

Formulation of *Padina australis* Extract Tablet as Antibacterial *Escherichia coli*

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Abstract. *Padina australis* (*P.australis*) is one of the seaweed that have a potential as antibacterial *Escherichia coli* (*E.coli*). Triterpenoids and steroids are secondary metabolites compounds contained in seaweed, and has a variety of activities as bactericide, and hypo-allergenic. This study aims to establish tablet formulation and determine the effectiveness against of *E.coli* bacteria causing diarrheal disease. The formulation was prepared with these treatment: formula I (20%), formula II (25%), formula III (30%), using a negative control in the form of tablet with no active ingredient, and a positive control using amoxycillin (10 ppm). The formulas' effectiveness was tested by calculating the inhibition zone width (IZW) –a clear zone resulting from the three formulas, negative, and positive control– using Kirby-Bauer method. The result shows that the formulation with active ingredient of 30% of *P. australis* extract has an inhibitory activity of 7.7 mm and effective as antibacterial *Escherichia coli*. Thus it can be concluded that the formulation of 30% of *P. australis* extract tablet is the most effective formula as antibacterial *E. coli* causing diarrhea disease.

Keyword: *Padina australis*, tablets, antibacterial, *Escherichia coli*.

1. Introduction

Padina australis is a seaweed that belongs to Phaeophyceae class (brown seaweed), it is often found on the shore and the large choppy beaches. It contains pigment fukosantin (brown), violasantin, chlorophyll a, chlorophyll c, β -carotene and xantofil. In the pharmaceutical industry, algin or alginic acid from *Padina australis* is used in the pills formation, ointments, dental cleansers, lotions and creams. Fukosantin is a part of carotenoids with formula $C_{42}H_{58}O_6$, orange color, including xantofil group of carotenoids. These pigments are found in several species of brown algae including *Padina australis*. (Putra, 2006, Sergiana, 2009). According Kim *et al.* (2010) research, the compound fukosantin has the ability as anti-carcinogenic, antibacterial, anti-inflammatory, protecting cells towards harmful materials such as H_2O_2 , and antidote free radicals or as antioxidants. As a health food supplement, fukosantin has been proven to have no toxic (Limantara and Heriyanto, 2010). Based on Haryani *et al.*, (2014) research result, active compounds can be derived from the extract of ethanol *P.australis* belonging to phenol class compounds namely triterpenoid, flavonoid and alkaloids, additionally from identification results using GC-MS produced *phytol* compounds that have activity as antibacterial and allergy.

Diarrhea is an infection disease caused by microorganisms which include bacteria, viruses and other parasites such as fungi, worms and protozoa. They are infectious disease that still become barrier in developing countries, especially Indonesia (Amiruddin, 2007). *Escherichia coli* is a bacterium that lives in the human digestive tract, it is also a Gram-negative bacteria that can cause diarrhea (Adnyana, *et al.*, 2004). Based on Haryani, *et al.* (2014) research result showed

that *P. australis* ethanol extract at 100% concentration (from 37.5 gram extract yield 15% yield) effectively inhibited the growth of *E. coli* bacteria with a resistor diameter of 14.37 mm. Tablets are solid, unified, made by pressing in the form of a flat tube, either flat or convex surface, containing a drug with or without a filler. Tablets have a variety of shapes, sizes, weights, hardness, thickness dissolution and disintegration. Widyani (2011) stated, *Turbinaria decurens* type seaweed can be made as tablet preparations which can be easy, comfortable and practical to consume by people. Besides containing active substances, it also contains additives that can be useful as fillers, binders, crushers, lubricants, flavorings, dyes and sweeteners (Lachman and Liebermann, 1994). Based on the discussion above, it is necessary to determine the formulation of tablet extract *P. australis*, as well as testing the effectiveness of tablets as antibacterial preparation *E. coli*.

2. Materials And Methods

2.1. Tools and Materials

The tools used in this test are analytical balance, Vacuum dry, Petri dish, ose needle, ALL AMERICAN autoclave, Fisher incubator, Whatman paper disc , AND MX-50 moisture balance, VULCAN A-560 furnace, mesh, tablet printer and microbiology laboratory equipment.

