Indonesian Journal of Applied Research IJAR

Antioxidant Activity and Tyrosinase Inhibitor of Red Betel Leaf Extract

Mustika Weni[1], Mega Safithri[2]

[1]Department of basic medical science, Faculty of Medicine, Universitas Swadaya Gunung Jati, Indonesia
[2]Department of Biochemistry, Faculty of Mathematic and Natural Sciences, Bogor Agricultural University, Indonesia

Volume 4 Issue 3
(December 2023)
e-ISSN 2722-6395
doi: 10.30997/ijar.v4i3.374

ARTICLE INFO

Article history:
Received: 09-07-2023
Revised version received: 09-14-2023
Accepted: 04-12-2023
Available online: 12-30-2023

Keywords:
Antioxidant activity; malondialdehyde MDA; piper crocatum; tyrosinase.

How to Cite:
Weni, M., & Safithri, M. (2023). Antioxidant Activity and Tyrosinase Inhibitor of Red Betel Leaf Extract. *Indonesian Journal of Applied Research (IJAR)*, *4*(3), 234-245. https://doi.org/10.30997/ijar.v4i3.374

Corresponding Author:
Mustika Weni
mustikaweni261192@gmail.com

ABSTRACT

Red betel leaf (*Piper Crocatum*) contains flavonoids, tannins, steroids, and alkaloids, known to act as antioxidants. This study aimed to determine the antioxidant activity of red betel leaf extract. The solvents used for the extraction process are ethanol and n-hexane. The thiobarbituric acid (TBA) method was used to determine antioxidant activity. The results showed that the extract of 200 ppm inhibited fatty acid oxidation by 52.13%. No significant difference ($\alpha = 0.05$) inhibition of unsaturated fatty acids oxidation between the sample and α-tocopherol at 200 ppm. The ethanol extract of red betel can inhibit the tyrosinase enzyme higher than the n-hexane extract of red betel, as seen from the IC_{50} value of the ethanol extract of red betel of 1655 ppm, while the IC_{50} value of n-hexane of red betel is 3090.56 ppm.

1. Introduction

The aging process is a natural process that occurs in the body and can be characterized by physical decline or physical, psychological, or social changes in interacting with others (Handayani & Rachma, 2013). However, this process can also be caused by a disease (Aunan et al., 2016). Social progress and industrialization have gradually led to the development of sociocultural factors that directly and indirectly influence aging, including ingested nutrients, UV radiation, smoking, and stress. Other causes include genetic factors, free radicals, tyrosinase enzymes, and hormone depletion. Oxidative stress is one of the contributing factors to the aging process. Oxidative stress is a condition where reactive oxygen species (ROS) production exceeds the body's antioxidant capacity (Liguori et al., 2018). ROS are produced naturally in the body from cellular respiration in mitochondria, phagocytosis, and drug hydroxylation processes in the liver. Excessive ROS can damage the body's cellular components and are irreversible, so that they can cause cell death through the intrinsic pathway and trigger mitochondrial DNA damage (Davalli et al., 2016). Our body has a natural pigment, namely melanin. Melanin protects the skin from UV damage and is a critical defense system for the skin against harmful factors. Besides its advantages, melanin is also involved in abnormal pigmentation and melanoma; therefore, different approaches to studying skin disorders have been developed.

Tyrosinase (EC 1.14.18.1) is a critical first two-step enzyme of melanin biosynthesis, catalyzing the hydroxylation of L-tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and the oxidation of DOPA to dopaquinone. Continuous UV irradiation induces increased accumulation of melanin, leading to skin hyperpigmentation (Jeon et al., 2018). Since tyrosinase is the limiting enzyme step in melanogenesis, inhibitors of this enzyme are significant in treating hyperpigmentation disorders. However, currently available tyrosinase inhibitors are constrained by toxicity and are less effective in inhibiting the tyrosinase enzyme. The constant search for better inhibitors from natural sources hopefully free from harmful side effects (Rashed et al., 2016), one of which is natural herbal plants. The community has carried out the use of natural materials for a long time because they contain many compounds that are efficacious in medicine, known as phytochemical compounds, which are natural groups of compounds that can be used to maintain health.

