Indonesian Journal of Applied Research IJAR

In Vitro Evaluation of Purple Sweet Potato Leaf Extract (Ipomoea batatas) as a Tyrosinase Inhibitor and Malondialdehyde Formation Inhibitor

Dewi Purnamasari[1], Mega Safithri[1,2], Dimas Andrianto[1]

[1]*Department of Biochemistry, Faculty of Mathematics and Natural Science, IPB University, Indonesia*

[2]*Tropical Biopharmaca Research Center, LPPM IPB University, Indonesia*

Volume 5 Issue 1
(April 2024)
e-ISSN 2722-6395
doi: 10.30997/ijar.v5i1.384

ARTICLE INFO

Article history:
Received: 10-02-2023
Revised version received: 11-06-2023
Accepted: 04-23-2024
Available online: 04-30-2024

Keywords:
antioxidant test; Ipomoea batatas; malondialdehyde inhibitor; melanogenesis; tyrosinase inhibitor.

How to Cite:
Purnamasari, D., Safithri, M., & Andrianto, D. (2024). In Vitro Evaluation of Purple Sweet Potato Leaf Extract (Ipomoea batatas) as a Tyrosinase Inhibitor and Malondialdehyde Formation Inhibitor. *Indonesian Journal of Applied Research (IJAR)*, *5*(1), 64-74. https://doi.org/10.30997/ijar.v5i1.384

Corresponding Author:
Mega Safithri
safithri@apps.ipb.ac.id

ABSTRACT

The study purple sweet potato is known to contain flavonoids, a class of natural polyphenols with the capability to mitigate skin pigmentation. The research aims to assess the antioxidant and tyrosinase inhibitory activities of purple sweet potato extracts obtained through hexane, ethyl acetate, ethanol, and water solvents. The results of phytochemical identification show that the extract contains various secondary metabolites which have the potential to act as antioxidants. The DPPH method IC50 values of 6948.12ppm for n-hexane, 3015.19ppm for ethyl acetate, 128.05ppm for ethanol, and 791.77ppm for water extract. The MDA inhibitor test IC50 values of 2067.02ppm for n-hexane, 1968.13ppm for ethyl acetate, 116.14ppm for ethanol, and 921.14ppm for water extract. In the tyrosinase inhibitor assay, IC50 values were 1328.29ppm for n-hexane, 1245.13ppm for ethyl acetate, 217.35ppm for ethanol, and 391.21ppm for water extract. Tuckey test statistics, ethanol extract was not significantly different from the positive control in the DPPH test, MDA inhibitor and tyrosinase test. These findings suggest that purple sweet potato extracts, particularly the ethanol extract, hold promise as natural ingredients with antioxidant and tyrosinase inhibitory properties, making them potential candidates for safe and effective skin brightening formulations.

1. Introduction

Appearing with bright white facial skin is a necessity for women in general. It is not surprising that many cosmetic manufacturers sell their products with claims to whiten skin. The bleaching agents that are often used are arbutin, hydroquinone, vitamin C, mercury, mulberry extract and so on. Of all the names of these bleaching agents, mercury and hydroquinone are not permitted to be present in preparations intended for skin or hair, referring to the requirements set by the RI POM Agency through Regulation of the Head of the RI POM Agency No. 18 of 2015 concerning Technical Requirements for Cosmetic Ingredients, due to their permanent damaging effects on the skin. However, based on the results of testing by the BPOM, as stated in public warning No. HM.01.1.2.10.21.48 and No. HM.01.1.2.10.21.49 on 13 October 2021 regarding Cosmetics Containing Prohibited/Dangerous Materials, cosmetics containing mercury and hydroquinone were still found. Therefore it is necessary to develop a natural compound that functions as a skin lightener so that it is safe to use.

The skin is the main protector from ultraviolet radiation because it is located on the outermost part of the body. UV light is needed in the process of melanin formation, however continuous exposure to ultraviolet light causes changes in the structure and function of the skin, ranging from acute side effects such as sunburn, tanning, hyperpigmentation, to chronic effects such as photoaging and skin cancer. Excessive UV exposure causes the formation of reactive oxygen species (ROS) or free radicals which increase protein oxidation and accumulation of lipid peroxidation in human skin and ultimately ROS will cause oxidative stress in the skin which can stimulate excessive melanin formation. The ROS that is formed will trigger the work of tyrosinase and end with the synthesis of melanin. Therefore, compounds inhibiting the formation of melanin are needed. Apart from determining the inhibitory activity of the tyrosinase enzyme, antioxidant activity was also determined. Antioxidants function as ROS scavengers so they can reduce hyperpigmentation (Chasanah, 2016). According to Parwata (2016), phenol compounds such as flavonoids can capture ROS, inhibit the work of enzymes that produce ROS and form chelates with metals that stimulate the formation of ROS so that ROS reactions with normal cells such as fat peroxidation and DNA damage can be prevented. or oxidative stress does not develop.