Materials used include seaweed (*Padina australis*) obtained from Bayah Beach, Banten, *Escherichia coli* strain test bacteria obtained from Department of Biology Bogor Agricultural Institute, comparative antibiotic amoxycillin tablet 500 mg, amylum manihot (amprotab), PVP, and Mg stearate.

2.2. Producing Simplicia *P. australis*

P. australis sample is cleaned from impurities stuck with running water. The sample used in this extract production was dry simplicia dried in an oven at 50 ° C for two days until it dried. Then dry simplicia is mashed (sodium powder) and sieved with mesh 20, then weighed and stored in a clean and sealed container.

2.2. Testing Water Content of *P. australis*

Determination of moisture content is done using moisture balance. The sample entered as much as 1 gram into the prepared tool, at the temperature of 105⁰C. Then the results listed on the moisture balance and noted. Measurements are done twice.

2.3. Testing powder Content of *P. australis*

Weighed as much as 2 grams of the powder of simplicia, inserted into the discharge cracked silicates and turrets. Gently emitted until the charcoal is exhausted, cooled, and then weighed. If the charcoal cannot be removed, hot water should be added, and filtered through filter paper in the same crucible. The filtrate inserted into the crucible, evaporated, permitted until the weight is fixed, and weighed, then the content of powder is calculated against the material that has been dried in the air (Depkes RI, 1995).

$$\text{Powder Content (\%)} = \frac{(\text{Crucible Weight} + \text{Simplisia Powder}) - \text{Empty Crucible Weight}}{\text{Powder Simplisia Weight}} \times 100\%$$

2.4. Phytochemical Analysis

Phytochemical tests are performed on nano propolis extract to determine the content of flavonoids, saponins, tannins, alkaloids, triterpenoids and steroids qualitatively.

2.4.1. Alkaloid Testing

A total of 0.5 grams of sample are added 1% ammonia bases solution and chloroform in the reaction tube, then the chloroform layer (bottom layer) piped and added HCl 2N then shaken. The solvent result divided into four, there are the blanks and the rest are reacted with each Mayer and Dragendorff. The positive result causes white deposits and mixtures with Dragendorff reagents causing turbidity and orange sediment (Soebagyo, *et al.*, 2007).

2.4.2. Tanin Testing

A total of 0.5 grams extract of *Padina australis* inserted into the reaction tube and dissolved with a small aquadest and heated over a water bath then dripped with 1% gelatin solution and 10% sodium chloride (1: 1). The positive result of white sediment formation (Soebagyo, *et al.*, 2007).

2.4.3. Saponin testing

A total of 0.5 grams extract of *Padina australis* dissolved with Aquadest and heated on the top of a water bath. After cooling, the solvent in the test tube is shaken tightly for \pm 30 seconds. The positive result of consistent foaming for several minutes with the addition of 1 drop of dilute HCl is still formed foam (Soebagyo, *et al.*, 2007)

2.4.4. Steroids and Triterpenoids Testing

As much as 1 mL extract of *P. australis* is added and shaken using ether. It is removed and evaporated with a vapor plate on the top of a water bath. The filtrate result is added to the Lieberman-Burchard reagent. Positive results of steroid compounds are the emergence of green color, while the positive triterpenoid compound results are characterized by the appearance of the color purple (Soebagyo, *et al.* 2007).

2.5. Producing Extracts of *P. australis*

The extraction uses the maceration method. The extraction process begins by taking 100 g sludge sample macerated with 96% ethanol at a 1:10 ratio for 3x24 hours. Subsequently immersed in 250 ml of ethanol (maceration) for 1x24 hours, and filtered using filter paper and filtrate accommodated in erlenmeyer, the filtrate result obtained filtrate 1. Residue result immersed with 750 ml of 96% ethanol for 1x24 hours, then filtered to obtain filtrate.

