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The Difference of Antioxidants Activity between Methanol Extract and Ethanol Extract of Bay Leaves (*Syzygium polyanthum* (Wigh) Walp) USED DPPH Method *(1,1-difenil-2-pikrilhidrazil)*

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ABSTRACT: Antioxidant are compounds that can inhibit oxidant by reacting with reactive of free radicals to form non reactive free radicals that was relatively stable. One of the natural antioxidants derived from plants is salam leaf. Salam leaf (Syzygium polyanthum (Wigh) Walp) has the chemical content of polyphenol compounds such as flavonoids and tannin its efficacious is as antioxidants. The aims of this research is to determine whether there is a reaction on antioxidant activity of ethanol extract and methanol extract of salam leaves, as well as to know whether there is a difference of antioxidants activity between the ethanol extract and methanol extract. The extraction of salam leaves is done by maceration method using liquid extraction 70% ethanol and methanol. The ethanol extract and methanol extract of salam leaves is made by the concentration 0,06%; 0,08%; 0,10%; 0,12%; 0,16% and its antioxidants power is being tested with DPPH. The antioxidants activity is determined by DPPH method with spectrofotometry UV-Vis till gets value EC_{50}. This value EC_{50} is reached through the linear regression equation which states the relationship between the concentration of test solution (x) with the antioxidant activity (y) of a series of replicate measurements. The quantitative test results show that the average value EC_{50} for the ethanol extract of salam leaves is 14,2168 µg/ml and methanol extract of salam leaves is 12,4338 µg/ml. From the test results of "t" test in EC_{50} shows significant value 0,188 > 0,05 difference between ethanol extract (α value) which means that there is no significant difference between ethanol extract and methanol extract of salam leaves.

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INTRODUCTION

Free radicals are chemical compounds that have one or more unpaired electrons. These free radicals are dangerous because they are highly reactive in seeking their electron partners. Free radicals formed in the body will produce new free radicals through a chain reaction which eventually continues to grow. Furthermore, it attacks body cells so that tissue damage will occur. These free radical compounds arise due to various complex chemical processes in the body, in the form of byproducts of oxidation or cell burning processes that take place at breathing time, cell metabolism, excessive exercise, inflammation or when the body is exposed to environmental pollution such as motor vehicle smoke, cigarette smoke, pollutants, and solar radiation or cosmic radiation (Indrayana, 2008). The high reactivity of free radicals causes a chain reaction. However, free radical reactivity can be inhibited by compounds that are antioxidants. So that the availability of antioxidants in the body must be maintained and increased to be able to ward off free radicals (Wirakusumah, 2000).

Antioxidants are substances that can neutralize free radicals so that they can protect the body's biological systems from adverse effects arising from processes or reactions that cause excessive oxidation (Hariyatimi, 2004).

Indonesian people have long known various types of plants that can be used to treat various diseases and are efficacious as antioxidants, one of which is bay leaves (Yuniarti, 2008). Bay leaf (*Syzygium polyanthum (Wigh) Walp)* apart from being a spice fragrance is also effective for the treatment of high cholesterol, diabetes, hypertension, gastritis, and diarrhea. Bay leaf (*Syzygium polyanthum (Wigh) Walp) contains many* essential oils (citral and eugenol), tannins, and flavonoids. Phenolic components found in plants have the ability to reduce which plays an important role in absorbing and neutralizing free radicals, and peroxyd

decomposition (Indrayana, 2008). Some of the chemical ingredients contained in bay leaves (*Syzygium polyanthum (Wigh) Walp)* can be used as a basis in the selection of solvents to be used for making extracts. Because the chemical content that is efficacious as an antioxidant in bay leaves is polar, the solvents used are polar as well, namely ethanol and methanol.

To find out more about the antioxidant power of ethanol extract and bay leaf methanol extract (*Syzygium polyanthum (Wigh) Walp*), it is necessary to test the antioxidant activity of bay leaves using the DPPH *method (1,1-diphenyl-2-picrylhydrazil)*. The antioxidant activity of ethanol extract and bay leaf methanol extract is expressed through the determination of EC_{50} values.

RESEARCH METHODS

The sample used was bay leaf (*Syzygium polyanthum (Wigh) Walp*). Samples were taken at Pasadena Housing, Ngaliyan District - Semarang, in the form of fresh plants which were then dried indirectly in the sun covered with black cloth. The chemicals used are 70% ethanol, p.a. ethanol, technical methanol, p.a. methanol, and DPPH *(1,1-diphenyl-2-picrylhydrazil).*

Extraction of antioxidant compounds from bay leaf *(Syzygium polyanthum (Wigh) Walp)*

The phenolic compounds contained in bay leaves are extracted by maceration method, by means of dry bay leaf powder soaked in filter liquid, namely 70% ethanol and methanol while being shaken 3-4 hours per day with a *shaker*, and allowed to stand 24 hours. After 24 hours of filtering, the filtrate obtained is collected and the solvent is replaced with a new solvent equal to the first solvent, this is done repeatedly for 5 days. The resulting filtrate is put together and allowed to stand for one day. After letting the solvent stand is evaporated using a *vacuum rotary evaporator* until a thick extract is obtained.