One of the plants that can be used as a medicinal and cosmetic plant is purple sweet potato (Ipomoea batatas). Based on Laksmiani's research in silico (Laksmiani et al., 2019), the compounds found in sweet potato namely cyanidin and peonidin have the potential to be depigmenting agents through inhibition of D-Dopachrome taumerase protein, however in vitro there has been no research related to tyrosinase inhibitors and their antioxidant activity with various solvents.

2. Methods

2.1. Tools and Materials

The tools and materials used in the research were a Memmert UM20 oven, Pyrex brand glassware, Eyela N1000 evaporator, microplate reader (Biotek Epoch), micropipette, Thermo Scientific HPSRT2 Advance hot plate, Memmert water bath, centrifuge, desiccator, shaker, 96 well plate, pH meter metrohm 691, analytical balance OHAUS PA214 , Spectrophotometer Agilent 8453, sweet potato leaves (Ipomoea batatas L.) obtained from the Research Institute for Spices and Medicinal Plants (Balitro) Bogor city, Ethanol 98% (Merck Germany), ethyl acetate (Merck Germany), n-hexane (Merck Germany), water, solvent DMSO (dimethyl sulfoxide), Tyrosinase enzyme (Sigma), L-Tyrosine, kojic acid, DPPH (2.2-diphenyl 1-picrylhydrazyl) (Merck), Tris-buffer solution HCL, ascorbic acid, methanol, TMP (1,1,3,3-tetramethoxypropane), TCA (trichloroacetic acid), TBA (thiobarbituric acid), acetic acid,

linoleic acid, phosphate buffer, Vitamin E.

2.2. Simplistic Setup

Samples were obtained from Balitro, Bogor City and determined by the Indonesian Research Agency (BRIN) Cibinong, Bogor. This test was carried out at the Pharmaceutical and Food Analysis Laboratory, Jakarta II Health Polytechnic, Ministry of Health, and the IPB Bogor Biochemistry Laboratory. Extraction of sweet potato leaves which are already in the form of fine simplicia using the maceration method. The extraction process in this study used 4 solvents (n hexane, ethyl acetate, ethanol and water), the first being N-hexane with a ratio of 1:10. Put it in an Erlenmeyer, then leave it for 24 hours on a shaker. The maserate was separated and the process was repeated three times with the same type and amount of solvent. Then the remaining macerated powder is macerated again with the second solvent, namely ethyl acetate (treated the same as the first solvent), doing so until the third solvent is ethanol. The results of all the macerates were collected and concentrated using a rotary evaporator at a temperature of 40-50 C until a thick extract was obtained.

2.3. Phytochemical Screening

Alkaloids. 20 mg of sample was put into a test tube and 2 ml of 5% HCl was added. Divided into 3 parts and put into a test tube. Test tube (I) is added to dragendorff to produce an orange red precipitate, test tube (II) is added to mayer to produce a yellowish white precipitate, and test tube (III) is added to Wagner to produce a brown precipitate. Flavonoids. 20 mg of sample was added to 6.25 ml of 70% ethanol. Heat until the volume becomes ½, add ethanol, magnesium powder and 5M HCL solution. Positive if it is bright red or violet. Saponins. 20 mg of sample was added to 10 ml of distilled water. Followed by heating 5 minutes 70C, let stand 5 minutes then for 10 seconds the solution is shaken vigorously then allowed to stand for 10 seconds. Foam 1-10 cm high is formed and the foam does not disappear even after adding 1 drop of 2N HCl if the extract is positive for containing saponin. Triterpenoids. 20 mg of sample was added to 5 ml of 30% ethanol, heated for 5 minutes at 50C, 2 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid were added. Glycosides (Rosyidah, 2021). A total of 0.1 g of sample was added to 1 mL of distilled water and then 5 drops of Fehling A and 5 drops of Fehling B were added to the mixture.

The mixture was boiled until a brick/orange yellow color was formed indicating the presence of glycosides. Temulawak as a positive control and table salt as a negative control. Tanin (Rosyidah, 2021). A total of 0.1 g of extract was added to 2 mL of distilled water then boiled for 5 minutes. The solution was filtered and the filtrate was added with 5 drops of 1% FeCl3 (w/v). A dark blue or greenish black color indicates the presence of tannins. Black tea as a positive control and fructose as a negative control. Phenol. 0.2 gram of sample extract was added with 1% FeCl3 solution. Results are indicated by the formation of green, red, purple, dark blue, blue, blackish blue, or blackish green. Quinone (Pratiwi, 2016), as much as 0.5 g of the extract was dissolved in 10 ml of water and heated over a water bath then added a few drops of 1 N sodium hydroxide, the presence of a red solution indicated the presence of quinone.

