Toxicity and Compound Identification of Padinaaustralis Extract

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Abstract: People awareness of the importance of health has increased significantly in the last decade, it forces people to find alternative treatments which are cheaper and safer when compared with the use of synthetic drugs. Research on antibacterial activity of Padina australis against Escherichia coli have been conducted, but it does not identify which active compounds in the Padina australis extract that has potential as an antibacterial. Identification phytol compounds from the extracts of Padina australis and toxicity tests have been conducted using BSLT and GCMS methods. The results of this research identified that Padina australis extract with ethanol has antibacterial activity value of LC50 177.83 ppm. Phytochemical test results show that active ingredient of Padina australis extract is terpenoid compounds, and that triterpenoids has potential as an antibacterial. GC-MS test result shows that active compound of Padina australis extract consists of phytol compound which has 90-99% similarity with steroids, phenols, fatty acids, carboxylic acids, hydrocarbons, and proteins. Compound identification test of active Padina australis extracts through GC-MS methods show that phytol compounds are useful as antibacterial and the toxicity test results show that phytol compounds are not cytotoxic.

Index Terms: Antibacterial, Extract Padina Australis, LC50.

I. INTRODUCTION

People awareness of the importance of health has increased significantly in the last decade [1]. It forces the public to find an alternative treatment that is economically cheaper and safer when compared with the use of synthetic drugs. Many people change their lifestyle by going back to nature and use medicines from natural ingredients. As an island country with coastline length of 81,000 km, Indonesia is an enormous source of many natural resources. In spite of that, land-based plant’s properties still tend to be explored more than water-based or sea-based plant’s, including seaweed’s. According to Rasyid (2004), several species of seaweed in Indonesia can be used as a medicine, but it is currently experiencing problems because the research is not developed yet [2]. Therefore, the use of seaweed as medicine is still limited. The research conducted by [3] showed that Padinaaustralis, has antibacterial activity against Escherichia coli, but which bioactive compounds from the extracts of Padinaaustralis that has potential as an antibacterial has not been tested.

II. MATERIALS AND METHODS

Identification of the active compound and Padinaaustralis toxicity extract tests were conducted in Bogor Agriculture Institute’s Laboratory of Microbiology and University of Pakuan’s Laboratory of Pharmacy, both located in Bogor. Materials that were used for this research are Padinaaustralis seaweed from Coastal Waters of Bayah, Banten, distilled water, ethanol 96%, materials for phytochemical test, and substances for GC-MS and BSLT tests. Tools used in this research are a set of GCMS (Gas Chromatography Mass Spectrophotometry) to identify active compounds inside Padinaaustralis extract, a set of BSLT (Brine Shrimp Lethality test) method toxicity test tools, and glass tools and other tools commonly used in microbiology laboratory and pharmacy.

A. Moisture and Ash Content Establishment

Moisture establishment was done by using a moisture balance tool. One gram of simplicia was set in the
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Temperature of 105°C for 10 minutes, and then the moisture content is measured. The result is a percentage of how much water contained in the simplicia.

Ash content was established by inserting 2 grams of dried simplicia into a crucible, and then put it in a kiln at a temperature of 700°C until it becomes ashes. After that, cool it down and weighed it to constant weight.

\[
\text{Total ash} = \frac{\text{Weight of ash}}{\text{Initial Weight of Simplicia}} \times 100\%
\]

B. *Padina australis* Extract Establishment

*Padina australis* utilized in this research was made by using a modified version of the research conducted by [9][10]. 250 grams of *Padina australis* simplicia was put inside a brown bottle, then a solvent is added until its volume reached 1000 ml with a ratio of 1:4 (weight/volume). The extraction procedure was done by soaking the sample with 96% ethanol. The result of maceration was then filtered using Whatman 42 filter paper to get the filtrate and residue. The soaking process was done 3 times until the filtrate appears to be clear. Concentration of the obtained filtrate was then increased by using a rotary vacuum evaporator at a temperature of 400°C to obtain a crude extract (crude extract) in the form of a paste or also known as the viscous extract. The yield (amount of reactant obtained through chemical reaction) of the extract is calculated by comparing simplicia extract’s initial weight and final weight.

\[
\text{Yield Extract} = \frac{\text{The Weight of Obtained Extract}}{\text{Simplicia Initial Weight}} \times 100\%
\]

C. Phytochemical Test of *Padina australis* Extract

Phytochemical test was conducted to identify chemical compound and to determine the classification of the active compound inside *Padina australis* extract in ethanol 96%. Phytochemical test was conducted on alkaloids test, tannins test, steroids/triterpenoids test, saponins test, and phenol test [11][12].

D. Active Compound Test using GC-MS Method

GC-MS Method is used to analyze the identified active compounds in *P. australis*. *Padina australis* extract samples in 96% ethanol solvent were analyzed by using GC-MS instrument Agilent 5975C to determine the organic compounds inside. GC-MS activity test results show there are 121 compound that has antibacterial activity, including terpenoids, triterpenoids, and pythol.

