Toxicity Test of Mathanol Fraction of Mentawan (Poikilospermum suaveolens Blume Merr) Stem Which Has Potential as Anticancer

P Salempa1, D E Pratiwi2, Ramdani3
1,2,3Chemistry Department, Universitas Negeri Makassar
e-mail: pince.salempa57@gmail.com

Abstract. Mentawan plant (Poikilospermum suaveolens Blume Merr) is a species of the genus poikilospermum, including the urticaceae family, which is traditionally used by the community for medicine. This plant has the ability to treat eye diseases, fever, malaria and itching and has the potential as an anticancer. This research method includes extraction, fractionation, and fraction bioactivity test with the brine shrimp lethality test (BSLT) against Artemia salina. From the results of the toxicity test using the BSLT method, the activity of methanol fractions with LC50 values were 4,8752; 62,8492; 85,5460; 45,7088; 28,1838; 11,985; 33.2123 µg / mL. Based on the LC50 value, it could be concluded that mentawan plant has potential as anticancer.

Keywords : Poikilospermum suaveolens, toxicity, anticancer, Artemia salina

1. Introduction

Indonesia is rich in various biodiversity that has the potential to be developed as medicine or medicinal raw materials. As many as 30,000 plant species found in Indonesia, 7,000 species are medicinal plants and 1,000 species have been used for treatment and overcoming health problems [1].

Medicinal plants are natural substances capable of producing certain active compounds. The active compound has different benefits depending on the type of compound, namely as an anticancer, antibacterial, antifungal and antioxidant [2]. One of the plants used by the community for traditional medicine is the Mentawan plant (Poikilospermum suaveolens Blume Merr).

Mentawan is a plant used to treat eye diseases, fever, malaria, and itching [3]. Mentawan is widely distributed in the Malesia region including India and South China. The leaves are used as an ulcer treatment. In Sabah, this species is used as a postpartum treatment, and in Negeri Sembilan it is used as an eye treatment [4]. In addition, Mentawan leaves have a toxicity of 24.7% which was tested on mice [3].

N-hexane extract of Mentawan fruit has an inhibitory effect on C. albicans bacteria by 0.50%, ethyl acetate extract of Mentawan fruit has an inhibitory effect of 3.35%, and ethanol extract of mentawan fruit is 8.86% [5]. While the methanol extract of P. suaveolens leaves has a CTC50 (cytotoxic concentration) value of 575.44 mg / L against viruses and bacteria [6]. The phytochemical test of P. suaveolens ethanol extract positively contained tannin compounds using FeCl3 reagent, triterpenoid using Liebermann-Burchard reagent, flavonoids using Shinoda reagent, alkaloids using Hager reagent, and cardiac glycosides using Keller Killiani's reagent [7].
Some of the compounds found in the Urticaceae family are 7-O-Routoside, β-sitosterol, diosmetin-7-O-Routine, phytol, stigmasterol, luzepol, maslinic acid, 7-oxo-β-sitosterol and 7-hydroxy sitosterol. Based on this description, it is necessary to carry out further research to study the chemical content contained in Mentawan plants.

2. Research Methods

2.1 Sampling
Mentawan (P. suaveolens Blume Merr) stem samples were obtained from Salenrang Village, Bontoa District, Maros Regency, South Sulawesi.

2.2 Extraction
Seven kilograms of P. suaveolens Blume Merr) stem was macerated with methanol for 3 x 24 hours. The methanol extract obtained was filtered and evaporated using an evaporator to obtain a concentrated extract in the form of a brown residue. Then analyzed by thin layer chromatography (TLC) and tested its activity.

2.3 Fractionation
The concentrated extract was fractionated using vacuum column chromatography into several fractions, the eluent used could be determined based on the TLC results. The fractions obtained were then monitored by TLC and fractions which had the same stain were combined into one fraction. Then one of the combined fractions is selected for column chromatography flash using the appropriate eluent. This process can be repeated until pure isolates are obtained. The purity of the isolates was analyzed by using three eluent system TLC and the melting point test was determined.

Bioactivity Test with BSLT (Brine Shrimp Letality Test)
One mg of sample in an Ependorf tube was dissolved with 100 µL of DMSO then diluted with 150 µL of aquabidest. The dilution was taken 200 µL and diluted again with 600 µL of aquabidest.

