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Antioxidant Activity of Nutmeg Mace (*Myristica fragrans*) Graded Extract

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Abstract: Nutmeg (*Myristica fragrans*) contains essential oils and can be applied in industrial scale because it has medicinal benefits such as stimulating the heart system, stomachache, rheumatism, muscle pain, toothache, eliminating toxins in the liver, flatulence, and antioxidant. The purpose of this study was to determine the antioxidant activity of the nutmeg mace graded extract. The research methods include plant determination, sample preparation, extraction by maceration method, phytochemical test, and antioxidant test using the DPPH method. The yield of Mace Nutmeg extract obtained was 30.3% *n*-hexane, 8.79% ethyl acetate, and 3.79% methanol and the IC_{50} value of each mace nutmeg extract, namely the *n*-hexane extract was 56.22±1.15 ppm, 20.39±0.13 ppm ethyl acetate extract and 17.89±0.10 ppm methanol extract. From these results, it can be concluded that mace nutmeg extract has antioxidant activity with a strong category.

Keywords: antioxidant, DPPH method, extraction, maceration, *Myristica fragran*

***Abstrak*:** *Tanaman pala (Myristica fragrans) menghasilkan minyak asiri yang digunakan dalam skala industri karena memiliki manfaat medis seperti untuk stimulus sistem jantung, mengatasi diare, rematik, nyeri otot, sakit gigi, menghilangkan racun dalam hati, perut kembung dan memiliki aktivitas antioksidan. Tujuan penelitian ini adalah untuk mengetahui aktivitas antioksidan dari ekstrak bertingkat fuli pala. Metode penelitian meliputi determinasi tanaman, preparasi sampel, ekstraksi dengan metode maserasi, uji fitokimia, dan uji antioksidan menggunakan metode DPPH. Dari penelitian ini diperoleh rendemen ekstrak fuli pala yang didapat yaitu: n-heksan sebesar 30,3%, etil asetat 8,79% dan metanol 3,79%. Nilai IC_{50} dari masing-masing ekstrak fuli pala yaitu ekstrak n-heksan sebesar 56,22±1,15 ppm, ekstrak etil asetat sebesar 20,39±0,13 ppm dan ekstrak metanol 17,89±0,10 ppm. Dari hasil tersebut dapat disimpulkan bahwa ekstrak fuli pala mempunyai aktivitas antioksidan dengan kategori kuat.*

Kata kunci: *antioksidan, metode DPPH, ekstraksi, maserasi, Myristica fragran*

INTRODUCTION

Nutmeg (*Myristica fragrans*) is used in the medicinal industry, the plant has various properties that are beneficial to the health of the body. In low doses, nutmeg can be used to reduce flatulence (stomach bloating), increase appetite, vomiting, nausea, and can treat stomachache (Agoez 2010). Nutmeg has good prospects because it is always needed, both in the food, beverage, drug industry, and others. This plant has been produced essential oil on an industrial scale because it has medicinal benefits such as stimulating the heart system, stomachache, rheumatism, muscle pain, toothache, eliminating toxins in the liver, and various other properties (Agaus & Agaus 2019). Nutmeg consists of 83.3% flesh, 9.54% seeds, 3.94% seed shell, and 3.32% mace (Citanirmala *et al*. 2016). The most important commercially in nutmeg is mace and nutmeg seeds because they can be processed in various products, namely oleoresin and essential oil (Agusta 2000).

At this time the use of natural antioxidant compounds is growing rapidly, both for food and for treatment, this is because the use of synthetic antioxidants is currently starting to be limited because they are suspected of causing cancer (Gupta *et al*. 2013). The main character of antioxidant compounds is their ability to capture and stabilize free radicals (Nurmilasari *et al*. 2017). The antioxidant activity of nutmeg leaf water extract can bind free electrons better than BHA, but is less active than BHT and has potential as an anticancer (Gupta

et al. 2013). Nurmilasari *et al*. (2017) reported that nutmeg leaf methanol extract showed antioxidant activity with an IC_{50} value of 24.60 ppm, ethyl acetate extract had an IC_{50} value of 26.17 ppm and *n*-hexane extract had a higher IC_{50} value of 27.67 ppm. Ginting *et al*. (2016) succeeded in elucidating compounds flavonoid from extract *n*-hexane nutmeg leaf the flavonoid group, that is dihydrocaemferol or 3,5,7,4'-tetrahydroxy dihydroflavonol which have antioxidant activity in a strong category with an IC_{50} 9.75 ppm (Ginting *et al.* 2016). Based on extract of *n*-hexane root, bark, fruit, and nutmeg seeds had IC_{50} values 0.216, 63.817, 44.022, and 11.61 ppm, respectively (Nurmilasari *et al.* 2017), while methanol and chloroform extracts of nutmeg leaf have IC_{50} values of 36.31 and 28.30 ppm, respectively (Nurmilasari *et al.* 2017).

