Toxicity and Identification of Compound Extract *Padina australis*

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Abstract. People awareness of the importance of health has increased significantly in the last decade, it requires the people to find alternative treatments which more economical and relatively safe when compared with the use of synthetic drugs. Research on antibacterial activity of *Padina australis* as *Escherichia coli* have been conducted, but it does not identified yet the active compounds in the extract *Padina australis* which potential as an antibacterial. Identification *phyto*l compounds from the extracts of *Padina australis* and toxicity tests have been conducted using BSLT and GCMS methods. The results of the research identification of *Padina australis* extract with ethanol has antibacterial activity with LC_{50} 177.83 ppm. From the test results of phytochemical, known active ingredient of the extract *Padina australis* is terpenoid compounds, and triterpenoids potential as an antibacterial. From the test results of GC-MS known active compound content of the extract *Padina australis* are compounds phytol 90-99% similarity compounds with steroids, phenols, fatty acids, carboxylic acids, hydrocarbons, and proteins. The conclusion of the test compound identification of active extracts of *Padina australis* through GC-MS methods obtained phytol compounds are useful as antibacterial and toxicity test results phytol compounds are not cytotoxic

Keywords: antibacterial, extract *Padina australis*, LC_{50}

1. Introduction

People awareness of the importance of health has increased significantly in the last decade (Golberg, 1994). This fact requires the public to find an alternative treatment that is more economical and relatively safe when compared with the use of synthetic drugs. Many people change their life style back to nature and take drugs from natural materials. Indonesia as an archipelagic country with a long coastline of 81,000 km is the large coastal and oceans that have a variety of biological resources. The tendency of properties terrestrial plants exploration is more than water plants from the sea, one of plants is seaweed. According to Rasyid (2004), several species of seaweed in Indonesia can be used as a drug, but it is currently experiencing problems because the research does not developed yet. Therefore, the use of the drugs are still limited. The research result conducted by Haryani, T.S. and Triastinurmiatiningsih (2008) showed that *Padina australis*, has antibacterial activity against Escherichia coli, but bioactive compounds from the extracts of *Padina australis* which has potential as an antibacterial *has not been tested*,.

Padina australis is a seaweed that comes from the class of *Phaeophyceae* (brown seaweed). *Padina australis* can be found on rocky coasts and choppy. This seaweed contains pigment fukosantin (brown), violasantin, chlorophyll a, chlorophyll c, β-carotene and xantofil, whereas in the pharmaceutical industry, algin or alginic acid types *Padina australis* is used in the formation of pills, ointments, toothpaste, lotions and creams (Susanto, 2008). Fukosantin is a part of carotenoid which has the formula of $C_42H_58O_6$, orange, including xanthophyl group of carotenoids. This pigment is found in some types of brown algae, including *Padina australis.*

According to Kim *et a*l. (2010), fucosantine compound has the ability as an anti-carcinogenic, antibacterial, anti-inflammatory, protecting cells against the harmful ingredients, such as H_2O_2, and free-radical scavengers or antioxidants. As a health food supplement, fucosantin has been shown to have no toxic properties (Limantara & Heriyanto, 2010).

Testing on the activity and toxicity of plant extracts can be done using Brine Shrimp Lethality Test (BSLT), the method used to isolate the bioactive compounds of plant extracts. BSLT method is often done in a preliminary test for screening or screening pharmacological activity on medicinal plants to support the use of medicinal plants in the traditional and modern treatment, to detect toxic effects of fungi, the toxicity of the plant extract, heavy metals, pesticides and cytotoxicity (Krishnaraju *et al*., 2005: Tamat, *et al*, 2007). Meanwhile, to identify active compounds in a material used GC-MS method (Gas Chromatography Mass Spectrophotometry), the method used for identifying a compound contained in the gas mixture and also determine the concentration of a substance in the gas phase.
Based on the problems above, the research conducted the test of the identification of active compounds *Padina australis* extract with ethanol using GC-MS method, and extract toxicity tests using BSLT.

