange hue, whereas steroids are indicated by the generation of a green or blue tint (Muhammad Ezzudin & Rabeta, 2018).

Phenolic Test

Phenolic test is done by dissolving the concentrated extract using distilled water and then placed in a test tube. Then added 1 mL of 1% $FeCl_3$ solution. When concentrated colors of purple, black, green, blue, or red form, the test indicates the presence of phenol chemical (Kuswandari et al., 2022).

Antioxidant Test

DPPH (2,2-diphenyl-1-picrylhydazyl) test for antioxidant activity is a technique that involves reacting antioxidant substances with DPPH free radical molecules (Ghozaly & Utami, 2017).

Preparation of DPPH Solution

2,2-diphenyl-1-picrylhydazyl (DPPH) was weighed and then dissolved in 50 mL of ethanol in a volumetric flask to create a 50 ppm solution (Nuryadi et al., 2019).

Preparation of Sample Solution

6.25 mg of n-hexane, ethyl acetate, ethanol, and methanol extracts of telang flowers were weighed, and they were dissolved with ethanol in a measuring flask to a volume of 25 mL to create a 250 ppm stock solution. The concentrations of 20, 40, 60, and 80 ppm were obtained by diluting the 250 ppm concentrated extract (Nuryadi et al., 2019).

Preparation of Comparison Solution

A stock solution of 40 ppm was made by weighing 1 mg of vitamin C then dissolved with ethanol until the volume was 25 mL using a volumetric flask, then diluted until a concentration variation of 2, 4, 6, and 8 ppm was obtained (Nuryadi et al., 2019).

Measurement of Antioxidant Power of Control Solution

The test was carried out by entering 4 mL of ethanol and 1 mL of 50 ppm DPPH into a test tube. It was then mixed and let to sit at room temperature for half an hour in a dark place. A UV-Vis spectrophotometer was used to measure the absorbance at a wavelength of 517 nm (Nuryadi et al., 2019).

Measurement of Antioxidant Power of Sample and Vitamin C

The test was carried out by entering each concentration of vitamin C sample extract as much as 4 mL and 1 mL of 50 ppm DPPH into a test tube. The absorbance was then measured three times at a wavelength of 517 nm using a UV-Vis spectrophotometer after it had been homogenized and let to stand at room temperature in a dark room for 30 minutes (Nuryadi et al., 2019).

Toxicity Test against *Artemia Salina*

Toxicity tests were conducted using the BSLT (Brine Shrimp Tetality Test) method. A 10 mL vial bottle was prepared and pipetted each test solution from concentrations of 200 ppm, 400 ppm, 600 ppm and 800 ppm. Then, 2 drops of DMSO (dimethyl sulfoxide) solvent were added to the vial bottle and continued with seawater added until the volume was 5 mL and 10 *Artemia Salina* shrimp larvae that were 2 days old. Each concentration was repeated 3 times. The test was conducted for 24 hours and the mortality rate of *Artemia Salina* shrimp larvae was observed. The hatching process of *Artemia Salina* shrimp larvae eggs uses seawater as the hatching medium. During the hatching process, oxygen levels must exceed 3 mg/L, so seawater must be aerated. Usually, within 24-36 hours, the eggs will hatch into larvae called nauplii. Nauplii that are active and 48 hours old are then used as test animals in experiments (Rosyadi et al., 2020).

C. Data Analysis

This study utilized phytochemical test, antioxidant test, and toxicity test. Phytochemical testing was conducted qualitatively by observing color changes to identify and determine the compound content of telang flowers. The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is used to test antioxidant activity, where the test involves a reaction between an antioxidant and the DPPH free radical molecule. In this study, the toxicity test used the BSLT (Brine Shrimp Mortality Test) method on *Artemia salina* shrimp larvae. Potential bioactive compounds found have antioxidant properties and are toxic to *Artemia salina*.