Red betel leaf cooking water contains alkaloids, flavonoids, and tannins (Safithri & Kurniawati, 2016). The highest Rancimat method was obtained from the ethyl acetate fraction with a protective factor value of 1.38. Ten compounds were identified in the ethyl acetate fraction of P. crocatum leaves. According to the CUPRAC method, antioxidant activity showed the highest in the sample of the n-hexane fraction with a value of 31.9 µmol Trolox/g extract (Safithri et al., 2022). Using the MDA-TBA method, this study used 70% ethanol extract and n-hexane to compare polar and non-polar compounds. The objective of this research is to study the possibility of using Piper crocatum as an antioxidant by examining its ability to inhibit unsaturated fatty acids oxidation to scavenge free radicals and to determine tyrosinase inhibitor activity extract of Red betel leaves (*Piper Crocatum*).

2. Methods

2.1. Tools and Materials

The materials used were red betel leaf simplicia, 99.8% ethanol, 70% ethanol, n-hexane, distilled water, Dragendorff reagent, Mayer reagent, Wagner reagent, methanol, ethyl acetate, 30% methanol, 30% ethanol, ammonia, chloroform, ether. , FeCl3, tyrosinase, L-3, 4-dihydroxyphenylalanine (L-DOPA), HCl 2 N, phosphate buffer pH 6.5, 0.1 M phosphate buffer pH 7, standard solution of 1,1,3,3-tetra methoxy propane (TMP) 6 M, sodium hydroxide (NaOH), acetic anhydride, sulfuric acid (H2SO4), linoleic acid, Dimethyl sulfoxide (DMSO),

kojic acid as positive control trichloroacetic acid (TCA) 20 and thiobarbituric acid (TBA) 1%. The tools used are a measuring flask, erlenmeyer, Ohaus GA 200 analytical balance, excitatory, Memmert oven, test tube, dropper pipette, knife, blender, porcelain cup,), rotav eyela n-1100, bulb, sanstat water bath, mohr pipette, filter paper, Genesys 10 UV spectrophotometer (190-1100 nm measuring cup, Hettich Universal centrifuge (0-6000 rpm), plastic funnel, pH meter.

2.2. Drying Process of Test Material

Red betel leaves are picked from the Bogor Raya Permai housing, obtained from 3-5 strands from the shoots, and taken as much as 2 kg. Then, the red betel leaves are washed and drained in the oven at 50°C for 4-5 days. The red betel leaves are blended into a fine powder and sieved through 60 mesh.

2.3. Determination of water content (Ikelle et al., 2014)

The process of determining content by the oven method is conducted. The porcelain cup is placed in the oven, then in the desiccator, and weighed as an empty weight. As much as 2 grams of red betel leaf powder was put into a cup; the weight was recorded, dried for 3 hours in an oven at 105°C, cooled in a desiccator, and weighed.

$$W\ (\%) = \frac{\mathrm{a-b}}{a} \text{ x } 100\%$$

Details:
W = water content (%)
a = sample weight before drying (g)
b = sample weight after drying (g)

2.4. Red Betel Leaf Powder Extraction *(P. crocatum)* (Safithri & Kurniawati, 2016)

Red betel leaf simplicia powder was weighed as much as 5 grams and added with solvent (1:10) (w/v), then shaken for 24 hours and filtered through filter paper. Maceration was carried out three times. The maserate obtained was concentrated using a rotary evaporator with different temperatures: 50ºC for 70% ethanol extract and 45ºC for n-hexane extract.

2.5. Extraction Yield

Yields of 70% ethanol extract and n-hexane extract of red betel leaves were calculated by comparing the initial weight of the simplicia with the final weight of the resulting extraction.

$$Yield\ (\%) = \frac{Final\ weight\ of\ extract}{\text{Initial weight of extract}} \text{ x } 100\%$$

2.6. Phytochemical Analysis (Harborne, 1987)

2.6.1. Alkaloid test

Identification was carried out with Dragendorff, Wagner, and Mayer reagents: 0.1 gram of extract added 10 mL of chloroform and three drops of ammonia. The chloroform fraction was added to 2 drops of 2 M H_2SO_4. The acid fraction was divided into 3 tubes, and 3 drops of Dragendorff, Meyer, and Wagner reagent were added to each tube. The formation of a white precipitate (Mayer), red precipitate (Dragendorf), and brown precipitate (Wagner) indicates that the sample contains alkaloids. Positive samples contain marked alkaloids.