2. Material And Method

Identification of the active compound and *Padina australis* toxicity extract tests conducted in the Laboratory of Microbiology, Bogor Agriculture Institute and Laboratory of Pharmacy, University of Pakuan, Bogor. Materials and tools used including seaweed *Padina australis* originating from Coastal Waters of Bayah, Banten, distilled water, ethanol of 96%, materials for testing phytochemicals, substances for testing of GC-MS and BSLT, the tools used in this study are a set of tools GCMS (Gas Chromatography Mass Spectrophotometry) to identify active compounds extract *Padina australis*, and a set of toxicity test extracts tool using BSLT method (Brine Shrimp Lethality test), as well as glass tools and other tools commonly used in microbiology laboratory and pharmacy.

2.1. Determination of Moisture and Ash Simplisia

Determination of water done using a moisture balance, by weighing 1 gram simplisia and set the temperature of 105^0C in 10 minutes, and then measured the moisture content, the result is a percent water content in the bulbs. Determination of ash content done by inserting 2 grams of dried botanicals into a crucible, then put in a kiln at a temperature of 700^0C to ashes, cooled and weighed to constant weight.

$$\text{Total ash} = \frac{\text{Weight of ash}}{\text{Initial Weight of simplicia}} \text{ x100 \%}$$

2.2. The Use of *Padina australis* Extract

Padina australis conducted based on the research of Juniarti *et al*. (2009) and Santoso *et al.* (2012) which is modified. Simplisia *Padina australis* weighed as much as 250 grams and put in a brown bottle, then added a solvent to achieve a final volume of 1000 ml with a ratio of 1: 4 (w / v). The extraction procedure done by soaking sample with 96% ethanol. The results of maceration filtered using Whatman filter paper 42, so the result is filtrated and residue. Soaking done 3 times up to be clear. The filtration obtained and concentrated by rotary vacuum evaporator at a temperature of 400C to obtain a crude extract (crude extract) in the form of pasta or a so-called viscous extract. The yield of extract is calculated by comparing the initial weight and final weight of botanicals extracts produced.

$$\text{Rendemen Extract} = \frac{\text{weight extract gained}}{\text{Initial weight Simplisia}} \text{ x 100\%}$$

2.3. Fitokimia Test of *Padina australis* Extract

Identification of chemical constituents in the extracts of *Padina australis* in the ethanol 96% done the test of phytochemical extracts to determine the classification of the active compound. Phytochemical test conducted on alkaloids test, tannins test, steroids/ triterpenoids test, saponins test, and phenol test (Harborne, 1987, Bhat, *et al*. 2009).

2.4. AnalysisTest of using GC-MS

Identification result of compounds active classes of *Padina australis* extract followed by analysis using GC-MS tool set. *Padina australis* extract samples in 96% ethanol solvent analyzed by GC-MS instrument Agilent 5975C to determine the organic compounds. The identification result shows that most resemble components of several components from the high molecular weight and intense peak, and the top is the closest resemblance (Pavia, *et al*. 2006).

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

Toxicity test done based on the method of Meyer *et al,(1982)* and McLaughlin & Rogers, and Carballo *et al*.(1998), with *Artemia salina* larvae as test animals. First, *A. salina* eggs incubated in artificial seawater (38 g of salt in a 1000 ml water) under a 20 watt 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* inserted into the vial which already contains a sample extract solution with 50,100,500 doses of the series, and 1000 ppm with three replications. All the vials were incubated at room temperature for 24 hours under a 20 watt fluorescent lamp illumination. Observations done after 24 hours to see the number of dead *Artemia salina* at each concentration. Pricing LC50 in ug / ml or ppm performed using probit analysis with the program MINITAB version 13.2 with 95% confidence interval (Zakaria *et al.*, 2011)

3. Result

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

Padina australis obtained directly from Banten Bayah Coastal waters in wet form as much as 2 kg. *Padina australis* dried under direct sunlight for five days. Total simplicia *Padina australis* obtained after drying as much as 250 grams, so it has a 83.33% drying shrinkage. Determination of ash content is done using a furnace. The ash content of simplicia *Padina australis* obtained by 14.53%, is higher than the research result value of Santoso, *et al* (2012) at 5.50%

3.2. Result of Phytochemical Test

The test result of phytochemical dry extract *Padina australis* ethanol 96% showed positive result in group triterpenes, alkaloids, tannins, phenols, quinone and saponin with qualitatively good result, but the positive result obtained in the compound of the steroid / triterpenoid.