Tablet Formulation

Table 1. Tablet Formulation Extract *Padina australis*

Materials	Formula (%)			
	I (20%)	II (25%)	III (30%)	IV (-)
Extract <i>Padina australis</i>	20	25	30	-
Amprotab (%)	70	65	60	90
PVP (%)	3	3	3	3
Avicel pH 102 (%)	5	5	5	5
Talk (%)	1	1	1	1
Mg stearat (%)	1	1	1	1

*1 tablet 250 mg

2.6. Granule Production Method (Syamsuni, 2006)

Granules production is done by wet granulation method. The extract is mixed with half the amprotab part, then drop the PVP that has been dissolved with 96% ethanol while stirring homogeneously to obtain a unified and clenched mass. The mixture then sieved with a mesh number 12. Additionally the wet granules are dried in the oven at 40°C. Dry granules are sifted back with mesh sieves 18. Performed granule evaluation which includes the determination of granule water content, Granul flow test, silent angle test, compressibility test. The granules that have been evaluated are mixed with half the starch, magnesium stearate and aerosil. Then mixed homogeneously. Form tablet using a tablet printing machine. Evaluation of the tablets conducted including weight uniformity test, uniformity test size, tablet hardness test, tablet fragility test, and Crushed Time Test

2.7. Effectiveness of Tablets Testing

After testing the activity of tablet extract *P. australis*, the effectiveness against tablet is tested. The effectiveness of tablet testing against *Escherichia coli* bacteria is performed using Kirby-Bauer diffusion test by scratch plate method (Lay, 1994). The media is used to scratch one lump culture *Escherichia coli* bacteria, then paper discs that already contain tablets extract *Padina australis* placed on the surface of the plate media and placed in order that tablet preparations can seep well. The result is performed after it was incubated at room temperature for 18-24 hours, by measuring the width of the obstacle area (clear zone) around the disc paper, using a slider or ruler. The treatments used in this test were tablets from the extract of *P. australis* which had been diluted with 100 ml of distilled water, which had been soaked in paper discs with concentrations of 40%, 50%, and 60%. For positive control used amoxicillin 20 ppm. The data analyzed using Completely Randomized Design with treatment of each formula of *P. australis* extract tablet, and replication as much as 3 times. Based on data analysis, derived an effective formula as antibacterial *E. coli*.

3. Results and Discussion

3.1. *Simplicia P. australis*

Seaweed of *P. australis* is taken directly from Bayah Banten coastal waters in the wet form of 1.5 kg. Seaweed dried in the oven with a temperature of 40-450 C for 2-3 days. It is

derived as much as 250 grams of *P. australis* seaweed. Moreover, it is extracted with 96% ethanol and dried again using vacuum dry.

3.2. Water Content and Powder Content of *P. australis* testing

The test result of water content of simplicia *P. australis* using moisture balance is as much as 6.11%. The value is close to results research of Fitrya (2010), related to water content is 6.4%. The purpose of measuring the moisture content of the simplicia is to minimize the growth of microorganisms that cause damage to the simplicia resulting in decreased quality of simplicia (Muchtadi and Ayustaningwarno, 2010).

The calculation of simplicia *P. australis* powder content as much as 14.53%. The value is more than Santoso (2003), research result as much as 5.50%. This can be due to the different treatments such as washing process of the sample. The length of washing can reduce the fine powder present in the sample. According to Fitrya (2010), the high powder content shows the amount of organic material contained in thallus *P. australis*.

3.3. Phytochemical Analysis

The results of phytochemical analysis of the positive *P. australis* extract contained alkaloid compounds, tannins saponins and triterpenoids. The statement above is based Harborne (1987), secondary metabolite compounds commonly found in the plants are alkaloids, flavonoids, steroids, saponins, terpenoids, and tannins. According to Robinson, (1995) alkaloid and terpenoid compounds act as antifungies, and phenol, quinone, tannin and saponin compounds act as antibacterials, meaning that *P.australis* has antifungal and antibacterial properties.

3.4. Evaluation of Granules

In this study, the granule water content of each formula I, II, III, and IV are 3.71%, 3.33%, 3.2%, and 3.41%. All of formulas have water content that fulfil the requirements as much as 2-5% (Lachman, 1989). The granular water content is performed to determine the condition of granules, wet or damp granules will affect the printing of tablets that will be attached to the wall punch, while the dry granules will cause the tablet fragile easily. From the flow test and the granular diameter angle, granular flow rate is obtained between 4-10 g / s with silence angle between 25-400, and has qualified in granule evaluation. The existence of different variations of the flow rate in the formulation is due to the concentration difference in the active substance contained in each of the formulas.