2.4. DPPH Method Antioxidant Test (Andrianto et al., 2015)

Preparation of Test Solutions. The DPPH solution was made by dissolving 0.8 mg DPPH in 10 mL methanol to obtain a concentration of 0.2 mM and homogenized for 30 minutes in the dark. A 1 M Tris-HCl buffer solution was made by dissolving 12,114 g of Tris-base in 100 mL of distilled water to which concentrated HCl was previously added to obtain a pH of 7.4. Next, the test mixture consisted of 10 mL 0.2 mM DPPH, 10 mL 1 M Tris-HCl buffer pH 7.4, and 10 mL 20% methanol. The sample stock solution was prepared with a concentration of 1000 µg/mL. A standard stock solution was prepared by weighing 0.25 mg of ascorbic acid and then dissolving it in 5 mL of methanol, to obtain a concentration of 50 µg/mL.

Ascorbic Acid Antioxidant Test. Ascorbic acid stock solution with a concentration of 50 µg/mL was prepared by dissolving ascorbic acid in methanol. Next, the standard stock of ascorbic acid was dissolved in 80% methanol to prepare solutions having final concentrations of 0, 1.25, 2.5, 3.75, 5, and 6.25 µg/mL. A total of 0.3 mL of a certain concentration of ascorbic acid solution was added to 0.9 mL of the test mixture solution that had been prepared beforehand. The mixture was vortexed and then incubated for 20 minutes in the dark. After that the absorbance was measured at a wavelength of 520 nm. Measurement of the positive control antioxidant with ascorbic acid was carried out three times.

Sample Antioxidant Test. The samples used were extracts of N-Hexane, ethyl acetate, ethanol and distilled water. The stock of each sample solution was prepared at a concentration of 50 µg/mL by dissolving the extract using methanol. Next, the stock solution was diluted to make solutions that had varying final concentrations, namely 0, 1.25, 2.5, 3.75, 5 and 6.25 µg/mL. A total of 0.3 mL of each sample solution with various concentrations was added to the test mixture solution of 0.9 mL. The mixture was vortexed and then incubated for 20 minutes in the dark. After that, the absorbance was measured at a wavelength of 520 nm. Three repetitions were carried out. Determination of Inhibition Percentage. Antioxidant activity is expressed by the IC50 (Inhibition Concentration 50) value, which is a number that indicates the concentration of the test sample that provides 50% reduction against free radicals from the equation produced by linear regression of percent inhibition. Percent inhibition of DPPH is calculated using the formula:

$$\text{Inhibisi (\%)} = \frac{\text{Absorbansi blanko} - \text{absorbansi sampel}}{\text{Absorbansi blanko}} \times 100$$

2.5. Malondialdehyde Formation Inhibition Test (Kikuzaki & Nakatani, 1993)

2.5.1. Determination of the Standard Curve of 1,1,3,3,-tetramethoxypropane (TMP) Solution

Standard curves were prepared using TMP solutions with concentrations of 1, 5, 8, 10, 13, 15, 18, 20 µM. Pipette 1 mL of each solution and add 2 mL of 20% TCA solution and 2 mL of 1% TBA solution in 50% acetic acid. the reaction mixture was placed in a 100 °C water bath for 10 minutes. After cooling, the solution was centrifuged at 1310 g (centrifuge rotor diameter 130 mm) for 15 minutes. Then the absorption is measured at a wavelength of 532 nm. The blank solution uses 1 mL of distilled water which is treated like any other TMP test solution (0 TMP concentration).

2.5.2. Determination of The Incubation Time of Linoleic Acid by The TBA Method

Determining the incubation time for linoleic acid using 6 mL of 0.1 M phosphate buffer pH 7, then adding 6 mL of 50 mM linoleic acid in 99.8% ethanol, and 3 mL of ion-free water. As much as 1 mL of the mixture was placed in a dark bottle then the mixture was incubated at 40°C. After incubation, 1 mL of the linoleic acid mixture was taken and added to 2 mL of 20% TCA solution and 2 mL of 1% TBA solution in 50% acetic acid. The reaction mixture was heated in a water bath at 100°C for 10 minutes. The solution was cooled, then centrifuged at 1310 g (rotor diameter 130 mm) for 15 minutes. The absorption intensity of the solution was measured at a wavelength of 532 nm. Measurements were made every day until maximum absorption was achieved. The blank solution used was 1 mL of mixture, 3 mL of 99.8% ethanol and 2 mL of 0.1 M phosphate buffer pH 7, then added 2 mL of 20% TCA and 2 mL of 1% TBA in 50% acetic acid. The blank solution was heated at 100°C for 10 minutes, then centrifuged at a speed of 1310 g rpm (rotor diameter 130 mm) for 15 minutes.