2.6.2. Flavonoid test

0.1 gram of plant extract was added to 5 mL of 30% methanol and heated for 5 minutes. The filtrate formed was added with three drops of H2SO4. A red precipitate indicates the presence of flavonoids.

2.6.3. Saponin test

0.1 gram of plant extract was added to 5 mL of distilled water and then heated for 5 minutes. The sample is shaken for 5 minutes. The formation of stable foam for 10 minutes indicates the presence of saponins. Test triterpenoids and steroids. Plant extracts: As much as 0.1 gram of extract was added to 5 mL of 30% ethanol, then heated for 5 minutes. The sample was filtered, and the filtrate obtained was evaporated to dryness. The residue was added with 0.5 mL ether and transferred to a test tube, then added with Liebermann Burchard reagent (3 drops of anhydrous acetic acid and one drop of concentrated H2SO4). Red or purple indicates the presence of triterpenoids, while the formation of green and blue indicates positive steroids.

2.6.4. Tannin test

0.1 gram of plant extract was added to 5 mL of distilled water and then boiled for 5 minutes. The sample is filtered, and the filtrate is separated. The filtrate was added with five drops of 1% (w/v) FeCl3. The formation of a dark blue or black color indicates the presence of tannins.

2.7. TBA-MDA Method Antioxidant Activity Test (Safithri et al., 2022)

A solution of 1,1,3,3-tetra methoxy propane (TMP) was used as the standard curve. Standard solution concentrations 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 heated for 10 minutes in a water bath 100oC and then centrifuged for 15 minutes at 3000 rpm (130 mm centrifuge rotor diameter). The wavelength used is 532 nm. A blank solution was prepared using 1 mL of distilled water, treated like other TMP concentration solutions (0 TMP concentration). Determination of the incubation time of linoleic acid with the TBA method. A total of 6 mL of 0.1 M phosphate buffer pH 7 plus 6 mL of 50 mM linoleic acid in 99.8% ethanol and 3 mL of deionized water were mixed. 1 mL of the mixture was incubated at 40oC in a dark bottle. A total of 1 mL of linoleic acid mixture was added to 2 mL of 20% TCA solution and 2 mL of 1% TBA solution in 50% acetic acid. The reaction mixture was heated for 10 minutes in a water bath at 100 degrees. After cooling, the solution was centrifuged at 3000 rpm (centrifuge rotor diameter 130mm) for 15 minutes and measured at a wavelength of 532 nm, which was used to measure absorption intensity. Measurements were made every day until maximum absorption was achieved. A blank solution was prepared by 1 mL of a mixture of 3 mL of 99.8% ethanol and 2 mL of 0.1 M phosphate buffer pH 7, then 2 mL of 20% TCA and 2 mL of 1% TBA in 50% acetate. The blank solution was heated for 10 minutes in a water bath 100oC and centrifuged for 15 minutes at 3000 rpm (130 mm centrifuge rotor diameter).

Analysis of the concentration of malondialdehyde (MDA) with the TBA method. The concentrations used for antioxidant analysis of red betel leaf extract were 25, 50, 75, 100, and 200 ppm. 1 mL of sample was taken and added to 2 mL of 0.1 M phosphate buffer pH 7 and 2 mL of 50 mM linoleic acid in 99.8% ethanol. α-tocopherol (200 ppm) was used as the positive control solution, 1 mL of α-tocopherol was taken, and 2 mL of 0.1 M phosphate buffer pH 7 and 2 mL of 50 mM linoleic acid in 99.8% ethanol were added. All solutions were incubated in a 40oC bath in a dark bottle during the optimum incubation time of the TBA

method. As much as 1 mL of each solution, then added 2 mL of 20% TCA solution and 2 mL of 1% TBA solution in 50% acetic acid and heated in a bath at 100ºC for 10 minutes; the solution was cooled and centrifuged at 3000 rpm for 15 minutes. Measured at a wavelength of 532 nm. A blank solution was prepared by adding 1 mL of a blank solution to a mixture of 3 mL of 99.8% ethanol and 2 mL of 0.1 M phosphate buffer pH 7, then adding 2 mL of 20% TCA and 2 mL of 1% TBA in 50% acetate. The blank solution was heated for 10 minutes at 100oC and then centrifuged for 15 minutes at 300 rpm (130 mm centrifuge rotor diameter).

