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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. The purpose of this study was to aim classify of antioxidant activity from ethanol extract of young and old namnam leaves by DPPH method. To obtain these antioxidants, the extraction process was carried out by using ethanol in a ratio of 1:5 through a maceration process for 3x24 hours. The samples used were young and old Namnam leaves each for 100 gram. The extract obtained was then tested for antioxidant activity using the DPPH method with measurements using an uv-vis spectrophotometer at a wavelength of 517 nm. The positive control used was ascorbic acid. After that, the absorbance measurement results obtained will be used to measure the IC50 value. This value shows the antioxidant activity from the extract that has been made. 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

Currently in developing countries there has been a shift in the main causes of death, from communicable diseases to non-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).

Most diseases are initiated by an excessive oxidation reactions in the cells of the human body. 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, fighting inflammation, killing bacteria, and controlling the smooth muscle tone of blood vessels and organs in the body. 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 number of free radicals in the body is an excessive, then the human body needs antioxidants from outside the body, namely natural antioxidants (3).

Phytochemicals can be divided into several categories such as alkaloids, phenolics, flavonoids, steroids, terpenoids, steroids and others. These phytochemicals have been shown to have antioxidant activity and reduce the risk of many diseases (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).

The purpose of this study was to aim classify of antioxidant activity from ethanol extract of young and old namnam leaves by DPPH method. This method uses a small amount of samples and also fast and simple method to determine the antioxidant activity of natural ingredients. This research used ethanol solvent because its properties safe and not toxic.

MATERIAL AND METHODS

1. Tools and Materials

The materials used in this study were young and old leaves of Namnam (*Cynometra cauliflora* L) from Rangkasbitung, Banten, West Java Province. The plant was identified by Indonesian Institute of Sciences (LIPI) with number B-994/IPH.3./KS/IV/2019. This plant was identified as *Cynometra cauliflora* L species and familie of Leguminosae/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 washed, dried, milled into powder, and then stored in a sealed container in cool, dry place for further use.

3. Preparation of extraction

The young leaves was dried grounded and weighed 100 gram to maceration using ethanol by ratio 1:5 solvent. The maceration process took 3x24 hours. 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. Uji Antioksidan

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. The reaction mixture was consisted of 100 μL sample and 100 μL DPPH. Negative control only added 100 μL ethanol p.a. After 30 incubation for room temperature in a dark, absorbance of the reaction mixture was measured at 517 nm by spectrophotometer UV-Vis (8). The absorbance measurement carried out will produce an absorbance values. This absorbance value is used to measure the IC_{50} value. This value indicates the antioxidant activity of the extracts that have been tasted. To get the IC_{50} value using the regression formula Y = A + Bx. The positive control used in this study was ascorbic acid. 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

The extraction results then tested for antioxidant activity using the DPPH method using a UV-Vis spectrophotometer at a maximum wavelength of 517 nm. The DPPH (1,1 Diphenyl-2-picrylhidrazyl) method is one of the tests used to determine the antioxidant activity of radical (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 simple, easy, fast, and sensitive and only requires a 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 unpaired electrons become paired in the presence of hydrogen donors, thus 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		IC_{50} (ppm)
	I	II	
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		IC_{50} (ppm)
	I	II	
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		IC_{50} (ppm)
	I	II	
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	

The concentration inhibition values for the ethanol extracts of young and old leaves can be seen in Tables 1 and 2. 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. The inhibition process is thought to occur when the DPPH radical reacts with antioxidant compounds by taking up hydrogen ions (16).

The results of concentration inhibition level of old namnam leaves ethanol extract had the highest concentration inhibition of antioxidant activity by 9,583 ppm and followed by young namnam leaves ethanol extract by 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. IC_{50} value is the concentration of the extract that can reduce or inhibit 50% intensity of DPPH radical uptake (17). The IC_{50} value is inversely proportional to the antioxidant ability of a compound contained in the test solution (18). The positive control or comparison used was ascorbic acid (vitamin C). Ascorbic Acid (Vitamin C) is a very strong source of antioxidants, can donate hydrogen atoms and form relatively stable ascorbyl free radicals. L(+) ascorbic acid is commonly used as a standard in determining antioxidant capacity (19). Ascorbic acid functions as a secondary antioxidant by capturing free radicals and preventing chain reactions from occurring (20).

Antioxidant activity is classified as :very powerful" if the IC_{50} value is less than 50 ppm, "strong" if the IC_{50} value is 50-100 ppm, if the IC_{50} is 101-150 ppm, and "week" if the IC_{50} is 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 difference in IC_{50} values in each young and old leave ethanol extract is caused by the distribution of species and number of groups of secondary metabolites which are as antioxidants based on the polarity of the solvent used (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. These

metabolites compounds have the ability to donate hydrogen atoms so that the DPPH radicals can be reduced to a more stable form. Free radical scavenging activity of phenolic compounds is influenced by the number and position of phenolic hydrogen in the molecule. The more the number of hydroxyl groups possessed by phenolic compounds, the greater the antioxidant activity produced (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. The IC50 values for the ethanol extract of young and old namnam leaves respectively were 28.932 ppm and 9.583 ppm, which indicated as a very strong category.

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