Phytochemical analysis and antioxidant activity of soursop leaf extract (*Annona muricata* Linn.)

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Abstract: Soursop (*Annona muricata* Linn) is one of herbal plants that are widely used as antidiabetic, anti-inflammatory, insecticidal, antimalarial, antibacterial and antioxidant. Soursop leaf has many benefits because it contains phytochemical compounds. This research aimed to identify the phytochemical compounds and antioxidant activity of soursop leaf. This research were carried out in a few steps which are extraction, evaporation, phytochemical tests and measurement of antioxidant activity. The result showed that the ethanol extract of soursop leaf was contained steroid, alkaloid, flavonoid, phenolic and saponin. The ethanol extract of soursop leaf has antioxidant activity by scavenging DPPH radical with IC₅₀ of 141.127 µg/mL.

1. Introduction

Indonesian is known for using medicinal plants to overcome the health problems since a long time ago. One of medicinal plant is soursop (*Annona muricata* Linn) with various health benefits which obtained from the fruit flesh, bark, flowers, root, seed and leaf [1]. This plant is reported very useful in various health disease treatment such as preventing and treating cancer, treating hemorrhoid, reducing cholesterol, eliminating acne [2], fever, respiratory illness, malaria, liver, heart and kidney infection [3]. Various studies have revealed about pharmacological activity of *A. muricata* L. such as antimicrobial, antiprotozoan, insecticide, larvicide, selective cytotoxicity to tumoral cells, anxiolytic, anti-stress, anti-ulceric, wound healing, anti-icteric, hepatoprotective, hypoglycemic and antioxidant [3].

Ethanol, water and n-hexane extract of soursop leaf show antioxidant properties by neutralizing free radicals using DPPH method [4,5]. The benefits of *A. muricata* L. leaf extract are reported as an antioxidant and correlate with secondary metabolites [6]. This is supported by research from phytochemical screening conducted, where the ethanol extract of *A. muricata* L. leaf contain compounds alkaloid, saponin, terpenoid, flavonoid, coumarin, lactone, anthraquinon, phenol, and phytosterol [5]. Other reports that soursop plant has powerful phytochemicals, that is *Annonaceous acetogenins* which are found only in Annonaceae family. These chemicals in general have been documented with antitumor, antiparasitic, insecticidal and...
anti-microbial activities. These acetogeneins are strong inhibitor of enzyme processes, that are found only in membran of cancerous tumour cells. The antioxidant activity is also related with their ability to quench reactive oxygen species such as singlet molecular oxygen and peroxyl radicals, thus acting as deactivators of excited molecules or as chain breaking agents respectively [6].

Antioxidants are chemical compounds that can contribute one or more electrons to free radicals. So, as to neutralize the increase in free radicals, protect cells from the toxic effects produce and contribute the prevention of diseases [8]. Antioxidant can protect the human body against damages which are caused by reactive oxygen compounds (ROS) and other free radicals. In addition, antioxidant can reduce oxidation of fats and oils, minimize process of damage in food, extend the usage period in food industry, increase the stability of fats that contained in food and prevent loss of sensory and nutritional quality [8,9].

The role of antioxidant is very important in neutralizing and destroying free radical which can cause cell damage and also damage inside biomolecules. In small amounts, free radical can be neutralized by the body's enzymatic systems such as the enzyme catalase, glutathione peroxidase, superoxide dismutase and glutathione-s-transferase. If amount of free radicals in body is excessive, antioxidants from outside the body are needed (exogenic) such as flavonoids, vitamin A, vitamin C and vitamin E [10]. So as to improve public health, this paper is focused to find out the phytochemical content and antioxidant activity of A. muricata L. leaf extract.

2. Materials and Methode

2.1 Material

Materials of this study were dried and wet A. muricata L. leaves from Tambangan village (Bulukumba regency, Indonesia), magnesium (Mg) powder, sulfuric acid (H2SO4), chloroform ammonia, dragendorff reagent, wagner reagent, chloroform (CHCl3), DPPH solution, vitamin C or ascorbic acid (C6H8O6), distilled water, whatman 42 filter paper.

2.2 Methode

2.2.1 Extract Preparation. Fresh A. muricata L. leaves were collected from Tambangan Village, Bulukumba Regency. The leaves were washed thoroughly in tap water and then cut into pieces. Leaves that were air dried in the shade and away from moisture during 4 weeks. These samples were crushed separately using a blender. The powdered samples were extraction with sonicator in ethanol. The obtained extract was filtered through Whatman No. 42 filter paper and the filtrate was concentrated using a rotary evaporator. The filtrate obtained was called crude extract for further studies.

2.2.2 Phytochemical Screening. Phytochemical screening for alkaloids, flavonoids, saponins, tannins, and terpenoids, were performed according to the standard procedures described by Harbone et al. (1987).

2.2.3 Antioxidant Activity Test

2.2.3.1 Making DPPH solution of 0.4 mM. DPPH solution with a concentration of 0.4 mM was made by weighing 7.9 mg DPPH and dissolved with methanol p.a up to 50 mL in a measuring flask.

2.2.3.2 Determination of the maximum wavelength (λ max) DPPH. Blank solution is made by piping 1 mL of DPPH solution and then increasing the volume to 5 mL with methanol p.a. Next, the absorbance is measured and maximum wavelength is determined.