III. RESULT AND DISCUSSION

Based on Table 1. The yield of telang flowers extract with maceration method using methanol solvent obtained a higher yield of 6.916% compared to methanol solvent of 5.22%, ethyl-acetate of 3.884% while *n*-hexane extract of 1.068%. Methanol solvents have polar properties that can attract or extract secondary metabolites compounds of telang flowers in comparison to others solvents (Esati et al., 2024; Saputri et al., 2023). According to research (Hadzri et al., 2014; Nguyen et al., 2020) stated that methanol extract has more yield compared to other extracts.

Secondary metabolite substances including alkaloids, flavanoids, steroids, triterpenoids, saponins, and phenolics are present in telang flower extract. The results of phytochemical screening of *n*-hexane extracts of telang flowers state that the extract contains secondary metabolites, such as alkaloids and flavanoids. The results of ethyl acetate telang flower extract contain alkaloid, flavanoid and phenolic compounds. The ethanol extract of telang flowers contains alkaloids, flavanoids, steroids, tritepenoids, saponins and phenolics. The methanol extract of telang flowers contains alkaloids, flavanoids, tritepenoids and phenolics. These results are in accordance with previous research (Ramdhini & Dewi, 2024; S Pertiwi et al., 2024; Ananda et al., 2024) which states that telang flowers positively contain alkaloid, flavanoid, steroid, triterpenoid, saponin, and phenolic compounds. compounds that are beneficial for health and cause the death of *Artemia Salina* larvae.

IV. FIGURE AND TABLE

The sample used in this study was the telang flower From Tenggarong Seberang, East Kalimantan.

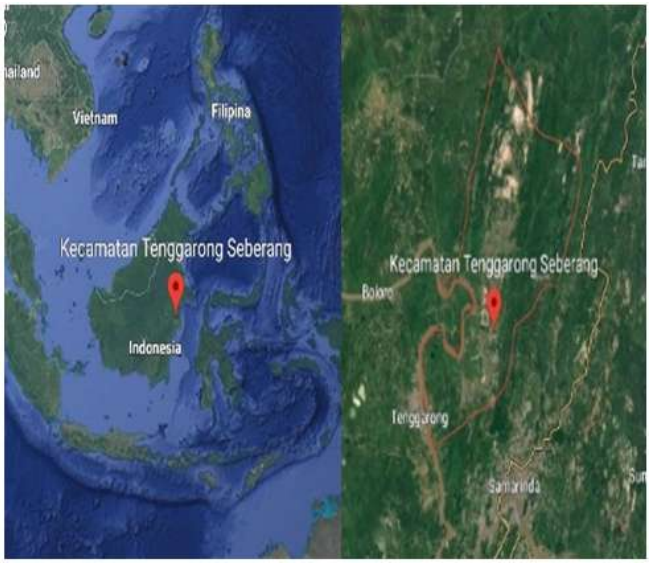

Figure 1. Sampling site Tenggarong Seberang sub-district, East Kalimantan

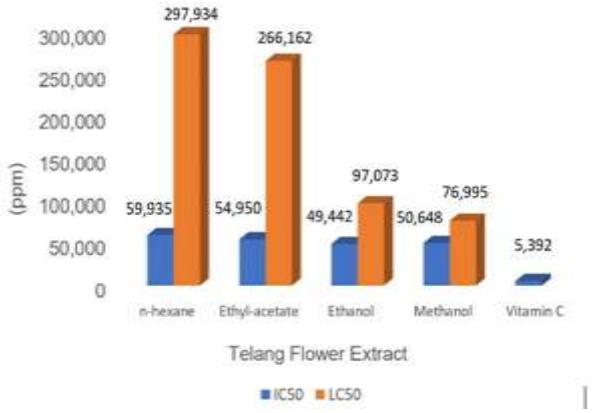

Figure 2. Antioxidant activity and toxicity of telang flowers extracts

The preparation of simplisia involves several steps, starting with collecting fresh telang flowers, followed by wet sorting, washing, drying, dry sorting, and grinding the simplisia. The extraction results of telang flowers are presented in **Table 1.**