Based on the description above, the antioxidant activity test of the gradual extract of nutmeg mace will be carried out through the stages of plant determination, sample preparation, extraction by maceration method, phytochemical test, and antioxidant test by DPPH method. Nutmeg mace samples were taken from Sindangwangi Village, Tasikmalaya.

MATERIALS AND METHODS

Materials and Equipments

The equipment used in this study are blender, Erlenmeyer flask, measuring cup, measuring flask, beaker glass, analytical balance, test tube, tube rack, maceration apparatus, oven, distillation equipment, pipette, and micropipette. The instrument used was UV-Visible spectrophotometer Cary 60 Agilent. The materials used in this research are technical grade *n*-hexane, technical grade ethyl acetate, technical grade methanol, metal magnesium, hydrocholric acid, chloroform, ammonium hydroxide, ferric chloride, Mayer's reagent (potassium tetra iodo mercurate), ethanol. The materials used for the antioxidant test were ethanol (p.a, Merck), DPPH powder (Merck), DMSO, and ascorbic acid.

Nutmeg mace was obtained from Sindangwangi Village, Tasikmalalaya, West Java, Indonesia. The plant was determined at Universitas Padjadjaran, Bandung, West Java.

Preparation and Extraction

As much as 2 kg nutmeg mace was dried and then after drying it was mashed using a blender until become powder. Furthermore, 800 g of the powdered sample were macerated with a gradual solvent with *n*-hexane, ethyl acetate, and methanol, consecutively, for 24 hours for each solvent. Maceration was repeated until a clear filtrate was obtained. Furthermore, the solution was filtered. The filtrate from the maceration was then evaporated using a rotatory evaporator to obtain a thick extract (Rudiana *et al*. 2021).

Phytochemical Screening

Flavonoids identification

To 0.5 g of sample, 2 mL of 50% methanol was added and heated at 50°C then cooled. To the solution solid magnesium was added followed by addition of 5 drops of HCl (hydrochloric acid). If a red/orange color appears, it is positive that it contains flavonoids (Hanani 2015).

Alkaloids identification

A total of 0.1 g of extract was dissolved in 10 mL of $CHCl_3$ (chloroform) and 4 drops of NH_4OH was added then filtered. The filtrate was put into a capped test tube. The $CHCl_3$ extract in the test tube was then shaken by adding 10 drops of 2 M H_2SO_4 until 2 layers were formed. The acid layer was separated into another test tube and Meyer's reagent was added which produces a white precipitate, while the addition of Dragendorff's reagent will cause a red precipitate (Hanani 2015).

Saponins identification

A total of 0.5 g of sample was put into a test tube and 10 mL of hot distilled water was added. After cooling, the solution was shaken vigorously for 10 seconds. If steady foam rises for 10 minutes as high as 1-10 cm, add 1 drop of 2 N HCl solutions. If the foam does not disappear, it indicates the presence of saponins (Hanani 2015).

Steroids and terpenoids identification

A total of 0.1 g of extract was dissolved in 10 mL of $CHCl_3$ (chloroform) and 4 drops of NH_4OH was added then filtered. The filtrate was put into a capped test tube. The $CHCl_3$ extract in the test tube was then shaken after adding 10 drops of 2 M H_2SO_4 until 2 layers were formed. The acid layer above is separated into another test tube and Meyer's reagent is added which produces a white precipitate, while the addition of Dragendorff's reagent will cause a red precipitate (Hanani 2015).

Tannins identification

A total of 0.5 g of sample was dissolved with 10 mL of distilled water. It was filtered and the filtrate was diluted with distilled water until it was colorless. Take 2 mL of the solution and add 1-2 drops of iron (lll) chloride reagent. A blue or blackish green color occurs indicating the presence of tannins (Hanani 2015).

Antioxidant activity test

The method used to analyze the antioxidant power is the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The DPPH test is based on the measurement of DPPH absorption determination at a maximum wavelength of 515 nm using a UV-Vis spectrophotometer. Activity is expressed by the effective concentration capable of reducing free radicals by 50% (Suparmajid *et al.* 2016; Rudiana *et al*. 2018).

