

Toxicity Test Toward Dichloromethane Fraction from White Frangipani Leaves (*Plumeria alba*)

Nurul Hidajati*, Qodriyah
Chemistry Department Faculty of Mathematic and Science
Universitas Negeri Surabaya
Surabaya, Indonesia
nurulhidajati@unesa.ac.id

Abstract— White frangipani (*Plumeria alba*) is a plant that is widely grown in Indonesia especially in Java and Bali. It has been conducted a research to know the chemical content of the plant leaf. The leaves of plant are extracted with dichloromethane solvent and then separated by using chromatographic techniques to obtain 4 combined fractions namely fraction A, B, C, and D. The chromatography results obtained 4 fractions namely A, B, C, and D. Except fraction D, after doing the phytochemical test, the three other fractions are known to be steroids. Using the same manner, fraction D is known containing alkaloid. Fraction D is then tested for knowing toxicity of it using BSLT method and resulted LC50 value to be 54,448 ppm.

Keywords— White frangipani, *Plumeria alba*, BSLT, Toxicity

I. INTRODUCTION

Frangipani is a type of flowering plants that are widely grown in Indonesia [2]. White frangipani including Apocynaceae family is known as one of medicinal plants in which leaves can be used as laxative and anti itch, fruit and stem bark can be used as anti-inflammatory [4]. Frangipani stems and leaves contain flavonoids and alkaloids and can be used as traditional medicine [3].

The chemical content of white frangipani include fulvoplumierin (antibacterial), in its essential oil there are compounds of eugenol, linalool, farsenol, geraniol, phenetolalcohol, and stigmasterol. It also contains serotonic acid, agoniadin, plumeric acid, plumerid, and lupeol [1]. In the hexane extract of the bark of the plant contains steroid, in dichloromethane extracts contain steroid and alkaloids compounds, whereas in the ethanol extract contain flavonoid compounds, tannins, saponins, alkaloids, phenolics, and steroids.

Toxicity study is a study method to examine the relevant effects on the biology system to determine the right dose. Toxicity is defined as drug parameters that can produce damage to living things. Brine Shrimp Lethality Test (BSLT) is a fast and inexpensive way to test a toxicity of samples using shrimp larvae *Artemia salina* Leach. by monitoring the mortality rate of the shrimp larvae.

II. METHOD

A. Equipment

Buchner funnel, Erlenmeyer flask, a set of Vacuum Liquid Chromatographic (VLC) tools, vacuum, rotary evaporator, micropipette, vial bottle, test tube, spatula.

B. Material

2 kg of leaves white frangipani plant, VLC silica, sea water, *Artemisia salina* larvae, aquades, dichloromethane, DMSO, HgCl₂, KI, Bi (NO₃)₂, HNO₃, I₂, filter paper whatman, HCl 2N, acetic anhydrous, concentrated H₂SO₄, FeCl₃ 1%, 70% ethanol, Mg band, concentrated HCl, HCl 1N, 10% NaCl, 1% gelatin.

C. Sample Preparation

As much as ± 1 kg of dried powder of white frangipani leaves is macerated by using dichloromethane solvent for 1x24 hours and repeated 3 times to gain macerates. It was then concentrated using a vacuum rotary evaporator. ± 20 grams of thickened extract. The extract is further separated by using VLC with hexane: ethyl acetate in graduating to yield 48 fractions. Based on VLC analysis, these fractions can further be combined to be four combined fractions, namely fraction A(1-15), B (16 - 30), C (31 - 37) and D (38 - 48). All of combined fractions was treated by phytochemical test to obtain the chemical components in each fractions as reported below.

D. Phytochemical Test

Phytochemical tests were performed on dichloromethane extract and fraction above.

1) Flavonoid Test

1 mL sample is inserted into a drop plate, then added 0.1 gram of Magnesium band and 2 drops of concentrated hydrochloric acid. The positive test is marked by the appearance of red.

2) Steroids and Triterpenoids Test

1 mL sample is inserted into the drop plate, added (CH₃CH₂)₂O and concentrated sulfuric acid. purple, orange, and golden yellow colors for triterpenoids meanwhile blue and green colors for steroids.

