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Determination of Total Phenolic Content and Antioxidant Activity of Red Ginger (Zingiber Officinale Roscoe) Ethanol Extract Using Dpph Method

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ABSTRACT: Red Ginger (Zingiber officinale Roscoe) is one of the plants from the Zingiberaceae family which is commonly used traditionally for the treatment of various types of diseases. Red ginger plants contain many bioactive compounds, one of which is from the phenolic group which has the potentialas an antioxidant. This study aims to determine the total phenolic content and antioxidant activity ofred ginger ethanol extract. From 600 g of wet red ginger, the extract yield was 8.393%. The results of organoleptic examination of the extract were thick extract form, blackish brown color, characteristicodor and bitter taste. Phytochemical screening examination of red ginger ethanol extract samples contained phenolics, flavonoids, andalkaloids.The method used in the determination of total phenolic content was the Folin-Ciocalteu method, with the maximum absorption wavelength of gallic acid at a concentration of 50g/Ml was 762.5nm and absorbance was 0.301. Antioxidant activity test using the DPPH method, with a maximum absorption wavelength of 35 g/mL DPPH is 516 nm and absorbanceis 0.666. The total phenolic content obtained from the ethanolic extract of red ginger (Zingiberofficinale Roscoe) which was measured using a UV-Vis Spectrophotometer was 7.74% w/w. The antioxidant activity obtained from the ethanolic extractofred ginger (Zingiber officinaleRoscoe) with IC_{50} of 144.64g/ml was classified as moderate (101-150 g/mL).

KEYWORDS: Zingiberofficinale Roscoe, Ethano lExtract, Phenolic, Antioxidants

I. INTRODUCTION

Free radicals are chemical compounds that are reactive and unstable because they contain one or more unpaired electrons, which can cause damage to body components. Free radicals are produced by various complex chemical processes in the body's cells, including exposure to sunlight and air pollution, which causes free radicals to rise. To overcome this, antioxidants from outside the body are required. Plants produce several antioxidants, including vitamins C, E, β-carotene, and phenols, particularly flavonoids [1].

Antioxidants are molecules that can counteract the negative effects of oxidants in the body. They function by giving one electron to oxidant compounds, thereby inhibiting their action. Antioxidants repair and protect skin cells from free radicals[2]. Indonesia has long been known for producing spices that are highly beneficial as flavors and medicines. Various ethnicities in Indonesia, including Java, practice traditional medicine using plants from the Zingiberaceae tribe. Red ginger (Zingiber officinale Roscoe) is a kind of plant in the Zingiberaceae family.

Red ginger has two components: volatile oil and non-volatile oil. Evaporated oil components, such as essential oils and ginger oil, contain a variety of chemicals. Essential oils contain phenolic compounds, including 1,8-cineol, eugenol, zingerone, and gingerdiols. Ginger's phenolic compounds are recognized to have antioxidant and anti-inflammatory properties.

According to the description above, researchers are interested in measuring the total phenolic content and antioxidant activity of red ginger. The total phenol content of red ginger was determined in two stages: extraction from samples and quantitative measurement with a UV-Vis spectrophotometer. Total phenol concentration was determined using the Folin-Ciocalteau reagent, and antioxidant activity was assessed using the DPPH method.

II. EXPERIMENTATION

UV-Vis Spectrophotometer PG T92+, rotary evaporator (Hettich zentrifugen), funnel,

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measuring cup, spatula, spatula, dropper, Erlenmeyer, measuring flask, dark bottle, beaker glass, watch glass, vial, test tube, poselin crucible, oven (Menmert), aluminum foil, analytical balance (Kern ABJ-nm/ABS-N), digital scale, measuring forceps, spray bottle, drop plate, evaporating cup, blender, Whatman filter paper, cuvette, furnace (Carboute), goiter pipette, stir bar and volume pipette. Next is red ginger (Zingiber officinale Roscoe), 96% ethanol, distilled water, DPPH, gallic acid, methanol, chloroform, Folin Ciocalteu reagent, FeCl3, Mg metal, concentrated HCl, anhydrous acetic acid, norite, ammonia chloroform, Mayer's reagent, sodium carbonate, sulfuric acid, H2SO4 2N and H2SO4(p).