Preparation of 125 µM DPPH Stock Solution

A total of 4.9 mg of DPPH (MW 394.32 g/mol) was put into a volumetric flask, then add ethanol p.a to reach 100 mL. To prevent sun exposure, the measuring flask was covered with aluminum foil and the solution was placed in a dark place.

Samples

A total of 10 mg of sample (thick mace nutmeg extract) and 10 mg of vitamin C were each dissolved with 1 mL of DMSO in a different test tube. Furthermore, it is sonicated until dissolved and vortexed so that the solution is homogeneous. For the concentration series of ethyl acetate and methanol extract samples, namely 1.95; 3.91; 7.81; 15.6; 31.25 ppm and for samples of *n*-hexane extract that is 4.88; 9.77; 19.53; 39.07; 78.13.

Samples Measurement

A total of 100 µL of the sample (thick mace nutmeg extract) and vitamin C were added to the microplate for two repeats. For repeats 1 and 2, 100 µL of DPPH was added, while for the negative control only 100 µL of 95% ethanol was added. Incubation was carried out at room temperature in the dark for 30 minutes. The solution was measured using a UV-Vis spectrophotometer at 515 nm. Free radical scavenging activity was calculated as the percentage of DPPH color reduction.

Calculation of Antioxidant Potential with IC_{50}

Calculation of antioxidant potential with DPPH reagent by calculating IC_{50} for each sample using the equation of the linear regression line obtained from the graph of the relationship between concentration and % DPPH reduction. The amount of % DPPH inhibition is calculated with equation (1).

$$\%\ Inhibition\ DPPH = \frac{(CA-SA)}{CA} \times 100\% \quad \ldots (1)$$

Where:
CA = Control absorbance
SA = Sample absorbance

The sample concentration and % inhibition were plotted on the sample absorbance curve and plotted on the x and y axes of the linear regression equation, respectively. Sample concentration as x-axis and % inhibition as y-axis. From the equation y = ax + b, the IC_{50} value can be calculated using equation (2).

$$IC_{50} = (50 - b) : a \quad \ldots (2)$$

The equation was used to determine the IC_{50} (50% inhibitor concentration) of each sample is expressed by the y value of 50 and the x value obtained as IC_{50}. The extract was declared to have antioxidant activity if the resulting IC_{50} value was less than 100 ppm (µg/mL).

RESULTS AND DISCUSSION

Nutmeg Mace Extraction

Extraction of nutmeg mace begins with sample preparation, where the sample was dried under the sun. The sample was dried to remove water content therefore will inhibit fungal growth, after drying the sample was then mashed with a blender until it become powder so that the cross-section is larger so that the compound is extracted optimally. The maceration process for solvent selection is based on the "like dissolves like" principle. Application of various types of solvents with different levels of polarity was carried out to obtain extracts with optimal results from unknown compounds that belong to polar, semi-polar and non-polar compounds (Jun *et al*. 2003). Extraction of secondary metabolites from nutmeg mace was carried out consecutively. The maceration process of nutmeg mace samples was carried out by immersion for 24 hours using *n*-hexane, ethyl acetate, and methanol, consecutively. Residual immersion was carried out until a clear filtrate was obtained. The filtrate was then concentrated by evaporation using a rotary evaporator to obtain a thick extract. The yield of viscous extract from nutmeg mace was obtained, namely *n*-hexane extract 30.3%, ethyl acetate extract 8.79%, and methanol extract 3.79%. The calculation of the yield of nutmeg mace extract is shown in Table 1.

Table 1. Nutmeg mace extract results (M. fragrans)

Samples	*Condenced extract weight (g)*	*Yield* (%)*
n-hexane extract	242.5	30.30
ethyl acetate extract	70.3	8.79
methanol extract	30.3	3.79

Note: *calculated against 800 g of nutmeg mace simplicia

Phytochemical Screening

A phytochemical test of a sample is carried out to determine the content of secondary metabolites contained in the sample so that it can be an important guideline in the isolation of secondary metabolite compounds. The results of phytochemical tests on fresh samples and extracts from nutmeg mace is presented in Table 2.

