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Antioxidant Activity of Young and Old Namnam (*Cynometra cauliflora* L.)Leaves Ethanol Extracts

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ABSTRACT

One of the natural defense systems that an organism had to prevent exposure from free radicals is by forming a number of compounds that act as antioxidants. One of the substances that can prevent these free radicals are enzymes produced in the body such as glutathione peroxidase enzymes, catalase, and others. The amount of these antioxidants can be added with intake from outside the body. This antioxidant is a type of natural antioxidant that can be found in plants. Namnam plants contain phytochemical compounds such as flavonoids, tannins, phenolics, and terpenoids. These compounds can act as antioxidants. This study's objective was to use the DPPH method to categorize the antioxidant activity of an ethanol extract of young and old namnam leaves. The extraction procedure used ethanol at a 1:5 ratio through a maceration process for three consecutive days in order to acquire these antioxidants. The samples used were young and old Namnam leaves each for 100 gram. The extract was measurements at a wavelength 517 nm by using an uv-vis spectrophotometer. The method was used DPPH for antioxidant activity. The positive control used was ascorbic acid. After that, the absorbance measurement results obtained will be used to measure the IC50 value. This number represents the antioxidant activity of the extracted substance. Old Namnam leaves generate IC50 results of 9.58 ppm, while young Namnam leaves generate IC50 results of 28.93 ppm. Based on this data, the antioxidants in old Namnam leaves give a greater IC50 value than young Namnam leaves.

Keywords: *ethanol extract, Namnam leaves, antioxidant*.

INTRODUCTION

The primary causes of death in developing nations are now non-communicable diseases rather than communicable diseases. This transitional tendency is influenced by changing lifestyles, urbanization and globalization. The World Health Organization (WHO) estimates that Non-Communicable Diseases (PTM) will cause 73% of deaths and 60% of all morbidity in the world at 2020. The prevalence of degenerative diseases in several developing countries increasing due to increased prosperity in the countries has recently been highlighted. The increase in per capita and the development of lifestyles, especially in big cities, has led to an increase in degenerative diseases (1).

Excessive oxidation reactions in the human body's cells initiate most diseases. Oxidation reactions occur at any time, including when breathing and metabolic processes in the body. This reaction can lead to the formation of free radicals. Free radicals in normal amounts are beneficial for health such as, to help host cell protect our body from pathogen cell and to regulate intracellular flow in some of type cells. Meanwhile, in excess amount of it could results in oxidative stress. This situation can cause damage from the cellular, tissue, until the organs of the body, which accelerates the aging process and the emergence of disease (2).

Free radical compounds that enter to the body can be inhibited by a compound known as an antioxidant. These an antioxidants have several functions, such as being able to reduce the risk of cardiovascular disease, increase endurance, prevent premature aging and inhibit the emergence of degenerative diseases due to premature aging. The human body itself actually has defenses in the body (endogenous antioxidants) against free radical attacks, if the body has an excessive amount of free radicals, the body will need external antioxidants, namely natural antioxidants. (3).

Flavonoids, phenolics, alkaloids, steroids, terpenoids, and steroids are phytochemicals compound that have antioxidant activity (4). One of the plants that contain these antioxidant compounds is namnam plant. Namnam (Cynometra cauliflora L) or often known as pukih, is a group of rare plants in Indonesia. Namnam fruit is used by the community as an ingredient for rujak, pickles and sweets (5).

Namnam plant methanol extract contains tannins, saponins, flavonoids, terpenoids, and glycosides which are useful as antioxidants. The study reported that the antioxidant activity in young Namnam leaves was 66.36 µg/mL. This amount indicates that the antioxidant compounds in the young leaves are greater than in the stems, bark, fruit (6). Namnam leaves ethanol extract has an toxicity value of LC50 is 125.89 µg/mL which category as toxic and has potential as an antioxidant (7).