Research procedures

The sample used in this research was red ginger (Zingiber officinale Roscoe) taken in the Sitiung District area, Dharmasraya Regency, West Sumatra.

Process for Making Red Ginger Simplicia

After properly cleaning 4 kg of red ginger under running water to get rid of any impurities, the ginger is drained, sliced into smaller pieces, and keptdamp. Finally, the ginger is pureed in a blender until simplicia powder is achieved.

Process for Making Red Ginger Ethanol Condensed Extract

Thick ethanol extract of red ginger is prepared by macerating 250 grams of wet red ginger powder with 90% ethanol solvent for 3 days while stirringoccasionally. After 3 days of soaking, the macerate was filtered through filter paper and evaporated using a rotary evaporator until a thick extract wasobtained. The extract is next examined, and a phytochemical screening test is performed to determine the quality of the extract.

Determination of Maximum Absorption Wavelength of Gallic Acid

Pipette 1 mL of the gallic acid stock solution into a 10 mL volumetric flask and then dilute with methanol: distilled water (1:1) to the mark to obtain aconcentration of 50 µg/mL. Then in a pipette, 0.5 ml is mixed with 5 mL of Folin-Ciocalteu reagent (diluted 1:10 distilled water). Then add 4 mL of 1M sodium carbonate solution, and shake homogeneously. Leave at room temperature for 15 minutes and measure the maximum absorption wavelength of gallic acid with a UV-Vis spectrophotometer [3].

Determination of Calibration Curve for Garlic Acid Solution

The 500 µg/mL gallic acid stock solution was pipetted into (0.8; 1; 1.2; 1.4; 1.6) mL respectively and diluted with methanol: distilled water (1:1) in a 10 mL volumetric flask until the mark limit so that the concentration is obtained (40, 50, 60, 70, 80) µg/mL gallic acid. Pipette 0.5 mL of each solution, then mix with 5 mL of Folin-Ciocalteu reagent (diluted 1:10 distilled water) add 4 mL of 1 M sodium carbonate solution, leave for 15 minutes, and measure the absorption at the absorption wavelength. maximum [4].

Determination of Total Phenolic Compound Content of Samples

Using a pipette, take 0.5 mL of the sample extract solution, then add 5 mL of Folin-Ciocalteu reagent (diluted 1:10 in distilled water), followed by 4 mL of 1 M sodium carbonate solution, shake thoroughly, leave at room temperature for 15 minutes, and measure the maximum wavelength absorption with a UV-Vis spectrophotometer. This method was repeated three times, and the total phenolic compound content was estimated using a linear equation formula derived from the standard solution calibration curve [3].

Determination of DPPH Maximum Absorption Wavelength

Using a pipette, take 4 mL of the freshly made 35 µg/Ml DPPH solution put it in a vial, and add 2 mL of a mixture of methanol and distilled water (1:1), then let it sit for 30 minutes in a dark place. Measure the absorption of the solution with a Uv-Vis spectrometer [4].

Determination of Standard Antioxidant Activity of Gallic Acid

Pipet 10 mL of gallic acid stock solution (500 µg/mL), then dissolve it in a mixture of methanol and distilled water (1:1) in a 100 mL volumetric flask to the mark, to obtain a gallic acid solution with a concentration of 50 µg/mL. From this solution, pipet each (0.4; 0.8; 1.2; 1.6; 2.0) mL into a 10 mL measuring flask, add a mixture of methanol and distilled water (1:1) to the mark so that The concentration obtained was (2; 4; 6; 8; 10) µg/mL. Pipette 2 mL of each solution then put it into a vial, add 4 mL of 35 µg/mL DPPH solution. Leave it for 30 minutes in a dark place until the yellow powder color decays from purple to yellow. The solution's absorbance was measured with a UV-Vis spectrophotometer. To find the linear regression equation, calculate the percentage

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inhibition for each at the highest absorption wavelength. The IC50 of gallic acid is the concentration of a reference solution that produces 50% inhibition and can be estimated using linear regression [3].

