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Standardization of Telang Flower Extract for Bioactive Compound Assessment using Solvent Variations

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ABSTRACT

Telang flower (Clitoria ternatea) is widely used in traditional medicine as an anticancer, anti-inflammatory, and antimicrobial. This study aims to standardize telang flower extract using maceration method with 96% ethanol, ethyl acetate, and n-hexane solvents. Parameters evaluated include yield weight, drying shrinkage, specific gravity, water content, ash content, and phytochemical analysis. The results showed that ethanol solvent produced the highest yield weight, specific gravity, drying shrinkage, ash content and moisture content, respectively, namely 11.83%, 0.90 ml, 3.55%, 7.3% and 2.95%. Likewise, with the results of phytochemical testing, 96% ethanol solvent produces more diverse types of secondary metabolites, namely alkaloids, flavonoids, tannins, and saponins, while n-hexane mainly extracts terpenoids and steroids. These findings indicate that ethanol is the most effective solvent for obtaining various bioactive compounds from Telang flowers. **Keywords**: Standardization, Solvent Variations, Telang Flowers

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1. INTRODUCTION

Indonesia has a tropical climate, making many kinds of plants that can live in it. Most of these plants have medicinal potential. Like the telang flower plant. This plant, which belongs to the *Leguminoceae* family, is a creeper tree with an age of 1-3 years. In general, it has bluish purple flowers, and some species are pink and white. The shape of the flower is like the wings of a butterfly. Telang flower seeds are like legumes. Lately, the utilization of telang flowers in the community tends to increase. This is because telang flowers have potential as a treatment that is easy to grow and obtain around the community.

In Aprillia E.N.'s research, 2023 showed that telang flower extract was able to reduce blood pressure in elderly patients suffering from hypertension. In addition, research by Fadel et al., 2023 showed that telang flower extract was able to reduce blood sugar in mice that had previously been induced with alloxan. Phytochemical analysis testing on ethanol extracts of telang flowers shows that there are flavonoids, alkaloids, saponins and tannins (Ramdani et al., 2021). In the research of Febrianti et al., 2022 showed that the secondary metabolite content of telang flower extract can function as an antimicrobial (Febrianti et al., 2022).

The many potentials of telang flower require standardization to ensure consistent quality, safety, and efficacy in medicinal applications. This study evaluated specific and non-specific standardization parameters of telang flower extracts using various solvents to determine the optimal extraction process. Specific parameter testing on telang flower plants includes determination and organoleptic test of the extract. Meanwhile, non-specific parameter testing includes weight yield, specific gravity, drying shrinkage, moisture content and ash content of the extract. The solvent variations used in this study were 96% ethanol, ethyl acetate and n-hexane.

2. RESEARCH METHODS

Determinasi

In the determination test of bayang flowers, plant parts such as leaves, stems, flowers, seeds and roots are needed. Almost all plant parts are identified with the plant classification book. Telang flowers used in this study were obtained from Glagah District, Banyuwangi.

Maceration Extraction Process

Fresh telang flowers as much as 2.500 gram were dried in an oven at 50° C for 12 hours, then blended and sieved with mesh 60, so that the simplisia powder was obtained. Then the maceration process was carried out for 3×24 hours at room temperature with occasional stirring. Then filtering is done with filter paper. The filtrate obtained was evaporated until a thick extract was obtained. This step was carried out on each solvent 96% ethanol, ethyl acetate and n-hexane.