This study's goal was to use the DPPH method to classify the antioxidant activity of an ethanol extract of young and old namnam leaves. A minimal number of samples are used in this quick, easy procedure to assess the antioxidant activity of natural substances. This research used ethanol solvent because its properties safe and not toxic.

MATERIAL AND METHODS

1. Tools and Materials

Young and old Namnam (*Cynometra cauliflora* L) leaves from Rangkasbitung, Banten, West Java Province served as the study's primary source of material. The analysis of this plant by Indonesian Institute of Sciences (LIPI) was identified as *Cynometra cauliflora* L. species, family of Leguminosae or Caesalpiniaceae, namnam. Other materials used were DPPH (Aldrich), ethanol (Merck), and ascorbic acid (Sigma Aldrich). In this research, we specifically used young and old Namnam leaves. The color of the new Namnam leaf is pale pink, while the old leaf is shiny dark green. Research toolsused were glass that commonly used in the laboratory. Rotary vacuum evaporator (Buchii B480) for extraction and Spectrofotometer uv-vis to antioxidant activity test.

2. Simplicia of Namnam leaves

The plant material of namnam (*Cynometra cauliflora* L) leaves were collected from Rangkasbitung, Banten, West Java. The leaves were cleaned, dried, ground into a powder, and then kept for future use in a sealed container in a cool, dry environment.

3. Preparation of extraction

To prepare for maceration, the young leaves were dried, crushed, and weighed 100 grams. Using ethanol as a solvent at a 1:5 ratio, this process took three times as long as usual. The maserate was dried with a rotary evaporator at 45-50^{o}C to produce a crude extract (7). Carry out this procedure to old namnam leaves.

4. Antioxidant Test

Antioxidant activity was carried out based on method (8), modified by BIOFARMAKA. The extract sample to be tested were dissolved in ethanol p.a with various concertrations. 100 L of sample and 100 L of DPPH made up the reaction mixture. Negative control only added 100 µL ethanol p.a. After 30 minutes of room temperature incubation in the dark, the substance was evaluated by a spectrophotometer using UV-Vis at 517 nm. (8). The absorbance measurement carried out will produce an absorbance values. This absorbance value is used to measure the IC_{50} value. This number represents the antioxidant capacity of the tasting extracts. To get the IC_{50} value using the regression formula Y = A + Bx. As a positive control, ascorbic acid was utilized. The percent inhibition is shown in the formula :

$$\% \text{ Inhibition } = \frac{Ac-At}{Ac} \times 100 \quad (9)$$

Information :

Ac = control absorbance (1 mL ethanol with 1mL DPPH solution) and At = sample absorbance (10).

RESULTS

The namnam leaves used are young namnam leaves and old namnam leaves. The solvent used for Namnam leaves extract is ethanol with a ratio of 1:5 using the maceration method for 3x24 hours (7). According to Dean 2009 in Pratiwi (2018) study states that the maceration method was chosen because it is easy and simple, it can also extract active compounds properly through immersion solvent without high heating, so as to avoid component damage (11). This causes the solvent to penetrate the cell wall so that it enters the cell containing the active compound, and the compound will dissolve in the solvent.

Ethanol solvent is one of the solvents used to dissolve organic compounds, because of its volatile property, and is polar (12).

Fig 1. Young and old namnam leaves exctract

A UV-Vis spectrophotometer was then used to assess the extraction results using the DPPH technique at a maximum wavelength of 517 nm. One test used to assess the antioxidant activity of a radical is the DPPH (1,1 Diphenyl-2-picrylhidrazyl) technique. (13). The advantage of using this tool is that it can quantitatively determine the absorbance of a substance, even though at very small amount, in a very simple way and the results obtained are accurate. This method was chosen because it has the advantage of being accurate method, easy procedure, and sensitive for small amount of samples (14).