Determination of Antioxidant Activity of Red Ginger Ethanol Extract

To get a concentration of 1 µg/mL, weigh 25 mg of extract and dissolve it in methanol in a 25 mL volumetric flask until the limit is reached. Pipette 0.2; 0.6; 1; 1.4; 1.8 mL of the sample solution. Then, in a 10 mL volumetric flask, add methanol and distilled water (1:1) until the limit is reached. Samples were collected at concentrations of (20, 60, 100, 140, 180) µg/mL. Pipette 2 mL of sample solution into a container. Add 4 mL of DPPH 35 µg/mL. The mixture was homogenized and placed in a dark area for 30 minutes before measuring absorbance using a UV-Vis spectrophotometer at the maximum absorption wavelength.

III. DATA ANALYSIS

1. Determination of Total Phenolic Content

The sample concentration is determined using the linear regression equation y = a + bx which is created based on absorbance and concentration data of the standard solution obtained from the standard solution calibration curve.

Total phenolic content was calculated using the following formula:

The content = C x V x Fp : Bs

Where:

C = Sample solution concentration (µg/mL)

V = Volume of sample solution (ml)

Fp = Dilution factor

Bs = Sample weight

2. Determination of Antioxidant Activity

The antioxidant activity of the sample was determined from the amount of DPPH radical absorption inhibition by calculating the percentage of DPPH absorption inhibition.

% Inhibition = AControl − Asample × 100% : Acontrol

where:

Acontrol = Absorption of DPPH radical solution 35 µg/mL

Asample = Absorption of the sample solution plus 35 µg/mL DPPH solution

3. IC50 value

The IC50 value is determined using a linear regression equation, and it represents the concentration of the extract that can inhibit the activity of a radical by 50%. To calculate IC50, a standard curve equation with percent inhibition on the y-axis and antioxidant extract concentration on the x-axis is required. The IC50 concentration is obtained by substituting the 50% value as the y-axis in the standard curve calculation and then determining the x value. The lower the IC50 value, the higher the antioxidant activity.

IV. RESULT AND DISCUSSION

Samples weighing 4 kg were collected in the Sitiung1 area of Dharmasraya Regency, West Sumatra Province, and identified at the Herbarium of the Biology Department, Faculty of Mathematics and Natural Sciences (FMIPA), Andalas University, Padang. Identification seeks to confirm the accuracy of the samples used in this study. According to the identification results, the sample utilized in this study was red ginger (Zingiber officinale Roscoe) from the Zingiberaceae family.

To clean or separate the rhizomes from soil pieces or other plants carried during the sampling process, 4 kg of fresh red ginger was removed straight away and wet sorted. The red ginger rhizomes are then cleaned under running water, drained, and sliced while still damp.

The simplicia is then cut into small pieces and pollinated to reduce the size of the simplicia and increase the surface contact of the red ginger rhizome with the solvent, allowing the solvent to penetrate the sample more quickly and making the extraction process more efficient. Red ginger rhizome powder is subsequently extracted using the maceration process. This approach was chosen since it has a more straightforward procedure than the others. It doesn't need any additional tools and is very successful at attracting the necessary elements. There is no heat involved. The maceration method is a cold method that does not include heating, which causes the active ingredient to break down at high temperatures. The advantage of maceration is that it is a simple approach that can be used for all sorts of samples, both wet and dry, as well as thermo labile samples.

The solvent for extraction is 96% ethanol. The extraction process was repeated three times for 24 hours, with the first maceration using 96% ethanol since the sample was wet, and the volume of water in the 96 ethanol solution was lower, 4%, than in the 70% ethanol solution, which was 30%. For the next repetition, 96% ethanol was employed

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to attract secondary metabolite compounds found in the samples. Ethanol is a universal solvent because it is inexpensive, nontoxic, and safe to use. Ethanol can extract the majority of the compounds found in simplicia, including polar and non-polar compounds. The filtrate is then filtered and collected, evaporated, and thickened using a high-pressure rotary evaporator, allowing for faster evaporation because the solvent evaporates at a temperature below boiling point, yielding a thick extract from red ginger rhizomes. From 250 g of dried red ginger rhizome, 50.36 g of thick extract was produced, yielding 20.14%. The yield data is related to the active compounds in a sample, therefore as the yield number increases, so does the quantity of active compounds in the sample.