2.8. Tyrosinase Inhibitor Test (Suganya et al., 2015)

The extract was made at a concentration of 10,000 ppm using DMSO. The stock solution was made at a concentration of 600 mg/mL by dissolving the concentrated extract in a 50 mM phosphate buffer with a pH of 6.5. Extract testing was conducted with different concentrations, namely 20, 200, 400, 800, 1600, and 3200 ppm. The positive control used kojic acid at a concentration of 200 ppm added with 30 µL of *Tyrosinase* enzyme (sigma, 333 units/mL in phosphate buffer) and incubated for 5 minutes. After that, 110 µL of substrate (12 mM L-DOPA) was added, and the mixture was incubated at 37°C for 30 minutes. The absorbance value of the solution was measured at a wavelength of 492 nm using a microplate reader to determine the percent inhibition and the value of 50% inhibitory concentration (IC50). The absorbance ratio of the sample without extract (A) with the addition of extract (B) was measured for its absorbance at a wavelength of 492 nm and calculated to obtain
% Inhibition = × 100 %.

2.9. Data Analysis

Statistical analysis used the variance test (ANOVA) analysis at the 95% confidence level and $\alpha = 0.05$, Post hoc test. The Statistical Program for Social Science (SPSS) PASW 18.0 software program was used for data analysis.

3. Results and Discussion

3.1. Results

The secondary metabolites contained in the ethanol extract include flavonoids, saponins, steroids, tannins, and alkaloids. The content of secondary metabolites in the n-hexane extract contains tannins and saponins. The analysis results of the red betel leaves' water content were obtained at 7.75%, which is below the maximum water content limit for simplicia, which is 10%. The yield obtained, respectively, 70% ethanol extract was 17.84%, while the n-hexane extract was 4.88%.

Table 1. Yield of 70% ethanol extract and n-hexane of red betel leaves

Type of Extract	Extract Yield (%)
Ethanol 70%	17.84% ± 0.66
n-hexane	4.88% ± 4.80

n = 2 repetitions

Table 2. Phytochemical test of 70% ethanol extract and red betel n-hexane

Compound	Extract	
	Ethanol 70%	n-hexane
Alkaloids	+	-
Flavonoids	+	-
Saponin	+	+
Tanin	+	-
Steroids and triterpenoids	+	+

The inhibition value of MDA formation of 70% ethanol extract was 52.13%; red betel extract had the potential as an antioxidant, while for n-hexane extract, it was 40.13%. The highest inhibition of the tyrosinase enzyme was the ethanol extract. The 70% ethanol extract has an IC_{50} value (1655 ppm); this value is lower than the IC_{50} of the n-hexane extract (> 2000 ppm), which means that the ability to inhibit the tyrosinase enzyme of the 70% ethanol extract is better than that of the n-hexane extract.