Table 1. **Results of Telang Flower Extract on Various Solvents**

Solvent	Powdered Powder (gram)	Extract Weight (gram)	Yield (%)	Characteristics		
				Texture	Color	Smell
n-hexane	250	2,67	1,068	Thrick	Brownish Yellow	Typical of *n*-heksana
Ethyl-acetate	250	9,71	3,884	Thrick	Brownish Yellow	Typical of etil-asetat
Ethanol	250	13,05	5,22	Thrick	Brownish Yellow	Typical etanol
Methanol	250	17,29	6,916	Thrick	Brownish Yellow	Typical of methanol

Qualitative phytochemical screening was carried out by observing color changes to identify secondary metabolite compounds contained in n-hexane, ethyl acetate, ethanol, and methanol extracts of telang flowers. The results of phytochemical analysis of telang flower extracts in detail presented in **Table 2.**

Table 2. **Phytochemical Screening Results of Telang Flowers (*Clitoria ternatea L*.)**

Chemical Compunds		Phythochemical Test Results of Telang Flower Extracts				Description
		n-hexane	Ethyl-acetate	Ethanol	Methanol	
Alkaloids	Dragendorff	+ (orange precipitate)	+ (orange precipitate)	+ (orange precipitate)	+ (orange precipitate)	(+) when orange precipitate is formed
	Wagner	+ (brown precipitate)	+ (brown precipitate)	+ (brown precipitate)	+ (brown precipitate)	(+) when brown precipitate forms
	Mayer	- (no precipitate formed)	- (no precipitate formed)	- (no precipitate formed)	- (no precipitate formed)	(+) when a white precipitate forms
Flavanoids		+ (cloudy yellow)	+ (cloudy yellow)	+ (green)	+ (yellow cloudy)	(+) when yellow, orange, red and green colors are formed
Steroids		- (clear)	- (clear)	+ (purple)	- (faint orange)	(+) when blue or purple color is formed
Triterpenoids		- (clear)	- (clear)	+ (purple)	+ (faint orange)	(+) when orange, red or purple color is formed
Saponins		- (white and no foam forms)	- (white and no foam forms)	+ (pink and foam formed)	- (white and no foam is formed)	(+) when stable foam and froth are formed
Fenolics		- (orange)	+ (blackish green)	+ (brownish red)	+ (black)	(+) when red, green, purple, blue, or solid black color is formed

Table 3. below shows the outcomes of tests for extract toxicity using the BSLT method against *Artemia salina* and antioxidant activity applying the DPPH method

Table 3. Antioxidant and Toxicity Test Results of Telang Flowers Extracts

Telang Flower Extracts	Characteristics			
	IC_{50} (ppm)	Category	LC_{50} (ppm)	Category
n-hexane	59,935	Strong	297,934	Weak Toxic
Ethyl-acetate	54,950	Strong	266,162	Weak Toxic
Ethanol	49,442	Very Strong	97,073	Strong Toxic
Methanol	50,648	Very Strong	76,995	Strong Toxic
Vitamin C	5,392	Very Strong	-	-

CONCLUSION

The Telang flower extract contains secondary metabolite compounds such as steroids, triterpenoids, flavonoids, saponins, and phenolics. The ethanol extract of telang flowers has a stronger antioxidant activity in the very strong category with an IC_{50} value of 49,442 ppm, while the methanol, ethyl acetate, and *n*-hexane extracts of telang flowers have IC_{50} values of 50,648 ppm, 54,950 ppm, and 59,935 ppm in the strong category, respectively. The toxicity of methanol and ethanol extracts of telang flowers against shrimp larvae (*Artemia salina*) is included in the strong category by having LC_{50} < 100 ppm. While n-hexane and ethyl acetate extracts of telang flowers are included in the less toxic category with LC_{50} values > 200 ppm..

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