DPPH can turn into a yellow color by reaction with antioxidants. By completing one electron to DPPH so free radicals are reduced. Free radical capture by antioxidants occurs when hydrogen donors forming stable DPPH. After measurements with a UV-Vis spectrophotometer, absorbance values from different concentrations will appear. Furthermore, the absorbance value obtained can produce a percentage of inhibition (inhibition) (15).

Table 1. Antioxidant activity of young namnam leave ethanol extract

Extract concentration (ppm)	Inhibition(%) Repetition I	II	IC_{50} (ppm)
10	29.178	22.379	28.932
50	67.422	66.289	
125	79.320	79.886	
250	86.119	86.402	
500	88.668	88.385	

Table 2. Antioxidant activity of old namnam leave ethanol extract

Extract concentration (ppm)	Inhibition(%) Repetition I	II	IC_{50} (ppm)
0.5	8	5.818	9.583
1.25	11.636	12	
2.5	18.909	16.727	
5	30.909	25.818	
10	53.818	50.181	

Table 3. Antioxidant activity of ascorbic acid

Extract concentration (ppm)	Inhibition(%) Repetition I	II	IC_{50} (ppm)
0.3125	4.739	6.635	
0.625	10.900	11.374	
1.25	21.800	22.748	3,100
2.5	44.075	45.971	
5	76.777	77.251	

Tables 1 and 2 show the concentration inhibition values for the ethanol extracts of young and old leaves, respectively. These results indicate that the percentage of inhibition obtained potential as an antioxidant with a higher level concentration of inhibition in the old ethanol extract than the ethanol extract of young Namnam leaves at different concentration variations. The greater the concentration inhibition value of the sample, the

higher the antioxidant activity. When hydrogen ions present from DPPH, the inhibition process was occur (16).

Old namnam leaves ethanol extract exhibited the highest concentration inhibitory level of antioxidant activity, which was 9,583 ppm, and was followed by young namnam leaves ethanol extract, which was 28,932 ppm.

To determine the Inhibition of concentration (IC_{50}) value by a regression equation. This value was obtained by x and y axis from the math equation. The IC50 value of 50 is calculated to inhibit DPPH radical uptake by 50% (17), and this value is inversely proportional (18). Ascorbic acid (vitamin C) was the positive control or comparison that was used. Because it contains hydrogen atoms and can form reasonably stable compounds, ascorbic acid (also known as vitamin C) is an extremely potent source of antioxidants. L(+) ascorbic acid is commonly used as a standard in determining antioxidant capacity (19). To capturing free radicals and stop chain reactions mechanism by using ascorbic acid as a secondary antioxidant (20).

Antioxidant activity is classified as very powerful, strong, and weak respectively IC_{50}< 50 ppm, 50-150 ppm, and 150-200 ppm (21). Based on the results of Tables 1 and 2, the young and old Namnam leaves extracts are included in the very strong category, but when the two Namnam leaves extracts are compared, the old namnam leaves extract is stronger in antioxidants than the young Namnam leaves extract and lower than ascorbic acid.

The amount of secondary metabolites compounds in extract could determine the difference in IC_{50} values (18). The content of namnam leaves consists of flavonoids, phenolics, and tannins which are compounds that are responsible for antioxidant activity. The antioxidant activity of flavonoids, phenolics and tannins is due to the fact that these three compounds are secondary metabolites. The DPPH radicals can be reduced to a more stable form by these metabolic molecules by donating hydrogen atoms. The amount of hydroxyl functional groups in phenolic compounds demonstrated the highest value antioxiddant activity (22). The value of total flavonoid in old namnam leaves was higher than young namnam leaves respectively 33,63±0,25 and 21,96±0,3. Flavonoids have been as protective antioxidants at various levels(6).

CONCLUSIONS

The ethanol extract of young and old namnam leaves has the ability to act as an antioxidant by the results of the IC50 value. Young and old namnam leaves had IC50 values of 28.932 ppm and 9.583 ppm, respectively, which suggested a very strong category.

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