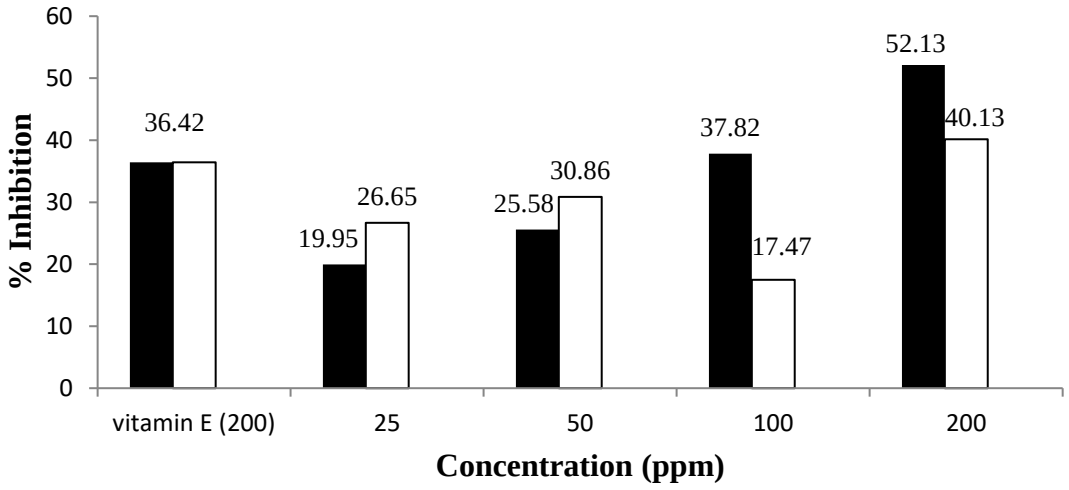

Figure 1. Antioxidant Activity of Red Betel Leaf Extract Using The TBA Method. ■70% Ethanol Extract and Extract □N-Hexane

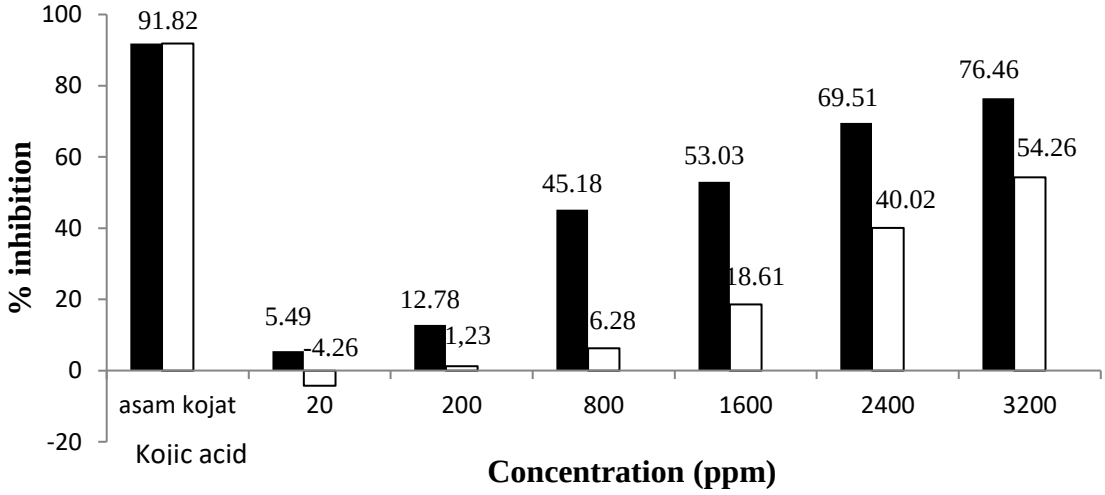

Figure 2. Tyrosinase Inhibitory Activity of Red Betel Leaf Extract. 70% Ethanol Extract and N-hexane extract

3.2. Discussion

Red betel leaves are analyzed for water content before being used for extraction. The analysis results of the red betel leaves' water content were obtained at 7.75%, proving that drying the oven for four days at 50^{o}C can reduce the water content. The lower the water content obtained, the better (Kumadoh et al., 2022). Microbial contaminants in some herbal products may pose a public health risk to consumers. The maximum moisture content limit for simplicia is 10% (Departemen Kesehatan Republik Indonesia, 2008). The water content obtained by the two simplicia in this experiment was below 10%, thus allowing long storage without any damage by microbes (Puspita et al., 2018). 70% ethanol extract and n-hexane extract in the rotary evaporator to obtain the yield. The yield obtained, respectively, 70% ethanol extract was 17.84%, while the n-hexane extract was 4.88% (Table 1). Compared to other studies, it is stated that the Ethanol Extract of Red Betel Leaves has a yield value of 20.8%. This value is higher; the difference in the yield value obtained can be due to the content of secondary metabolites in the extract. The higher the content of secondary metabolites attracted to the extract, the higher the yield value. In addition, the method used in extraction can also affect the value of the yield (Puspa et al., 2018).