Weight Yield Calculation Process

The 1 gram thick extract obtained from each solvent was then weighed. The weight of the dried simplisia was compared to the weight of the thick extract, then multiplied by 100%, until the percentage yield was obtained. Formula of yield weight (Kementerian Kesehatan RI, 2017):

Percentage Weight Yield =
$$\frac{\text{Weight of Extract}}{\text{Weight of Simplisia}} \times 100\%$$

Drying Shrinkage Calculation Process

The 1 gram of extract was put into a porcelain cup that had previously been heated in an oven at 105°C for 30 minutes. Then the porcelain cup containing the thick extract was put into the oven at 105°C until a constant weight was obtained. Drying shrinkage formula (Kementerian Kesehatan RI, 2017):

Percentage drying shrinkage=
$$\frac{\text{Weight of extract after treatment}}{\text{Weight of simplisia}} \times 100\%$$

Specific gravity calculation process

Weigh the empty pycnometer (m1). Then weigh the pycnometer that has been filled with solvent to the brim (m2). Dilute the extract with solvent until get an extract concentration of 5%, put it in a pycnometer, then weigh it (m3). Specific gravity formula (Kementerian Kesehatan RI, 2017):

Specific gravity =
$$\frac{\text{m3-m1}}{\text{m3-m1}}$$
 x Specific gravity solvent

Water Content Calculation Process

Put the empty porcelain cup into the oven at 105°C for 15 minutes, then weigh (m1). Put 2 gram extract into the porcelain cup, then weigh (m2), then put it in an oven at 110°C for 2 hours or until a constant weight is obtained (m3). Formula of water content (Kementerian Kesehatan RI, 2017):

Percentage water content =
$$\frac{\text{m2-m3}}{\text{m2-m1}} \times 100\%$$

Ash Content Calculation Process

Put the empty porcelain cup into the oven at 110°C for 15 minutes, the weigh (m1). Put 2 grams of extract into the porcelain cup then weigh (m2) then put it in a furnance at 80°C for 3 hours, the weigh the cup containing the ash extract (m3). Formula for ash content (Kementerian Kesehatan RI, 2017):

Percentage ash content =
$$\frac{\text{m2-m3}}{\text{m2-m1}} \times 100\%$$

Phytochemical Testing Alkaloids

Thick 0.5 gram extract add 2 ml chloroform, 5 ml NH3 and 2 ml H2SO4 2N then shake until homogeneous. Transfer into 3 test tubes, each 2.5 ml. solution in the tube and add Mayer reagent 3 drops. Tube II add Dragendorf reagent 3 drops and tube III add Wagner reagent 3 drops. The presence of alkaloids in the Mayer reagent tube is characterized by the formation of a white precipitate. In the second tube Dragendorf reagent will form a red to orange precipitate if there are alkaloids. In the third tube Wagner reagent will form a brown precipitate if there are alkaloids. This test is done three times repetition on each solvent ethanol 96%, ethyl acetate and n-hexane.

Flavonoids

A thick extract of 0.5 grams was added with 2 drops of 10% NaOH. The presence of alkaloids is indicated by an orange color change. This test was done three times repetition of each solvent ethanol, ethyl acetate and n-hexane.

Steroids

A thick extract 0.5 gram extract add 3 drops of chloroform and 3 drops of anhydrous acetic acid. Stir until homogeneous. Add H2SO4 as much as 3 drops. The presence of steroids is indicated by a blue color. This test was repeated three times on each solvent 96% ethanol, ethyl acetate and n-hexane.

Saponin

A thick extract of 0.5 grams is added to 9 ml of distilled water and heated until bubbles form then add 2 N HCl, shake vigorously until foam forms. The presence of saponins is indicated by the presence of foam. This test was repeated three times for each solvent 96% ethanol, ethyl acetate and n-hexane.

Tanin

A thick extract 0.5 grams of extract was added to 4 ml of hot water and homogenized then filtered. The filtrate obtained was then filtered using filter paper. Then the filtrate is mixed with 1-3 drops of FeCl3 1%. There is tannin if a dark brown or bluish black color is formed. This test was repeated three times on each solvent 96% ethanol, ethyl acetate and n-hexane.

Terpenoid

A thick extract 0.5 g of extract is added 1 ml of chloroform, 1 ml of anhydrous acetate and 0.4 ml of concentrated H2SO4. If terpenoids are present, a purple, red or orange color will form. This test was repeated three times in each solvent 96% ethanol, ethyl acetate and n-hexane.