2.5.3. Analysis of the Concentration of Malondialdehyde (MDA) with the TBA Method

Antioxidant analysis of purple sweet potato leaf extract was carried out at concentrations of 25, 50, 75, 100, 200 ppm. 1 mL of each solution was taken, then 2 mL of 0.4 M phosphate buffer pH 7 and 2 mL of 50 mM linoleic acid in 99.8% ethanol were added. The solution was

put into a dark bottle and incubated at 40°C for several days (based on the optimum temperature). After incubation, 1 mL of each solution was taken, 2 mL of 20% TCA solution was added and 2 mL of 1% TBA in 50% acetic acid. The mixed solution was heated at 100 °C for 10 minutes. After heating, the solution was cooled and centrifuged at 1310g (rotor diameter 130 mm) for 15 minutes. The solution was measured for its absorption at a wavelength of 532 nm. The blank solution used distilled water.

2.6. Tyrosinase Activity Test (Batubara et al., 2010)

Determination of tyrosinase inhibitory activity on sample extracts was carried out using L-Tyrosine as a substrate and kojic acid as a positive control. The extract was dissolved in 1 ml DMSO until the concentration reached 10,000 g/mL. Solutions were made in several series of concentrations with 50 mm phosphate buffer (pH6.5) so that concentrations of 500, 1000, 2000, 3000, 4000 g/mL were obtained. 40 L of kojic acid was added. A total of 40 L of the sample was put into a 96-well microplate then 40 L of tyrosinase enzyme (sigma, 333 Unit/mL) was added and incubated for 5 minutes. Then 40 L (L-tyrosine) was added and incubated for 40 minutes at 37 C. The Abs value of the solution in each well was measured using a microplate reader at a wavelength of 492 nm. To determine the percent inhibition and 50% inhibitory concentration value (IC50). Percent inhibition is calculated by comparing the absorbance of the sample without the addition of extract (A) and with the addition of extract (B) at a wavelength of 492 nm with the following equation:

$$\text{Inhibisi (\%)} = \frac{A - B}{A} \times 100$$

The IC50 value is obtained from the logarithmic curve of the sample equation and % inhibition. The logarithmic equation obtained is, Y=a In X + b, to calculate the IC50 value by stating y=50 the x value will be obtained as IC50.

2.7. Statistic analysis

Statistical analysis was carried out using the Statistical Program for Social Science (SPSS) PSWT 26.0 program using One Way ANOVA and continued with further analysis in the form of the Duncan test. This analysis compares the activity of each sample in the form of an IC50 value using a 95% confidence interval.

3. Results and Discussion

3.1. Results

3.1.1. Content of Secondary Metabolite Compounds in Sweet Potato Leaves

The results of phytochemical screening for each solvent extract of n hexane and ethanol have similarities and differences in their secondary metabolite content. The n hexane and ethyl acetate solvents contain secondary metabolites in the form of triterpenoid steroids and quinones. In ethanol and water solvents contain secondary metabolites in the form of alkaloids, flavonoids, glycosides, tannins and phenols. Where the results of the identification of phytochemicals are in Table 1.

Table 1 Phytochemical test of sweet potato leaf extract

Phytochemical Identification	n-hexane	Ethyl acetate	Ethanol	Water
Alkaloid Test	-	-	++	+++
Flavonoid test	-	-	++	+++
Saponin test	-	-	-	+
Glycoside Test	-	-	+	-
Triterpenoid SteroidTest	+	+	+	-
Tannin test	-	-	+	+
Phenol test	-	-	+	+
Quinone test	+	+	-	-

3.1.2. DPPH Method Antioxidant Activity

The antioxidant activity of the results of this research is expressed in IC50, namely the concentration of antioxidant substances that produces a DPPH inhibition percentage of 50%. The IC50 value was obtained through a linear equation between the inhibition percentage and the sample concentration. The lower the IC50 value, the higher the inhibitory power of antioxidants against free radicals. The average IC50 result of the DPPH antioxidant test was 3.07a ± 0.0051 ppm for ascorbic acid as a positive control and for samples with N hexane, ethyl acetate, ethanol and water extracts it was 6948.11d ± 572.82 ppm , 3015.19c ± 55.02, 128.05 ab ± 1.78 and 791.86b ±4.15 ppm. The smallest IC50 result in the sample was produced by the extract with ethanol solvent which was 128.05ab ± 1.78 ppm from the triplo test (Table 2).

Table 2 IC50 values of ascorbic acid and samples (n hexane extract, ethyl acetate, ethanol and water) of sweet potato leaves using the DPPH method

Sample	Ascorbic acid	N-hexane	Ethyl acetate	Ethanol	Water
IC_{50}(ppm)	$3,07^{a}$±0,0051	$6948,11^{d}$±572,82	$3015,19^{c}$±55,02	$128,05^{ab}$±1,78	$791,86^{b}$±4,15

Different letters (a, b, c and d) indicate statistically significant differences (0.05) in the Tukey test.