The content of secondary metabolites in plants strongly influences antioxidant activity. Phytochemical analysis was carried out to know the content of secondary metabolites that have potential as antioxidants in red betel leaves. The results of the phytochemical test showed that alkaloids, flavonoids, saponins, tannins, and steroids were present in the 70% ethanol extract. In contrast, the red betel n-hexane extract contained saponins and steroids (Table 2). The results of other studies explain that the 30% ethanol extract of red betel leaves contains alkaloids, steroids, and tannins (Di Petrillo et al., 2016). This result is not much different from the phytochemical content in the 70% ethanol extract; this proves that 70% ethanol can attract chemical compounds that are relatively the same as boiling red betel leaf water. Flavonoid compounds are known to act as antioxidants and are reported to be able to inhibit the tyrosinase enzyme (Mohammed et al., 2014). Tannin compounds also function as antioxidants because they inhibit the formation of free radicals (Tsikas, 2017). Based on research conducted by Mega Safithri, the ethyl acetate fraction of red betel leaves identified by LC-MS (Liquid Chromatography-Mass Spectrometry) contains Schisandrin B (C_{23} H_{28} O_6) (Safithri et al., 2022). Sch B (Schisandrin B) is one of the most abundant and active dibenzo cyclooctadiene derivatives found in Schisandra chinensis fruit. Sch B compounds have been shown to prevent or reduce the breakdown of fat molecules (oxidized fat) due to CC14 exposure to liver cells. Sch B has also been shown to reduce hepatic transaminase levels. The mechanism of the hepatoprotector function possessed by this compound is by increasing liver function as an anti-inflammatory and antioxidant (Kwan et al., 2017).

Lipids in cell membranes are one of the targets of free radical attack by living things. Polyunsaturated fatty acids (PUFA) in cell membranes are targets of free radical attack, so they will form new free radicals that are very sensitive to oxygen (lipid peroxyl radicals). Malondialdehyde (MDA) results from lipid peroxidation, which results from the breakdown of polyunsaturated fatty acids. MDA is a good indicator of lipid peroxidation and a biomarker of oxidative stress (Muzzalupo, 2013). Unsaturated fatty acids (linoleic acid) were used in this study because free radicals quickly attack them in their double bonds to produce lipid peroxide. Temperature, light, metal ions, and oxygen can oxidize fatty acids so that the fatty acids become damaged (Kartika, 2013). Therefore, linoleic acid's oxidation rate is accelerated by keeping the temperature constant at 40^{o}C, and stored in a screw-down dark bottle to avoid other factors besides temperature and oxygen.

Before the antioxidant test using the TBA method, the primary oxidation product of linoleic acid, namely hydroperoxide, was measured using the conjugated diene method to determine the maximum incubation time. The length of time for the linoleic acid incubation process was determined from the results of hydroperoxide measurements. The most excellent

absorbance occurred on day 6, according to Alfarabi's research (Alfarabi, 2010). The antioxidant potential was measured two days after the hydroperoxides were formed on the eighth day, assuming all the hydroperoxides had been decomposed on the eighth day (Kartika, 2013). Antioxidant compounds in red betel leaves, such as tannins, alkaloids, and flavonoids, can inhibit oxidation by adding electrons to radical linoleic acid compounds so that MDA is not formed or formed in small quantities. Red betel extract concentrations of 25, 50, 75, 100, and 200 ppm were chosen because they were based on the maximum limit of antioxidant content in food, which is 200 ppm. A vitamin E concentration of 200 ppm was used as a positive control.

Incubation in the experiment was carried out for eight days, and MDA levels were measured at a wavelength of 532 nm using a spectrophotometer. Solutions of 1,1,3,3-tetra methoxy propane (TMP) with various concentrations were used as standard curves. The linear line equation obtained is y=0.086x+0.006 with a value of R_2= 99.8%. The absorbance measurement value at a wavelength of 532 nm is not directly proportional to the amount of MDA formed. The results showed that red betel leaf extract could inhibit MDA formation, as evidenced by the absorbance value of the extract being smaller than the negative control absorbance value from this study. The test results of 70% ethanol extract and n-hexane extract with various concentrations showed non-linear results between the concentration and the percentage of inhibition. This is because the extract used is still crude extract. The crude extract still contains carbohydrates, proteins, and fats, which can inhibit the scavenging of free radicals, causing the antioxidant activity of the crude extract to be lower than that of the pure compound.