3. RESULTS AND DISCUSSION

Standardisation of Specific Parameters Determination

The results of the determination test started that the pants used were true telang flowers (*Clitoria ternatea*) belonging to the Fabaceae family. The determination test aims to determine the classification of plants and avoid sampling errors.

Organoleptic

The organoleptic test aims to determine the sensory properties of an extract from the five senses such as shape, aroma, taste and texture. This test is carried out for the initial acceptance of a product that is in accordance with good sensory quality. The results of the organoleptic test are shown in table 1

Table 1. Organoleptic Test Results of Telang Flower Extract (*Clitoria ternatea*)

Pelarut		Organoleptic Test Results			
	Shape	Aroma	Color		
Ethanol 96%	Viscous	Solvent typical	Deep brown		
N-hexane	Viscous	Solvent typical	Intense green		
Ethyl acetate	Viscous	Solvent typical	Intense yellow		

Standardisation of Non Specific Parameters Weight Yield

The yield in ethanol 96% solvent was higher when compared to n-hexane and ethyl acetate solvents, which was 11.83% (Table 2). This value is in accordance with the standard weight yield \geq 10% (Kemenkes RI, 2017). The calculation of yield weight aims to determine the quantity of extract produced as well as the efficiency during the extraction methods, the amount of solvent and the ratio of solvent to simplisia. The ethanol solvent produces the highest yield weight because the polarity of the solvent affects the quantity of the extract. Ethanol is a polar solvent that has the ability to dissolve organic sompounds more widely when compared to non polar and semi polar solvents (Senduk et al., 2020)

Table 2. Weight yield, specific gravity and drying shrinkage of telang flowers (*C. ternatea*)

Solvent	Simplisia Weight (g)	Weight of Thick Extract (g)	Yield Weight (%)	Weight Type (g/mL)	Drying Shrinkage (%)
Etanol	200	23,67	11,83	0,90	3,55
Etil Asetat	200	10,24	5,12	0,87	1,02
N-heksana	200	7,62	3,81	0,68	0,22

Specific gravity

The calculation of specific gravity is the chemical content dissolved in the extract as a ratio of the density of a substance to the density of water with a mass value per unit volume. The specific gravity values in ethanol, ethyl acetate and n-hexane solvent were 0.90 g/Ml., 0.87 g/ml and 0.68 g/ml respectively. The highest value is found in ethanol solvent. The results of specific gravity test have met the standard which is \leq 10% (Suhendy et al., 2022)

Drying Shrinkage

The maximum limit of compounds lost in the extraction process can be known by calculating the drying shrinkage. The principle of calculating drying shrinkage is done by calculating the value of the extract after being given treatment in an oven 105°C until a constant weight isobtained. Drying shrinkage in all solvent has met the standard which is ≤10% (Kemenkes RI, 2017). Drying shrinkage in ethanol, ethyl acetate and n-hexane solvents were 3.55%, 1.02% and 0.22% respectively. The highest value of drying shrinkage was found in ethanol solvent. This is because the polarity in ethanol has a tendency to attract water in the extract, therefore the drying shrinkage value in ethanol extract is higher when compared to ethyl acetate and hexane solvents (Nadhira et al., 2024). Drying shrinkage testing is important to do because it relates to the moisture content and residual solvent in the extract.

Water Content

The moisture content of an extract will determine its quality, stability and shelf life. If the moisture content exceeds the standard limit of \leq 10% (Kemenkes RI, 2017) then it is

feared that contamination can occur whuch affect the quality of the extract. Water content testing in this study met the standard for all extract. The water content of the ethanol, athyl acetate and n-hexane extract was 7.3%, 6.3% and 3% respectively (Wijaya & Mutia Rissa, 2024).