3.1.3. Malondialdehyde Inhibitor (TBA-MDA)

MDA measurement is the reaction of one MDA molecule with two thiobarbituric acid (TBA) molecules to form a pink color which is measured on a spectrophotometer at a wavelength of 532 nm. The standard used in the calculation of MDA levels is tetramethoxypropane (TMP). Standard TMP in acidic conditions can be hydrolyzed to produce semiacetal and methanol, the hemiacetal formed will then decompose into methanol and aldehyde which can react with TBA (Prasetyo, 2021; Wresdiyati et al., 2002). The resulting linear regression equation for measuring the standard curve is y = 0.0406x – 0.0133 with a value of r = 0.9961. The IC_{50} result of vitamin E as a positive control was 59.54a ± 0.17 ppm. In the n hexane extract sample, the IC_{50} was obtained at 2067.02c ± 86.59 ppm, ethyl acetate extract 1968.13c ± 91.87, ethanol extract 116.14a ± 0.38 ppm and extract 921.14b ± 45.52 ppm. Of the 4 samples, the smallest IC_{50} was found in the ethanol extract, which was 116.14a ± 0.38 ppm from the triplo test (Table 3).

Table 3 IC_{50} values of vitamin E and samples (n hexane extract, ethyl acetate extract, ethanolextract and water extract) of sweet potato leaves in the MDA inhibitor test

Sample	Vitamin E	N-hexane	Ethyl acetate	Ethanol	Water
IC50(ppm)	$59,54^{a}$±0,17	$2067,02^{c}$±86,59	$1968,13^{c}$±91,87	$116,14^{a}$±0,38	$921,14^{b}$±45,52

Different letters (a, b and c) show statistically significant differences (0.05) on the tuckey test.

3.1.4 Tyrosinase Inhibitors

The principle of testing the activity of tyrosinase inhibitors is the inhibition of the formation of dopachrome products resulting from the reaction of the L-tyrosine substrate and the tyrosinase enzyme. The inhibition of dopachrome formation is characterized by a decrease in color intensity as measured using a microplate reader at a maximum wavelength of 510 nm. Microplate reader is a spectrophotometer measurement technique that passes light of a certain wavelength through a well containing a sample and then measures the intensity of the transmitted light, so that the absorbance is obtained. The absorbance is used to calculate the amount of inhibition of the L-Tyrosine reaction. The IC_{50} result of kojic acid as a positive control was 12.33a ± 0.66 ppm. In the n hexane extract sample, IC_{50} was 1328.27c ± 92.26 ppm, ethyl acetate extract was 1245.13c ± 55.23 ppm, ethanol extract was 217.35ab ± 38.42 ppm and water extract was 391.21b ± 5.49 ppm. Of the k-4 samples, the smallest IC_{50} was found in the ethanol extract, which was 217.35ab ± 38.42 ppm (Table 4).

Table 4 IC_{50} value of kojic acid and samples (n hexane extract, ethyl acetate extract, ethanol extract and water extract) of sweet potato leaves in the tyrosinase inhibitor test

Sample	Kojic acid	N-hexane	Ethyl acetate	Ethanol	Water
IC50(ppm)	$12,33^{a}$±0,66	$1328,27^{c}$±92,26	$1245,13^{c}$±55,23	$217,35^{ab}$±38,42	$391,21^{b}$±5,49

Different letters (a, b and c) show statistically significant differences (0.05) on the tukey test.

3.2. Discussion

3.2.1. Content of Secondary Metabolite Compounds in Sweet Potato Leaves

Phytochemical components, based on the results of testing for alkaloid compounds, flavonoids, tannins and phenols are found in extracts with ethanol and water solvents. This is because these secondary metabolites dissolve in semi-polar solvents, namely ethanol and water. Triterpenoid steroid compounds are found in extracts with n hexane, ethyl acetate and ethanol solvents, this is because triterpenoid steroids are soluble in semipolar solvents which tend to be non-polar. Glycoside compounds are only found in extracts using ethanol solvent, this is because glycosides dissolve in semi-polar solvents, namely ethanol. Quinone compounds are present in extracts with n hexane and ethyl acetate solvents. This can mean that quinones soluble in semi-polar compounds tend to be non-polar (Table 1).