According to Harborne (1987), a sample's high yield indicates the presence of a large number of active compounds. The following step is an organoleptic examination, which tries to assess the shape, smell, color, and taste of the sample extract. The extract obtained in this study was thick, had a distinct scent, was blackish brown in color, and had a bitter taste when tested using the five senses. Aside from organoleptic tests, phytochemical analysis revealed that the ethanol extract of red ginger included phenolic chemicals, flavonoids, and alkaloids. The drying shrinkage study revealed that it was around 19.73%. This drying loss value is high, indicating that the sample contains a high concentration of volatile compounds such as essential oils and resins. In addition to volatile chemical components, the sample is thought to contain a high concentration of water, which does not meet the standard of no more than 10%[5].

The goal of drying shrinkage is to calculate the percentage of compounds lost during the heating process, including water and other evaporated compounds [5].The drying process involves heating to 105℃ to evaporate water and compounds with lower boiling points. The determined ash content was approximately 4.85%. The goal of measuring ash content is to offer an overview of the internal and external mineral and metal content, such as heavy metals (Cu, Pb, Fe), that arise from the starting process till the production of a thick extract [6].

Total phenolic content is determined using the Folin-Ciocalteu method, which includes the use of the Folin-Ciocalteu reagent. Total phenolic content is determined by forming a blue complex product using the Folin-Ciocalteu reagent and measuring it at a maximum wavelength of 765 nm [7].First, the maximum absorption wavelength of the standard solution of gallic acid is determined. Gallic acid is a class of phenolic compounds that are stable and pure, cheap, and have a stability of no more than 5% when stored for a long period of ± 2 weeks in a cupboard, cooling, and closed(Waterhouse,1999).At a concentration of 50 µg/mL, the maximum absorption wavelength is 762.5 nm and the absorbance value is 0.301.

After obtaining the maximum wavelength, we can continue measuring the absorbance of gallic acid with concentrations of 40, 50, 60, 70, and 80 µg/mL. This was done to determine the gallic acid calibration curve with the Folin-Ciocalteu reagent to obtain a regression equation for determining total phenolic content. From the work carried out, a calibration curve was obtained with the regression equation $y = -0.0792 + 0.00779x$ and a value of $r = 0.999$. The calibration curve's findings with the obtained data were linear, as demonstrated by a r value close to one. The phenolic content of the ethanol extract was measured at a concentration of 50 µg/mL, yielding 7.74% w/w. The phenolic chemicals found in the ethanol extract of red ginger are the consequence of secondary metabolites that function as antioxidants. Aside from that, this can occur because the phenol group is polar or semi-polar[8].Not only are phenolics antioxidants, but the largest group of phenolic chemicals, flavonoids, may also be polar.

The detection and quantitation limits for total phenolic levels were 1.9709 µg/ml and 6.5699 µg/ml. The detection limit is the lowest concentration of analyte that can be measured using UV-Vis spectrophotometry, whereas the quantitation limit is the lowest concentration that can be determined using the Folin-Ciocalteu method. The concentration of the test solution in the ethanol extract is 77.41 µg/ml, exceeding both the detection and quantitation limits. The DPPH method is used to test antioxidant activity because it is simple, quick, and sensitive, requiring just a small sample and taking only a short amount of time. DPPH is a free radical that is stable at room temperature and is easily oxidized by light and air. Compounds that have antioxidant activity will react with DPPH as indicated by a color change from violet to yellow due to the donor of hydrogen atoms from the antioxidant to DPPH. The amount of antioxidant activity is indicated by the IC50 value, namely the sample solution required to inhibit 50% of DPPH free radicals. The IC50 value is obtained from the regression equation of percent inhibition (y) and sample extract concentration (x) by entering the value 50 as the y-axis into the regression equation and then calculating the x value as the IC50 concentration. Percent inhibition is the

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ability of a material to inhibit free radical activity which is related to the concentration of the material.