The concentration of 200 ppm was the highest inhibitory concentration of the 70% ethanol and n-hexane extract. The statistical analysis proved that the 70% ethanol extract and n-hexane extract at a concentration of 200 ppm had no significantly different inhibition ($p<0.05$) from vitamin E as a positive control at a concentration of 200 ppm. Vitamin E (200 ppm) inhibited MDA formation by 36.42% (Figure 2); compared to other studies conducted by Bella, this value was lower. Bella reported that the positive control with a concentration of 200 ppm produced an inhibition of 85.88% (Bella, 2021); based on Kartika's research, Vitamin E with a concentration of 200 ppm was able to inhibit MDA formation by 49.82%. Another study said that the inhibition of ethanol extract was lower than the ethyl acetate fraction but higher than the n-hexane and water fractions, namely 51.51% (25 ppm), 63.40% (50 ppm), 67.02% (75 ppm), 70.39% (100 ppm), and 79.83% (200 ppm) (Zaelani, 2021). The difference in the results obtained in the study was because vitamin E, as the positive control, was easily oxidized, thereby reducing its ability as an antioxidant. One of the fat-soluble vitamins is vitamin E, which quickly oxidizes, especially at temperature and storage. In addition, using different methods and lengths of incubation time for linoleic acid can also affect the results obtained. Incubation can affect the antioxidant value obtained.

The antioxidant activity of red betel leaf extract compared to the antioxidant activity of noni and Physalis peruviana, ethanol, and n-hexane solvents of red betel leaf was better than noni (*Morinda citrifolia* L.) extracted with methanol 33 because red betel leaf extract showed no effect prooxidant. Another similar study showed no different results, namely mulberry ethanol extract at a concentration of 200 μg/mL (200 ppm) with an inhibition of 44.19% (Danuri et al., 2020). According to Alfarabi (2010), red betel leaf at a concentration of 200 mg/10 mL with water as a solvent was able to inhibit MDA formation by 81.78%; when compared to this study, 70% ethanol extract and n-hexane extract of red betel leaves had lower antioxidant activity. Meanwhile, at the same concentration, the ethanol extract and n-hexane extract were only able to inhibit 52.13% and 44.13%, respectively (Alfarabi, 2010). The antioxidant activity of the water and ethanol extract was 70% higher than that of the n-hexane extract. This is because secondary metabolites such as flavonoids, saponins, alkaloids, tannins, and steroids, known to have potential as natural antioxidants, dominate in 70% ethanol extract

and aqueous extract. The antioxidant activity of the water extract is higher than the 70% ethanol extract even though the content is relatively the same; this is presumably because the amount of antioxidant compounds in the two extracts is different.

The tyrosinase enzyme inhibition test results showed that the concentration increase was directly proportional to the increase in the tyrosinase enzyme inhibition value. The inhibition result of 70% ethanol extract with a concentration of 200 ppm was 12.78%, while the n-hexane extract with the same concentration had an inhibition value of 1.23% (Figure 3). The inhibition of 70% ethanol extract is better than that of n-hexane extract. Compared with kojic acid as a positive control at a concentration of 200 ppm, it has an inhibition value of 91.82%. When compared with this study, the 70% ethanol extract of red betel leaves at a concentration of 1600 ppm inhibited the tyrosinase enzyme by 53.027%, the n-hexane extract had an inhibition value of the tyrosinase enzyme by 54.260% at a concentration of 3200 ppm.

The content of flavonoids in red betel leaves, including 15 phenolic compounds, two monoterpenes, four sesquiterpenes, a neolignane, and a flavonoid C-glycoside. flavanones, flavonols, isoflavones and auron groups (Li et al., 2019). Based on its structure, the flavonoid group (Phenol hydroxyl) of red betel leaf is similar to L-Tyrosine and L-Dopa, which are substrates of the tyrosinase enzyme, so their inhibition leads to competitive inhibition. Several studies have shown that the amount and location of phenolic hydroxyl in flavonoids will significantly affect the inhibition of tyrosinase activity (Zuo et al., 2018). The more phenolic hydroxyl numbers, the more hydrogen bonds and the enhanced antioxidant activity (Zuo et al., 2018). According to research conducted by Aziz et al. (2022), the secondary metabolites of Wungu leaves were able to inhibit the tyrosinase enzyme. These compounds had the best inhibitory power, namely linolenic acid, and phytol, with affinity energies of -5.8 kcal/mol and -5.4 kcal/mol, respectively, as well as inhibition constants of 55.718 μM and 109.489 μM. Linoleic acid interacts with tyrosinase through hydrogen bonds (Aziz et al., 2022).