3.2.2. DPPH Method Antioxidant Activity

The antioxidant activity of the results of this research is expressed in IC50, namely the concentration of antioxidant substances that produces a DPPH inhibition percentage of 50%. The IC50 value was obtained through a linear equation between the inhibition percentage and the sample concentration. The lower the IC50 value, the higher the inhibitory power of antioxidants against free radicals. The average IC50 result of the DPPH antioxidant test was found to be 3.07 ± 0.0051 ppm for ascorbic acid as a positive control and for samples with N hexane, ethyl acetate, ethanol and water extracts, namely 6948.12 ± 572.82 ppm ; 3015.19 ± 55.02 ppm; 128.05 ± 1.7837 ppm and 791.86 ± 4.15 ppm. The smallest IC50 result in the

sample was produced by the extract with ethanol solvent which was 128.05 ± 1.7837 ppm from the triplo test. These results indicate that ascorbic acid can be regarded as a compound that has very strong antioxidant activity. The IC50 value of the extract with ethanol solvent can also be said to have moderate antioxidant strength, because the IC50 value is 128.05 ppm.

Molyneux (2004) states that a compound is said to be a very strong antioxidant if it has an IC50 value of less than 50 ppm, strong if the IC50 value is between 50-100 ppm, while if the IC50 value is between 100-150 ppm, weak if it has an IC50 value between 150- 200 ppm, and very weak if it has an IC50 value of more than 200 ppm. The results showed that the best IC50 value was found in the extract using ethanol solvent of 128.05 ppm and the highest IC50 was in the extract using n-hexane solvent which was 6948.11 ppm. Based on Sifa (2023), that the IC50 antioxidant DPPH of betel leaf water extract was 1098.99 ppm and that of ethyl acetate extract was 1378.59 ppm, this could mean that antioxidant compounds are in non-polar solvents that tend to be polar.

The ability of antioxidants to reduce DPPH is determined by the chemical structure, number and position of hydroxyl groups on the ring. If the molecule is substituted with more hydroxyl groups, the stronger it is to capture DPPH free radicals because its ability to donate hydrogen atoms is greater (Iflahah et al., 2016). The highest antioxidant activity research was found in ethanol solvent. Antioxidant activity is closely related to the content of secondary metabolites that function as antioxidants such as flavonoids and phenols. Phenolic compounds are known to have antioxidant properties that are formed under stress conditions. The ability of flavonoids as antioxidant compounds is due to their properties as good acceptors against free radicals. Phenolic and flavonoid compounds from plants have the ability to act as antioxidants because they are able to scavenge reactive oxygen species (ROS) which produce free radicals (Prasetyo, 2021). From the results of the identification of samples with ethanol solvent which contain secondary metabolite compounds in the form of flavonoids and the results of the Tukey statistical test, it can be concluded that the ethanol extract and ascorbic acid as a positive control are not significantly different and the ethanol solvent is not significantly different from the water solvent.

3.2.3. Malondialdehyde Inhibitor (TBA-MDA)

MDA measurement is the reaction of one MDA molecule with two thiobarbituric acid (TBA) molecules to form a pink color which is measured on a spectrophotometer at a wavelength of 532 nm. The standard used in the calculation of MDA levels is tetramethoxypropane (TMP). Standard TMP in acidic conditions can be hydrolyzed to produce hemiacetal and methanol, the hemiacetal formed will then decompose into methanol and aldehyde which can react with TBA (Wresdiyati et al., 2002). The resulting linear regression equation for measuring the standard curve is $y = 0.0406x – 0.0133$ with a value of $r = 0.9961$. The IC50 result for vitamin E as a positive control was 59.54 ± 0.17 ppm. In the n hexane extract sample, the IC50 was 2067.02 ± 86.59 ppm; ethyl acetate extract 1968.13 ±91.87 ppm; ethanol extract 116.14 ± 0.38 ppm and water extract 921.14 ± 45.52 ppm. Of the 4 samples, the smallest IC50 was found in the ethanol extract, namely 116.14 ± 0.38 ppm from the triplicate test (Table 3). This can be said to be the best extract in ethanol solvent which can inhibit MDA. Likewise, this research uses sweet potato leaves.

The MDA inhibitor test used the TBA method which has the principle of MDA and TBA condensation to form a pink color complex and was measured using spectrophotometry at a wavelength of 532 nm (Weitner et al., 2016). The MDA-TBA method has the advantage of being able to determine lipid oxidation levels which are relatively simple but have good accuracy, so this study used extracts of n hexane, ethyl acetate, ethanol and sweet potato leaf water as antioxidants to inhibit MDA using the TBA method. The standard curve that has been determined becomes a reference for the linear equation for the determination of linoleic acid incubation and the MDA test from sweet potato leaf samples. The diene conjugation method