E. Toxicity Test of *Padina australis* Extract Using BSLT Method

Toxicity test was conducted based on the method of [13][14], with *Artemia salina* larvae as test animals. *A. salina* eggs were first incubated in artificial seawater (38 g of salt in a 1000 ml water) under a 20watt fluorescent lamp. After 48 hours the eggs hatch into nauplii instar III / IV and ready to be used as test animals. The larvae of *A. salina* then inserted into the vial which already contains a sample extract solution with 50, 100, and 500 doses of the series, and 1000 ppm with three replications. All the vials were incubated at room temperature for 24 hours under the light of a 20watt fluorescent lamp. Observations were conducted after 24 hours to see the number of dead *Artemia salina* at each concentration. LC50 value in ug/ml or ppm was determined by using probit analysis with MINITAB version 13.2 software with 95% confidence interval [15].

III. RESULT

A. Moisture Concentration and Ash Content of *Padina australis*

The amount of *Padina australis* obtained directly from Banten Bayah Coastal waters in wet form was 2 kg. *Padina australis* was dried under direct sunlight for five days. After being dried, the amount of *Padina australis* simplicia that was obtained is 250 grams. This means it has 83.33% drying shrinkage. Ash content was determined by using a furnace. The ash content of *Padina australis* simplicia that was obtained is 14.53%, and is a higher value than the result in the research conducted by [10] at 5.50%.

B. Phytochemical Test Result

Phytochemical test result of dry *Padina australis* extract in ethanol 96% showed positive result in triterpenes, alkaloids, tannins, phenols, quinone and saponin group with qualitatively good result.

C. Active Compound Test Result using GC-MS Method

Testing dry *Padina australis* extract in 96% ethanol using GC-MS method is the next stage to identify the compound present in the sample. The compound listed in Table 1 is the compound that has similarity percentage ranges between 90-99%.

D. Analysis of Toxicity Extract Using BSLT

The toxicity test results of *Padina australis* extract in ethanol 96% to shrimp larvae is obtained by counting the number of dead shrimp larvae. By knowing the number of dead larvae, LC50 value can be calculated by conducting probit analysis. LC50 is concentration of a substance that can cause death to 50% population of test animals.

<p>| Table 1. Identification Result of Active Compound In <em>Padina australis</em> Extract |</p>
<table>
<thead>
<tr>
<th>No</th>
<th>Name of Compound</th>
<th>Retention Time (minutes)</th>
<th>% Area</th>
<th>Devolution</th>
<th>Molecular Weight</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phyrol</td>
<td>11.962</td>
<td>2.13</td>
<td>91</td>
<td>296.31</td>
<td><img src="image" alt="Structure" /></td>
</tr>
</tbody>
</table>
IV. DISCUSSION

A. Moisture Concentration and Ash Content of Padina australis

The obtained moisture concentration of Padina australis at the temperature of 105°C is 6.68%. This is almost equal to the value in the research conducted by [16], which is 6.4% extraction of Padina australis is conducted by maceration method using ethanol 96%.

The amount of dry Padina australis extract in ethanol 96% obtained through that method is 26.72 grams. The amount obtained is used to calculate the amount of yield extract. The obtained amount of yield extract of dry Padina australis extract in ethanol 96% is 10.68%. This indicates that Padina australis contains bioactive components which tend to dissolve in polar solvents. The process of extracting some herbal plants using different solvents conducted by [17] produced the highest yield in polar solvents. The magnitude of the yield extract shows that a large number of active components are being extracted by the compound during the maceration process. This is similar to the report of [18] that the high yield value indicates the number of bioactive components.

B. Phytochemical Test

These results are consistent with the research of [19]-[20] who argued that the phytochemical compounds detected in Padina australis extract—alkaloids, phenols, steroids, triterpenoids, tannins and saponins—are effective as an antibacterial and antifungal.

C. Active Compound Test Using GC-MS Method

Active compound test of Padina australis extract in 96% ethanol by using GC-MS method produces 17 compounds. The test result of positive activity against dry extract Padina australis in ethanol 96% is the activity as an antibacterial namely class terpenoids, alkaloids, and steroids. Terpenoids compounds that have antibacterial activity are monoterpenoid, linalool, diterpenoid, phytol, triterpenoids, and saponins [21]-[23]. Based on the previous pychochemical test results, the possible compounds contained in the sample are terpenoids, phenolics, saponins, alkaloids, and tannins. The GC-MS test indicates that the sample containing 96% ethanol has a good concentration of terpenoids, saponins, alkaloids, phenolics, steroids and fatty acids, from all of the possible compounds found in the sample.

D. Analysis of Toxicity Extract Using BSLT

This research shows that LC50 value of Padina australis extract samples with 96% ethanol is 177.83 ug/ml. [24], explained that chemical compound is potentially bioactive if it has LC50 values less than 1000 pg/ml, and has potential as an antibacterial when it is less than 200 ug/ml. Therefore, Padina australis extract in 96% ethanol can be said to have potential as an antibacterial.

V. CONCLUSION

The phytochemical test of dry Padina australis extract in 96% ethanol shows strong positive results in triterpenoid, and shows that Padina australis is effective as an antibacterial.

The Active compound test of Padina australis extract in 96% ethanol by using GC-MS method produces 17 compounds and compounds suspected phytol potential as antibacterial. LC50 value obtained through toxicity test of Padina australis extract samples in 96% ethanol by utilizing BSLT method is 177.83 g/ml. Therefore, Padina australis extract in 96% ethanol can be said to have potential bioactivity as an antibacterial.

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