2.2.3.3 Making ascorbic acid solution (positive comparison/control). Ascorbic acid is weighed as much as 10 mg and dissolved in 10 mL of methanol p. So that 200 ppm of concentration is obtained as a stock solution. Then from the stock solution of a series of concentrations of 10, 20, 40, 80 and 160 μl/mL were made. Each concentration is replicated 3 times. The DPPH solution was added as much as 1 mL of the test tubes, and the volume was sufficient to 5 mL with p.a methanol in a measuring flask. The mixture is left for 30 minutes. Then, it was measured using a
UV-Vis spectrometer with maximum wavelength 515 nm. The ability to reduce DPPH free radicals (inhibition) is calculated using the following equation 3 as below:

\[
\text{Barriers to free radical activity (\%) = \frac{(Ab-As)}{Ab} \times 100\%}
\]  

3. Result and discussion
3.1 Phytochemical analysis
This research used ethanol solvent to extract metabolite compounds in the sample where ethanol solvents able to penetrate the cell wall in sample. So that polar compounds such as flavonoids and acetogenin which have antioxidant abilities that can be obtained. \textit{A. muricata} L. leaf were extracted using sonicator which is known to have advantages over the maceration method that were safer, shorter, increases the number of crude yields and extraction process that is faster than thermal extraction or conventional [11]. The extract obtained in the form of dark green thick extract with weight of 3, 8723 grams.

The results of the research showed that ethanol extract of \textit{A. muricata} L. positively contained several types of secondary metabolites. This can be seen in Table 1 as below.

| Phytochemical tests | Reagent | Description |
|--------------------|---------|-------------|
| Steroid            | Liebermann-Burchard (+) steroid |
| Terpenoid          | Liebermann-Burchard (-) terpenoid |
| Alkaloid           | Wagner (+) alkaloid |
| Flavonoid          | Mg + HCl (+) flavonoid |
| Phenolic           | Acetic anhydride + FeCl$_3$ 1% (+) phenolic |
| Saponin            | Distilled water (+) saponin |

3.2 Antioxidant Analysis with DPPH Reagent
DPPH antioxidant activity testing was used DPPH reagent. The use of this reagent was because in addition to the most common reagents and it was widely used by previous researchers and also process is simple, easy, fast and requires only a few samples [12], then testing is done using a UV-Vis spectrophotometer at a wavelength of 515 nm. Absorption measurements were carried out after 30 minutes incubation so that a reaction between DPPH as free radicals could be occurred and the samples to be tested. Measurement of absorbance of leaf extract of \textit{A. muricata} L. and positive control in the form of ascorbic acid that were made in five series of concentrations, namely concentrations of 10 ppm to 180 ppm, so that the results as shown in Figure 1 were obtained as below.
Figure 1. Antioxidant activity of *A. muricata* L. leaves

Figure 1 shows that *A. muricata* L. leaf extract for a concentration of 10 ppm showed free radical inhibition of 9.62%, concentration of 20 ppm at 11.62%, concentration of 40 ppm at 21.14%, concentration of 80 ppm at 41.62 % and 160 ppm concentration of 51.24%.

Test the antioxidant activity used comparative solution (vitamin C) because this vitamin is very good at inhibiting free radicals and widely used by researchers as positive control on antioxidant testing. Measurement of antioxidant activity from vitamin C can be seen in Figure 2.

Figure 2. Antioxidant activity of vitamin C

The results of the analysis of antioxidant activity of vitamin C at a concentration of 0.25 ppm showed free radical inhibition of 27.10%, concentration of 0.5 ppm of 30.70%, concentration of 1 ppm at 35.25%, concentration of 2 ppm at 42.69%, and concentration 4 ppm at 64.03%.

The parameters showed antioxidant activity of *A. muricata* L. leaf extract that were inhibitor concentration (IC$_{50}$), which was the concentration of sample solution needed to inhibit 50% of
DPPH free radicals (Andayani et al., 2008). If the antioxidant activity was higher, the IC\textsubscript{50} value would be smaller [13].

The IC\textsubscript{50} value of \textit{A. muricata} L. leaf extract and the comparative solution in the form of vitamin C were 141.127 \mu g / mL and 2.597 \mu g/mL respectively. The intensity of antioxidant activity of \textit{A. muricata} L. leaf extract included moderate antioxidants. While the positive control (comparative) namely vitamin C has the intensity of the ability of strong antioxidant activity. Where a compound was said to be an antioxidant was very strong if the IC\textsubscript{50} value is less than 50 \mu g/mL, strong antioxidant if the IC\textsubscript{50} value was 50-100 \mu g/mL, moderate antioxidant if the IC\textsubscript{50} value was 100-150 \mu g / mL, and the antioxidant is weak if the IC\textsubscript{50} is more than 150 \mu g / mL [13].

The mechanism of DPPH radical capture by antioxidants is in the form of proton donations to radicals (Sari, 2012) and this antioxidant test is based on the measurement of discoloration of DPPH after reacting with samples ([12], This can be observed in the color changes that occur which were originally concentrated violet to pale yellow. This change shows the antioxidant activity of the sample.

The higher the concentration of the sample containing antioxidant compounds, the more yellow the color would be DPPH's color change that is related to the energy that free radicals have. When DPPH tended to be unstable in a radical state. This is indicated by the existence of purple fading from DPPH solution, because diphenylpicrylhydrazyl (DPPH) has one N atom whose electrons are unpaired and when reacting with compounds that can reduce free radicals that there can be binding of one electron with atoms that can donate electrons (H atom) forms stable diphenylpicrylhydrazine (DPPH-H) [13]. The reaction can be seen in Figure 3.

![Figure 3. DPPH free radical inhibition reaction by antioxidants](image)

### 4. Conclusion

Ethanol extract of \textit{A. muricata} L. leaf have contains secondary metabolites which are steroid, alkaloid, flavonoid, saponin, phenolic and have medium potential as an antioxidant activity with IC\textsubscript{50} value are 141.127 \mu g / mL.

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