for determining the incubation time of linoleic acid was carried out before testing the antioxidant activity of the samples by measuring the hydroperoxides which were measured every day to obtain the maximum absorbance value (Ratnawati et al., 2013). Linoleic acid incubation was carried out from day 0 to day 10 with the result that on day 6 the maximum absorbance value was obtained which then decreased on day 7. The maximum absorbance value indicated that the formation of conjugated dienes had reached the maximum amount. The maximum absorbance value can produce different results in several studies due to the quality factors of linoleic acid, incubation temperature that is not constant, hydrogen ions, pH, lipid radicals, oxygen, and the environment (Hęś et al., 2017). Vitamin E served as a positive control where vitamin E gave an IC50 value of 59.54 ppm. The results showed that the best IC50 value was found in the extract using ethanol solvent of 116.14 ppm and the highest IC50 was found in the extract using n-hexane solvent which was 2067.02 ppm. The extract was tested by statistical analysis using ANOVA compared to the positive control of vitamin E. Statistical analysis was obtained, namely sweet potato leaf extract n hexane, ethyl acetate extract, and water extract which yielded significantly different results from the positive control vitamin E except for the ethanol extract sample which did not significantly different (Table 3). Ethanol has semi-polar properties so that flavonoid compounds will dissolve in semi-polar compounds. These properties cause semi-polar flavonoid compounds to come out of plants due to the degradation of plant cell walls by ethanol (Tiwari et al., 2011).

3.2.4. Tyrosinase Inhibitors

In the tyrosinase inhibitor test, the IC50 of kojic acid with the substrate L-Tyrosine was 12.33 ± 0.66 ppm. In the n hexane extract sample, the IC50 was 1328.27 ± 92.26 ppm; ethyl acetate extract 1245.13 ±55.23 ppm; ethanol extract 217.35 ± 38.42 ppm and water extract 391.21 ± 5.49 ppm. From the k-4 samples, the smallest IC50 was found in the ethanol extract, namely 217.35 ± 38.42 ppm (Table 4). The higher the concentration of kojic acid used, the higher the inhibitory ability. High inhibition ability is characterized by a decrease in dopachrome formation and a decrease in the intensity of the color formed. Tests for the inhibition of tyrosinase enzyme activity in 4 sweet potato leaf extracts were carried out to see the ability of various solvents to inhibit the tyrosinase enzyme. Of the 4 extracts, the smallest IC50 was found in the ethanol extract, which was 217.35 ± 38.42 ppm, this was directly proportional to the results of the antioxidant activity of the DPPH method, the MDA inhibitor test and the results of the phytochemical screening which stated that the ethanol extract contained flavonoid compounds. Based on Safithri (2022) the IC50 value of the ethanol extract of red betel leaves was 7,565 ppm and in silico in Mustopa (2022) the ethanol extract of red betel leaves contained catechin compounds in the anti-tyrosinase test while in (Laksmiani et al., 2019) peonidin and cyanidin compounds were suspected role in the inhibition of tyrosinase in sweet potato leaves.

Tyrosinase inhibition testing was carried out in vitro using the substrate L-Tyrosine. The tyrosinase inhibitory activity test was carried out to determine whether or not there was an inhibitory effect on the bioactive compounds contained in sweet potato leaf extract. The test results stated that the IC50 of sweet potato leaf extract was > 200 ppm. This result is much greater than the IC50 value of kojic acid as a positive control, namely 12.32 ppm. The smaller the IC50 value, the more potential the compound is as a tyrosinase inhibitor. These results indicate that sweet potato leaf extract is an inactive inhibitor of tyrosinase. Statistical analysis obtained that sweet potato leaf extract n hexane, ethyl acetate extract and water extract produced significantly different results ($P<0.05$) with the positive control kojic acid but the ethanol extract produced results that were not significantly different from the water extract and kojic acid as positive control (Table 4), because the IC50 test results are much greater than the positive control value, it can be said that sweet potato leaf extract does not have good activity in tyrosinase inhibition. Tyrosinase has an important role in melanin biocatalysis as it

gives brown color to the skin and protects it from ultraviolet (UV) rays (Hoogduijn et al., 2004). Therefore, when the tyrosinase inhibition value in a sample is low, in other words a sample is less effective as a skin whitener.

4. Conclusion

Sweet potato leaf extract (Ipomoea batatas L.) has tyrosinase enzyme inhibitor activity with the smallest IC50 value of 217.35 ppm in ethanol extract. In the antioxidant activity test with the DPPH method, the smallest IC50 value was 128.05 ppm in the ethanol extract and in the MDA formation inhibitor test, the smallest IC50 was 116.14 ppm in the ethanol extract. From the results obtained, sweet potato leaf extract (Ipomoea batatas L.) has antioxidant activity and tyrosinase inhibitors in ethanol solvents.

Acknowledgment

The authors express their gratitude to Institut Pertanian Bogor, Politeknik Kesehatan Kementerian Kesehatan Jakarta II, and the Ministry of Health of the Republic of Indonesia for their invaluable support and facilitation of this research endeavor.

References

Andrianto, D., Katayama, T., & Suzuki, T. (2015). Screening of antioxidant and antihyperlipidemic potencies of Indonesian underutilized fruits. *J Forest Biomass Utilization Soc*, *1*, 19–25.