3.5. Tablet Evaluation

Based on tablet evaluation result of each formulas derived the tablet measurement diversity test around 0.8634 cm - 0.8674 cm (thickness) and 0.4308 cm - 0.4396 cm (diameter); the result of weight diversity test ranged from 255.8 - 272.9 mg; tablet hardness test results ranged from 5.38 - 6.82 (Kg / Cm³); the test results of the crushed time range between 10.39 - 10.50 minutes / tablet. According to the results of tablet evaluation on all formulas give different results, this is due to the pressure on the tablet machine during printing, the particle size of the material of each printed tablet, the length of time release of the active substance from the tablets preparation and the effect of additional fillers on the tablets. The results showed that tablets compounds is fulfilled to the tablets production. The

result is supported by Suryadi (2004) who states the evaluation of tablets preparations that include uniformity of size, uniformity of weight, hardness of tablets and tablets crushed time.

3.6. Effectiveness Test of Tablet as Antibacterial

Effectiveness tablet testing of *P. australis* against antibacterial *E. coli* done by measuring the width of the inhibitory area (LDH), the research result obtained the clear zone. The effectiveness level of a material using Kirby-Bauer method is stated sensitive if clear zone is formed around the disc paper. In other words, clear zones around disc paper show antibacterial activity (Rosyidah *et al*, 2010). Testing of antibacterial activity in this study used tablets of four formulas by increasing dosage, where those formulas used as negative control because they did not contain active substances, and the positive control used was amoxicillin solution with a concentration of 20 ppm. The result of the clear zone formed can be seen in the following figure:

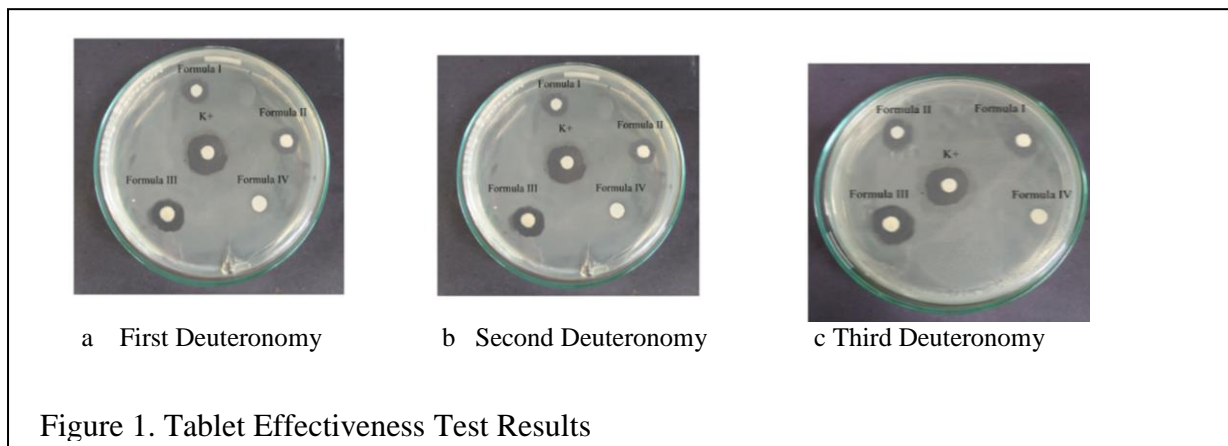


Figure 1. Tablet Effectiveness Test Results

The pictures show the inhibit zone formed on each formula. The inhibit zone of the 1st formula 2mm, 3mm for the 2nd formula, the 4th formula has 1mm inhibit zone, and a positive control has a 4.5mm inhibitory zone. The 4th formula is used as a negative control. The inhibitory force formed is a clear area adjacent to the treatment and there is no colony growth from bacteria. Each of the formulas has a difference in the inhibit zone, it states that as higher as an active substance concentration in the formula it will increase the antibacterial power activity. It can be seen from the width of the inhibit zone that is formed on a disc paper filled with a formula with high concentration compared to a paper disk filled with a low concentration formula. Amoxycillin with a concentration of 20 ppm used as a positive control gives the largest inhibition zone. With the calculation of the width of the resistor area formed by 4.5mm. The presence of active substances contained in the *P. australis* such as alkaloids and terpenoids that have bacteriostatic activity, it can be stated that the *P. australis* has antibacterial activity.

4. Conclusion

As a conclusion of the research result is that *Padina australis* tablet extract has an antibacterial effectiveness to *Escherichia coli*. As big as the concentration of the active substance it will increase the inhibitory diameter produced very much.

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