Gallic acid was utilized as a comparison in this study since it is a low-cost, pure chemical with great stability when maintained in a refrigerator for two weeks[9].Gallic acid had an IC50 of 5.631 µg/mL and red ginger ethanol extract had an IC50 of 144.64 µg/mL, indicating medium antioxidant activity. According to Maulidha et al. (2015), antioxidant activity is classified into four categories: very strong (<50 µg/mL), strong (51-100 µg/mL), medium (101-150 µg/mL), and weak (>150 µg/mL) [10]. The ethanol extract of red ginger exhibits moderate antioxidant activity. This is possible because various bioactive compounds function as antioxidants, one of which being phenolic compounds, which are drawn to the extraction process utilizing semi-polar and polar solvents. The findings of the equivalence between the antioxidant activity of red ginger ethanol extract and the comparison, namely gallic acid, have a value of 1:25.6863 mg, indicating that 1 mg of gallic acid is equivalent to 25.6863 mg.

The findings of assessing total phenolic content and antioxidant activity demonstrate a link between the two, with higher total phenolic content indicating higher antioxidant activity.

V. CONCLUSION

According to results of the research that has been carried out, it can be concluded that the total phenolic content obtained from the ethanol extract of red ginger (Zingiber officinale Roscoe) using the Follin-Ciocalteu method as measured using a UV-Vis Spectrophotometer is 7.74% w/w and the anti-oxidant activity obtained from ethanol extract of red ginger (Zingiber officinale Roscoe) with IC50 = 144.64 µg/mL is classified as medium (101-150 µg/mL).

REFERENCES

[1]. Muchtar,H.,Juwita,A.,Putri,K.2020.UjiAntioksidan Ekstrak Etanol KulitBuah danDagingBuahMenteng(Baccaurearacemosa(Blume)Mull.Arg.)denganMetodeDPPH(2,2diphenyl-1-picrylhydrazyl).JurnalFarmasidanKesehatan.

[2]. Ulfa,A.M.,Chusniasih,D.,&Bestari,A.D.2019.PemanfaatanPotensiAntioksidandariLimbahKulitBuahKakao(Theobromacacao L).JurnalFarmasiMalahayati,2(1).

[3]. Pourmorad,F.,Hosseinimehr,S.J.,danShahabimajd,N.2006.AntioxidantActivity, Phenol and Flavonoid Contents ofSomeSelectedIranianMedicinalPlants.AfricanJournalofBiotechnology;5(11):1142–1145.

[4]. Mosquera, O. M., Corra, Y. M., Buitrago, D. C.,and Nino J. 2007. Antioxidant Activity ofTwentyFivePlantsfromColombianBiodiversity.Mem.Inst.OswaldoCrus;102(5):631-634.

[5]. Departemen Kesehatan Republik Indonesia. 2008. Farmakopeherbal Indonesia EdisiI,Jakarta, DepartemenKesehatan RI.

[6]. Isda,M.N.,Wahyu,L.,danDiana,A.2013.Optimasi Konsentrasi Ekstrak Alang-alang(ImperatacylindricaL.)UntukMemacuPertumbuhandanProduksiJagungManis(ZeamaysSacharataSturt).Al-KauniyahJurnalBiologi; 6(1):1-6.

[7]. Prior,R.L.,Wu,X.,andSchaich,K.2005.StandardizedMethodsforTheDetermination of Antioxidant Capacity and PhenolicsInFoodsandDietarySupplements. Journal of Agricultural and Food Chemistry;53(10):4290-4302.

[8]. Hayati,E.K.,Fasyah,A.G.,danSa'adah,L.2010.Fraksinasi dan Identifikasi Senyawa Taninpada Daun Belimbing Wuluh (AverrhoabilimbiL.).JurnalKimia;4(2):193-200.

[9]. Waterhouse,A.1999.Folin-CiocalteuMicroMethodForTotalPhenolInWine.DepartementOfViticulture&EnologyUniversityOf California:David

[10]. Maulidha.N.,Fridayanti.A.,danMasruhim.M.A.2015.UjiAktivitasAntioksidanEkstrakDaunSirihHitam(Pipersp.)TerhadapDPPH(1,1-Diphenyl-2-picrylHidrazyl).JurnalSains dan Kesehatan;1(1):16-20.