Qualitative determination of antioxidant activity

This test was carried out by means of bay leaf extract (*Syzygium polyanthum (Wigh) Walp)* tolerated on the KLT plate, *then eluted with n-butanol eluent: glacial acetic acid: water (4: 1: 5). After the elution is complete, the plates are dried and sprayed with a 0.1 mM DPPH solution.*

Determination of antioxidant activity by DPPH method *(1,1-diphenyl-2-picrylhydrazil)*

Determination of antioxidant activity was carried out by means of 4.0 ml of 0.1 mM DPPH solution plus 0.05 ml of test solution. *Next, the mixture is homogenized with vortex for 1 minute and allowed to stand for a certain time according to the results of the operating time. Then the absorbance of the solution is measured on a UV-Vis spectrophotometer and read at each of the maximum wavelengths. A reading of the absorbance of the control solution was also carried out, namely a 0.1 mM DPPH solution without the addition of a test solution.*

RESULTS AND DISCUSSION

Extraction of antioxidant compounds from bay leaf (*Syzygium polyanthum (Wigh) Walp)*

Antioxidant compounds contained in bay leaves are obtained through the process of extraction by maceration method, because maceration is a fairly effective and relatively easy to implement method and besides that also to minimize the occurrence of damage to antioxidant compounds when compared to using the hot method. Maceration is carried out in a closed vessel which aims to minimize the evaporation of volatile ethanol and methanol at room temperature, while also intended to prevent contamination from outside. Maceration vessels are placed in a dark place to avoid direct light and sunlight. This is intended to prevent the reaction of compounds by the presence of UV sunlight, sun or light.

Qualitative determination of antioxidant activity

The results of qualitative antioxidant activity testing obtained chromatogram spots that are pale yellow on a purple background at ± 30 minutes after spraying with the appearance of 0.1 mM DPPH solution spots. This shows that ethanol extract and bay leaf methanol extract have antioxidant effects. This effect occurs by the presence of compounds contained in ethanol extract and methanol extract which have the potential as antioxidants.

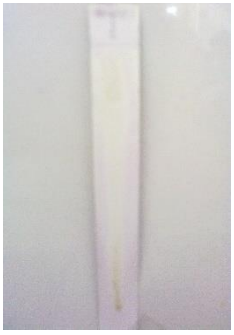

Fig. 1 Chromatogram ethanol Extract

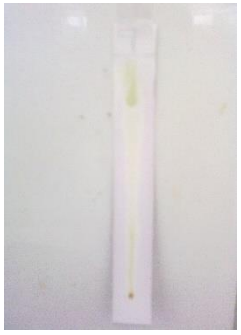

Fig. 2 Chromatogram methanol Extract

Determination of antioxidant activity by DPPH method *(1,1-diphenyl-2-picrylhydrazil)*

Antioxidant activity testing was carried out by mixing 0.05 ml of ethanol extract or 0.05 ml of methanol extract with 4.0 ml of 0.1 mM DPPH solution in a test tube that was protected from light and tightly closed to prevent the influence of light during the DPPH radical damping reaction by the extract. Each mixture is vortex for ± 1 minute to homogenize the mixture after divortex, the mixture is allowed to stand for a certain time according to the results of the *operating time* of each mixture. The results obtained showed that the settling time for ethanol extract was 25 minutes, while for methanol extract 10 minutes.

Testing antioxidant activity with the DPPH method is based on the ability of an antioxidant to reduce the intensity of the DPPH radical color, which was originally purple to yellow. The decrease in color intensity illustrates a decrease in DPPH concentration, which causes a decrease in absorbance when compared to the DPPH control absorbance of 0.1 mM without the addition of a test compound, bay leaf extract. The decrease in color intensity is produced by the reaction of a molecule of diphenyl picrylhydrazil (DPPH) with a hydrogen atom released by one component molecule of the test material so that picrylhydrazine compounds will form.

One of the result parameters of the DPPH method is EC50. EC50 is an effective concentration of the test compound that can decrease absorption intensity by 50% when compared to controls. The EC50 value is obtained through a linear regression equation that states the relationship between the concentration of the test compound (x) and the antioxidant activity (y) of a measurement replication series. With the smaller value of EC50, the test compound has the potential as a better antioxidant. The average data of EC50 values of ethanol extract and methanol extract can be seen in the table below:

Table 1. Average EC50 Value

Test Solution	Average EC50 value
Bay Leaf Ethanol Extract	14.2168 µg/ml
Bay Leaf Methanol Extract	12.4338 µg/ml

So when viewed from the EC50 value between ethanol extract and bay leaf methanol extract, it is known that the EC50 value of ethanol extract is greater than the EC50 value of methanol extract. When viewed from the results of the linear regression equation, an EC50 value is obtained between ethanol extract and different bay leaf methanol extract. To ensure the difference, it is necessary to do a t test. Before the t test is carried out, it is necessary to do a normality test based on *the Shapiro-Wilk* formula, because the number of samples used is less than 50.

Based on the normality test, it produces normally distributed data with the value of calculated significance (P) for ethanol extract is 0.438 (P>0.05), while for bay leaf methanol extract the calculated significance (P) is 0.337 (P>0.05). After the data is declared normally distributed, it is necessary to perform a parametric test by doing a t test. As for the "t" test, the significance value for ethanol extract and bay leaf methanol extract was 0.188 (P>0.05), so it can be concluded that the EC50 value of ethanol extract and bay leaf methanol extract is not significantly different. There is no difference in EC50 values between ethanol extract and bay leaf methanol extract because most of the compounds have antioxidant activity, in this case polyphenolic compounds that dissolve well in polar solvents. Therefore, between ethanol solvents and methanol solvents their ability to attract or dissolve compounds that have antioxidant activity (polyphenolic compounds) is the same even though methanol solvents are more polar than ethanol solvents.

CONCLUSION

From the results of the research that has been done, it can be concluded that bay leaf ethanol extract has antioxidant activity in vitro with an average EC50 value of 14.2168 µg / ml, and bay leaf methanol extract of 12.4338 µg / ml, and there is no significant difference between the antioxidant activity of ethanol extract and bay leaf methanol extract.

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