Batubara, I., Darusman, L., Mitsunaga, T., Rahminiwati, M., & Djauhari, E. (2010). *Potency of Indonesian medicinal plants as tyrosinase inhibitor and antioxidant agent.*

Chasanah, N. (n.d.). *Formula Campuran 3 Daun Tanaman Obat sebagai Penghambat Tirosinase serta Sidik Jari Kromatogramnya*.

Hęś, M., Gliszczyńska-Świgło, A., & Gramza-Michałowska, A. (2017). The effect of antioxidants on quantitative changes of lysine and methionine in linoleic acid emulsions at different pH conditions. *Acta Scientiarum Polonorum Technologia Alimentaria*, *16*(1), 53–67.

Hoogduijn, M. J., Cemeli, E., Ross, K., Anderson, D., Thody, A. J., & Wood, J. M. (2004). Melanin protects melanocytes and keratinocytes against H2O2-induced DNA strand breaks through its ability to bind Ca2+. *Experimental Cell Research*, *294*(1), 60–67.

Iflahah, M. A., Puspawati, N. M., Suaniti, N. M., Terapan, M., Udayana, P., Udayana, U., & Jimbaran, B. (2016). Aktivitas antioksidan biji kakao (Theobroma Cacao L.) dalam menurunkan kadar 8-hidroksi-2'-deoksiguanosin dalam urin tikus setelah terpapar etanol. *Cakra Kimia*, *4*(2), 113–119.

Kikuzaki, H., & Nakatani, N. (1993). Antioxidant effects of some ginger constituents. *Journal of Food Science*, *58*(6), 1407–1410.

Laksmiani, N., Putra, I., Nugraha, I., Suwartawan, I., & Ani, N. (n.d.-a). Studi Potensi Sianidin Dan Peonidin Dari Ubi Jalar Ungu (Ipomoea batatas L.) Sebagai Agen Depigmentasi Secara In Silico. *Jurnal Kimia (Journal Of Chemistry)*, *13*(1), 34–39.

Laksmiani, N., Putra, I., Nugraha, I., Suwartawan, I., & Ani, N. (n.d.-b). Studi Potensi Sianidin Dan Peonidin Dari Ubi Jalar Ungu (Ipomoea batatas L.) Sebagai Agen Depigmentasi Secara In Silico. *Jurnal Kimia (Journal Of Chemistry)*, *13*(1), 34–39.

Molyneux, P. (2004). The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for

estimating antioxidant activity. *Songklanakarin J. Sci. Technol*, *26*(2), 211–219.

Mustopa, S. (2022). *Kajian In silico dan In vitro Ekstrak dan Fraksi Daun Sirih Merah (Piper crocatum) Sebagai Inhibitor Tirosinase*.

Parwata, I. (2016). Bahan Ajar Antioksidan, Kimia Terapan Program Pascasarjana Universitas Udayana. *Bukit Jimbaran*.

Prasetyo, B. F. (2021). Uji Aktivitas Antioksidan dan Daya Hambat Enzim Tirosinase Ekstrak Etanol Azolla filiculoides Lam. *JSFK (Jurnal Sains Farmasi & Klinis)*, *8*(1), 53–59.

Pratiwi, R. (2016). Uji Kualitatif fitokimia daun Ruta angustifolia. *Faktor Exacta*, *9*(3), 200–206.

Ratnawati, D., Martono, A., & Fitriyanti, W. (2013). Study on the potency of methanol extracts from Xanthosoma nigrum stellfeld as natural anti oxidant by thiobarbituric acid method. *Aceh International Journal of Science and Technology*, *2*(3), 82–87.

Rosyidah, R. A. (n.d.). *Aktivitas Antidiabetes In Vitro dan Uji Organoleptik dari Kombinasi Ekstrak Teh Hitam, Kunyit, dan Jahe*.

Safithri, M. (n.d.). *Formulasi dan pengujian aktivitas anti aging kulit sediaan serum berbahan aktif nano sirih merah dan nano propolis*.

Sifa, R. A. (n.d.). *Analisis Fitokimia, Aktivitas Antioksidan, dan Inhibisi Kolagenase Ekstrak Daun Sirih Merah (Piper crocatum Ruiz & Pav.) In Vitro*.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: A review. *Internationale Pharmaceutica Sciencia*, *1*(1), 98–106.

Weitner, T., Inić, S., Jablan, J., Gabričević, M., & Domijan, A.-M. (2016). Spectrophotometric determination of malondialdehyde in urine suitable for epidemiological studies. *Croatica Chemica Acta*, *89*(1), 133–139.

Wresdiyati, T., Mamba, K., Adnyane, I. K. M., & Aisyah, U. S. (2002). The effect of stress condition on the intracellular antioxidant copper, zincsuperoxide dismutase in the rat kidney: An immunohistochemical study. *Hayati*, *9*(3), 85–88.