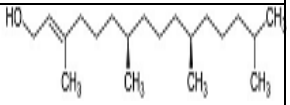
3.3. Result Analysis Extract Using GC-MS Method

GC-MS analysis Agilent 5975C dry extract *Padina australis* in 96% ethanol is an advanced stage to identify the compound present in the sample. The compound listed in Table 1 that have a similarity percentage ranges between 90-99%.

3.4. Analysis of Toxicity Extract Using BSLT

The toxicity test *Padina australis* extract in ethanol 96% of the shrimp larvae can be determined by counting the number of dead shrimp larvae. The results of shrimp larvae that die can be calculated LC_{50} value using probit analysis. LC_{50} is the concentration of a substance that can cause death of 50% of the population of test animals.

Table. Results Identification of Active Compounds Extract *Padina australis*

No	Name of Compound	Retention Time (minutes)	% area	Devolution	molecular weight	Structure
1	phytol	11.962	2.13	91	296,31	

4. Discussion

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

The water content of *Padina australis* at the temperature of 105˚ is obtained to 6.68%. This value is almost equal to the water content of the research result of Fitrya, *et al* (2010) that is equal to 6.4%. Extraction *Padina australis* done by maceration method using ethanol 96%. From the results obtained maceration *Padina australis* ethanol dry extract 96% to 26.72 grams. Based on the result obtained, dry extract calculated by yield of extract. The result of yield dry extract *Padina australis* 96% ethanol obtained a value of 10.68%. This indicates that the *Padina australis* contains bioactive components which tend to dissolve in polar solvents. The process of extracting some herbal plants using different solvents conducted by Suryanto *et al*, (2008) produced the highest yield in polar solvents. The magnitude of the yield of the extract showed the large number of active components extracted compounds during the maceration process. This is similar to the report of Nurhayati *et al*, (2009) that the high yield value indicates the number of bioactive components.

4.2. Phytochemical Test

These results are consistent with the research of Fitriany. (2012) and Suganda (2007) who argued that the phytochemical compounds were detected in *Padina australis* extract, namely alkaloids, phenols, steroids, triterpenoids, tannins and saponins are efficacious as an antibacterial and antifungal.

4.3. Analysis Extract Using GC-MS Method

The results of GC-MS activity test against *Padina australis* extract 96% ethanol to produce 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, antineoplasticibiotik steroids, terpenoids have antibacterial activity that is monoterpenoid, linalool, diterpenoid, phytol, triterpenoids, saponins (Grayson, 2000; Bigham *et al*., 2003; Lim *et al* 2006). Based on the test results, it can be expected phytochemical compounds contained in the sample is a class of compounds terpenoids, phenolics, saponins, alkaloids, and tannins. All of the suspected compounds in the sample, the GC-MS test indicates a group of terpenoids, saponins, alkaloids, phenolics, steroids and fatty acids in a solvent containing 96% ethanol in the presence of compounds in the sample concentration.

4.4. Analysis of Toxicity Extract Using BSLT

From this experiment LC_{50} value *Padina australis* extract samples with 96% ethanol at 177.83 ug / ml. Restasari, *et al* (2009) explained that the chemical compounds potentially bioactive if it has LC_{50} values of less than 1000 pg / ml, and when less than 200 ug / ml potential as an antibacterial. Therefore extract *Padina australis* in 96% ethanol can be said to have potential as an antibacterial bioactivity.

5. Conclusion

The test results of phytochemical dry extract *Padina australis* 96% ethanol showed strong positive results in the steroid / triterpenoid, and efficacious as antibacterial.

The results of GC-MS activity test against *Padina australis* extract 96% ethanol produces 17 compounds, and compounds suspected phytol potential as antibacterial.

The toxicity test *Padina australis* extract in 96% ethanol by BSLT method LC_{50} values obtained sample of 177.83 g / ml. Therefore extract *Padina australis* in 96% ethanol can be said to have the potential bioactivity as antibacterial

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