Ash Content

Ash content the amount of minerals or inorganic residues still present in the extract after the ash process is the purpose of the ash content test. The presence of minerals such as calcium, magnesium and iron is very important for human biological activity (Badaring et al., 2020). However high levels of minerals in the extract are also not allowed, because it is feared that there is heavy metal contamination in the extract. Standard ash content in extract $\leq 10\%$ (Kemenkes RI, 2017). The results of ash content in this study have met the standard, ethanol, ethyl acetate and n-hexane solvents respectively are 2.95%, 1.34% and 0.75% (Table 3).

Phyrochemical Screening Test Results

Phytochemical testing aims to determine secondary metabolites. The results of phytochemical screening in this study showed that ethanol solvent can dissolve almost all secondary metabolites tested expect terpenoids and steroids (Tabel 4)

Alkaloid test with Mayer, Dragendorf and Wagner reagents showed positive results in 96% ethanol and ethyl acetate solvents. Positive results on alkaloids are characterized by the presence of a precipitate. In n-hexane solvent there is precipitate (Badaring et al., 2020). This is because n-hexane solvent is a non polar solvent while alkaloids are mostly nitrogen which is partially positive which makes it polar (Klau & Hesturini, 2021). Therefore, n-hexane solvent is not suitable for dissolving polar secondary metabolite compounds

Screening	Solvent			
Test Results	Etanol 96%	Ethyl Acetate	N-hexane	
Alkaloid:		<u> </u>		
- Mayer	+	+	-	
- Dragendorf	+	+	-	
- Wagner	+	+	-	
Flavonoid	+	+	-	
Terpenoid	-	+	+	
Steroid	-	-	+	
Tanin	+	+	-	
Saponin	+	+	-	

Table 3. Phytochemical Test Results of Telang Flower Extract (Clitoria ternatea)

Descriptions:

(+): Secondary metabolites present(-): No Secondary metabolites present

The flavonoid test in 96% ethanol and ethyl acetate solvents showed positive results indicated by a colour change to red or orange. However in n-hexane solvent there was no colour chane, thus showing negative results. This is because flavonoids are polyphenolic compounds that contain many hydroxyl groups (-OH) which make them polar. These hydroxyl groups can dissolve with polar solvents (Yolanda Putri et al., 2023).

The terpenoid test in n-hexane and ehyl acetate solvents ahowed positive results,

marked by a colour change to purple, red or orange. The non polar nature of terpenoids is due to the dominant hydrocarbon group, therefore terpenoids are easily dissolved in non polar solvents such as n-hexane (Mierza et al., 2023).

The steroid test showed positive results marked by blue colour changes found in n-hexane and ethyl acetate solvents. Steroids are secondary metabolite compounds that are non polar, so that polar solvents such as ethanol show negative results (Siddiqui et al., 2024).

The tanin tes in ethanol 96% and ethyl acetate solvents showed positive results marked by change in colour to brown or black. Tanin are polar making it difficult to dissolve in non polar solvents such as n-hexane (Suhaila et al., 2024)

The saponin test in ethanol 96% and ethyl acetate solvent showed positive results characterized by foam. Saponins have polar and non polar parts, so they are amphipathic. However, saponins are more difficult to dissolve in non polar solvents such as n-hexane (Anggraeni Putri et al., 2023).

4. CONCLUSION

This study successfully standardized Telang flower extract using various solvents. Ethanol is the most effective solvent because it successively produces the highest yield weight, specific gravity, drying shrinkage, type, moisture content and ash content, namely 11.83%; 0.90 g; 3.55%; 7.3 g/ml; 2.95%. Likewise, with the results of phytochemical tests, 96% ethanol solvent is able to extract more diverse secondary metabolites such as bioactive compounds such as alkaloids, flavonoids, and tannins. These findings suggest ethanol as the optimal solvent for future pharmaceutical applications of Telang flower extract. Further research is recommended to explore its bioactivity in vivo.

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