Furthermore, the dilution was carried out in microplate with various concentrations and the sample volume per hole was 100 µL by triplo. Shrimp fry that were 48 hours old were pipetted as much as 100 µL with 7-15 shrimp fry, put in the microplate containing the sample, then incubated for 24 hours. For control, the same treatment was carried out without using samples. Furthermore, the dead and living shrimp were counted and the LC50 was determined.

The LC50 values representing the toxicity of the extract were less than 500 µg / mL and 200 µg / mL, respectively. This toxicity value is divided into two categories, high toxicity for LC50 <100 µg / mL and low toxicity for LC50> 100 µg / mL.

3. Results and Discussion

3.1 Extraction of Bioactive Compounds
The concentrated methanol extract was fractionated by KKV using n-hexane, EtOAc, acetone and methanol as eluent with increased polarity order to obtain 22 fractions. Based on TLC analysis, fractions with the same RF were combined to obtain seven main fractions (A-G). The fractions were then evaporated at room temperature so that a creamy powder-shaped crystal was obtained in fraction G. The crystals were recrystallized using n-hexane solvent to produce an isolate weighing 0.0536 g with a melting point of 155-156 °C. The purity of the isolates was analyzed by using TLC with three eluent system.
3.2 Bioactivity Test with BSLT (Brine Shrimp Letality Test)
The toxicity test result of methanol fractions of Mentawan (*P. suaveolens* Blume Merr) plants on *Artemia salina* shrimp larvae or BSLT (Brine Shrimp Lethality Test) can be seen in Table 1.

**Table 1.** Activity data of methanol fraction of *P. suaveolens* Blume Merr with the LC$_{50}$ value

| Number | Fraction Code | LC$_{50}$ (ppm) |
|--------|---------------|-----------------|
| 1      | A             | 4,8752          |
| 2      | B             | 62,8492         |
| 3      | C             | 85,5460         |
| 4      | D             | 45,7088         |
| 5      | E             | 28,1838         |
| 6      | F             | 114,4985        |
| 7      | G             | 33,2123         |

**Table 2.** Observation Data of Brine Shrimp (*Artemia salina* Leach.) Larvae Mortality in Fraction A after 24 Hours of Treatment

| Sample (ppm) | Absis (log of sample) | Amount of Dead Shrimp | Mortality Percentage (%) | Ordinate (Probit value) |
|--------------|-----------------------|-----------------------|--------------------------|-------------------------|
| 1            | 0,00                  | 13                    | 43                       | 4,75                    |
| 10           | 1,00                  | 17                    | 57                       | 4,92                    |
| 100          | 2,00                  | 30                    | 100                      | 5,84                    |

Relationship between logs (samples) to the probit value of fraction A of methanol fraction of *P. Suaveolens* Blume Merr stem can be seen in Figure 1.

**Figure 1.** Relationship between log (sample) and probit value of A fraction of methanol extract of *P. suaveolens* Blume Merr stem

Based on the picture, the linear regression equation obtained was $y = 0,545x + 4,625$, so that the LC$_{50}$ value is calculated based on the formula, then:
\[ y = 0.545 \times + 4.625 \]

obtained LC\textsubscript{50} (probit 1):
\[
\frac{y - 4.625}{0.545} = x
\]
\[\frac{\log x}{0.545} = 0.688\]
\[x = 4,8752 \text{ ppm}\]

LC\textsubscript{50} value for methanol fractions of \textit{P. suaveolens} Blume Merr plant stem against \textit{Artemia salina} Leach shrimp larvae respectively: 4,8752; 62,8492; 85,5460; 45,7088; 28,1838; 11,985; 33,2123 µg / mL which is included in the high toxicity category (high toxic). The LC\textsubscript{50} values that represent the toxicity of the extract are less than 500 µg / mL and 200 µg / mL. This toxicity value is divided into two categories, namely high toxicity for LC\textsubscript{50} <100 µg / mL and low for LC\textsubscript{50} > 100 µg / mL [12].

4. Conclusion
Based on the results obtained, it could be concluded that from the results of the toxicity test using the BSLT method, the activity of methanol fractions was obtained with the respective LC\textsubscript{50} values: 4,8752; 62,8492; 85,5460; 45,7088; 28,1838; 11,985; 33,2123 µg / mL which has potential as an anticancer.

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