3) Tanin Test

1 mL sample is inserted into the drop plate, added 1 drop of 10% sodium chloride, 1% gelatin and sodium chloride again. White sediments for tannin.

4) Phenolic Test

1 mL sample is inserted into the drop plate then added 2 drops of 1 iron (III) chloride. Red, blue, green, red, purple, or solid black formed indicate a phenolic.

5) Alkaloid Test

1 mL sample was inserted into the test tube and added 1 ml of chloroform and 1 mL of ammonia, subsequently heated in a water bath. The next mixture is added 3 drops of 2N sulfuric acid and is divided into 3 parts. Each mixed portion is added 3-drop test reagent of the Mayer reagent (yellow precipitate indicates positive test), 3 drops of Dragendorff reagent (red sediment indicates positive test), and 3 drops of Wagner reagent (brown or reddish deposition indicates positive test).

6) Saponin Test

1 mL sample was put into the test tube and added 2 mL of aquades while shaken for 1 minute, then added 2 drops of 1N hydrochloric acid. The positive test is characterized by a stable foam formed.

E. Toxicity Test Using Brine Shrimp Lethality Test Method

1) Preparation of *A. salina* Leach larvae

A gram of *A. Salina* Leach shrimp is soaked in seawater and illuminated and aerated for 48 hours.

2) Preparation of Test Solutions

The test solution came from a predetermined fraction and prepared a solution of concentration 0, 10, 25, 50, 75, and 100 ppm

3) Toxicity Test

Prepare a container that has been filled with each of 10 shrimp larvae *A. salina* aged 48 hours. Each container was then given each solution test with a volume of 6 mL. Furthermore after 24 hours calculated the number of dead shrimp larvae.

III. RESULT AND DISCUSSIONS

The dichloromethane extract of *P. alba* was separated by using VLC with eluent hexane : ethyl acetate in graduating polarity to obtain 48 fractions. Based on TLC analysis, it was obtained 4 combined fractions namely fractions A(1-15), B(16-30), C (31-37) and D (40-48). Furthermore extracts and each fraction were treated for phytochemical test and the results can be shown in the Table I.

TABLE I. THE RESULT OF PHYTOCHEMICAL TEST OF DICHLOROMETHANE EXTRACT (EDM) AND FRACTION

Secondary Metabolites	EDM	Fractions			
		A	B	C	D
Alkaloid					
- Mayer	+	-	-	+	-
- Dragendorff	+	-	-	-	+
- Wagner	-	-	-	-	+
Flavonoid	-	-	-	-	-
Tannin	-	-	-	-	-
Phenolic	-	-	-	-	-
Steroid	+	+	+	+	-
Triterpenoid	-	-	-	-	-

In the Table I, it was seemed that dichloromethane extract is known that it contains alkaloids and steroids. While the fraction A, B, and C contain steroid. Fraction C also

shows the content of alkaloid in Mayer test but there is no alkaloids for two other reagents. The last fraction D contains alkaloids and then especially taken as a sample for the toxicity test using BSLT method. The result of toxicity test of D is shown in Table II.

TABLE II. THE RESULT OF TOXICITY TEST FOR FRACTION D

Test Sample (ppm)	Number of Death			% of Death			Average Number of Deaths (15)
	1	2	3	1	2	3	
0	0	0	0	0	0	0	0
10	3	2	2	30	20	20	23,33
25	4	3	4	40	30	40	36,67
50	6	7	6	60	70	60	63,33
75	7	6	5	70	60	50	60
100	10	8	9	100	80	90	90

Based on probit analysis, it is obtained LC50 value of fraction D with 54.448 ppm. BSLT method is used to determine the toxicity level of an extract or isolates before the sample is applied to cancer cells treatment. If a sample (e.g. isolate) has a LC50 value to be <1000 ppm, then thus the compound is toxic [5].

IV. CONCLUSION

The separation of dichloromethane extract was of *P. Alba* leaf followed by doing phytochemical test was allegedly to contain alkaloids. The fraction containing alkaloid was then treated by toxicity test to yield LC50 value is 54.448 and included as toxic compounds.

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