Table 2. Phytochemical test results of nutmeg mace

Secondary metabolites types	*n-hexane extract*	*ethyl acetate extract*	*methanol extract*
Alkaloids	+	-	-
Tannins	-	-	-
Saponins	-	-	-
Terpenoid	+	+	+
Flavonoids	+	+	+
Steroids	+	-	-

Antioxidant Activity

In the present study, the antioxidant activity of mace nutmeg extract was tested using the DPPH test method. The test method using DPPH is one of the conventional quantitative methods and has long been used to determine the activity of antioxidant compounds. In addition, the process is also easy, fast, and sensitive to test the antioxidant activity of plant extracts using DPPH by spectrophotometer (Suparmajid *et al.* 2016). The principle of quantitative measurement of antioxidant activity using the DPPH method is that there is a change in the intensity of the purple color of the DPPH which is proportional to the concentration of the DPPH solution. The free radical DPPH which has an unpaired electron will give a purple color. The color will turn yellow when the electrons are paired. This change in the intensity of the purple color occurs due to the reduction of free radicals produced by the reaction of the DPPH molecule with the hydrogen atoms released by the sample compound molecules to form diphenyl picryl hydrazine compounds and cause a change in the color of DPPH from purple to yellow. This color change results in a change in absorbance at the maximum wavelength of DPPH using UV-Vis spectrophotometry so that the value of free radical scavenging activity will be known which is expressed by the inhibitory concentration (IC_{50}) value (Cahyani 2017). The IC_{50} value is defined as the concentration of the test compound that can reduce free radicals by 50%. The smaller the IC_{50} value, the higher the free radical scavenging activity. The IC_{50} value is obtained from the linear regression equation. The measurement of extract absorbance with DPPH using a UV-Vis spectrophotometer was previously carried out to determine the maximum wavelength of DPPH where in this study the maximum wavelength obtained at 0.5 mM DPPH is 515 nm. The molecules being analyzed so that UV-Visible spectrophotometer is more widely used for quantitative analysis than qualitative. The concentration of the analyte in the solution can be determined by measuring the absorbance at a certain wavelength using Lambert-Beer's law (Suparmajid *et al.* 2016). The antioxidant activity test was carried out on nutmeg mace extract repeat test and the absorption was measured at the optimum wavelength of 515 nm because at that wavelength the absorption was maximum. The absorbance value obtained can then be calculated as the percentage value of DPPH radical inhibition (% inhibition). Then a linear regression curve was plotted and an equation was developed with concentration as the x-axis and absorbance as the y-axis. The IC_{50} value can be calculated from the previously obtained linear regression equation by replacing y with 50 in the equation. The IC_{50} value is a number that indicates the concentration of the test sample (ppm) which can inhibit the oxidation process by 50%. The antioxidant activity test of mace nutmeg extract was carried out by varying the concentration of *n*-hexane extract, i.e. 4.88, 9.77, 19.53, 39.07, and 78.13 ppm. As a positive control ascorbic acid was used. The result of the antioxidant activity test nutmeg mace extract is presented in Table 3.

Table 3. IC_{50} value of graded extract nutmeg mace

Sample	*IC_{50} (ppm)*
n-hexane extract	56.22 ± 1.15
ethyl acetate extract	20.39 ± 0.13
methanol extract	17.89 ± 0.10

From the doubling of the antioxidant activity test of the *n*-hexane extract of mace nutmeg, the average IC_{50} value was 56.22 ppm with a standard deviation of 1.15. According to Awe *et al*. (2013) the activity is categorized as very strong, strong, moderate, and weak depending on the IC_{50} value as shown in Table 4. Based on Table 4, the antioxidant activity of the *n*-hexane extract of mace nutmeg is classified as strong with an IC_{50} value in the range of 50-100 ppm.

Table 4. Antioxidant power level based on IC_{50} value (Awe *et al*. 2013).

IC_{50} (ppm)	*Antioxidant activity*
<50	Very strong
50-100	Strong
100-150	Medium
150-200	Weak

Testing the antioxidant activity of the thick extract of ethyl acetate and methanol mace nutmeg was carried out by varying the concentration which are 1.95, 3.91, 7.81, 15.6, 31.25 ppm and as a positive control ascorbic acid was used with the same concentration series as *n*-hexane extract activity test. The results of the antioxidant activity test of the ethyl acetate extract and methanol extract of nutmeg mace is shown in Table 3. The values presented are means of two repetitions. The antioxidant activity test of ethyl acetate and methanol extracts of nutmeg mace gave the average IC_{50} values 20.39 and 17.89 ppm, respectively, with standard deviations of 0.13 and 0.10. Based on Table 4, the antioxidant activity of the ethyl acetate and methanol extracts of nutmeg mace is categorized at a very strong level with IC_{50} values in the range of <50 ppm.

CONCLUSIONS

The yield of nutmeg mace extract was 30.3%, 8.79%, and 3.79% for *n*-hexane, ethyl acetate, and methanol. The *n*-hexane, ethyl acetate, and methanol extracts of nutmeg mace has a strong antioxidant activity with IC_{50} values of 56.22, 20.39, and 17.89 ppm, respectively.

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