Based on these values, it can be observed that 70% ethanol extract has the lowest IC_{50} value (1655 ppm), indicating a very weak inhibition of tyrosinase activity. N-hexane extract has a slightly higher IC_{50} value (3090.56 mg/mL), suggesting weak tyrosinase inhibition, which means that the inhibitory ability of the 70% ethanol extract is better than that of the n-hexane extract. This is presumably because the content of flavonoid compounds found in the 70% ethanol extract has more potential as an inhibitor of the tyrosinase enzyme than the essential oil in the n-hexane extract. Generally, copper-chelating aromatic compounds inhibit tyrosinase by mimicking the substrate of tyrosinase (Zolghadri et al., 2019). Since several polyphenols are accepted as substrates by tyrosinase, whether a polyphenol may act as an inhibitor depends on the presence and position of additional subsistents. Flavonoids are among the most numerous and best-studied polyphenols, benzo-γ-pyrone derivatives consisting of phenolic and pyrene rings. The number of phenolic hydroxyls on the two aromatic rings of the flavonoid structure can significantly impact the inhibition of the tyrosinase enzyme. The hydroxyl groups of A and B rings, separated by the etherical bond of the C (pyran) ring, mainly contribute to the tyrosinase inhibitory activity. The docking result on the prepared tyrosinase enzyme showed that the catechol group and copper ions complex well, inhibiting enzyme activity. Further activity is lost or decreases with additional hydroxyl groups, which add to steric hindrance (Kothapalli et al., 2021).

Some steroids were also determined to be tyrosinase inhibitors. The research group of Choudhary et al. (2012) contributed many studies in this field. Three steroids isolated by these authors from the aerial parts of Trifolium balansae showed higher diphenolase inhibitory activity toward mushroom tyrosinase than that of kojic acid. Among the steroids, stigmast-5-ene-3β,26-diol (Figure 5b) was 7-fold more active. The authors also found that a long-chain ester, 2β(2S)-hydroxyl-7(E)-tritriacontenoate (Figure 5a), from Amberboa ramose exhibited 12.3-fold more inhibition against the diphenolase activity of mushroom tyrosinase compared to the standard kojic acid. On the other hand, the derivative containing a d-galactopyranosyl

moiety at C2 lost one order of magnitude on its tyrosinase inhibitory activity. Like most cases of tyrosinase inhibitors, the bulky and hydrophilic d-galactopyranosyl moiety interferes with the molecule's entrance into the enzyme's active site, thus reducing its inhibitory activity.

4. Conclusion

The inhibition value of MDA formation of 70% ethanol extract was 52.13%; red betel extract had the potential as an antioxidant, while for n-hexane extract, it was 40.13%. The ethanol extract of Red betel leaves (*Piper crocatum*) has fragile tyrosinase enzyme inhibitor activity with a total IC_{50} value of the ethanol extract of red betel of 1655 ppm, while the IC_{50} value of n-hexane of red betel is 3090.56 ppm.

Acknowledgment

Special gratitude is extended to Dr. Mega Safithri, S.Si., M.Si, for her invaluable role as the primary supervisor and to Syaefudin, Ph.D., for his insightful guidance as the co-supervisor, both of whom played an instrumental role from the inception of the research to the finalization of this publication manuscript. We sincerely thank the esteemed Dean of FK UGJ and the dedicated members of his team for their unwavering support. Our appreciation is also extended to the diligent personnel of the IPB biochemistry lab and the IPB Biopharma Study Center, whose contributions significantly enriched the research endeavor.

References

Alfarabi, M. (2010). *Kajian Antidiabetogenik Ekstrak Daun Sirih Merah (Piper crocatum) in vitro*.

Aunan, J., Watson, M., Hagland, H., & Søreide, K. (2016). Molecular and biological hallmarks of ageing. *Journal of British Surgery*, *103*(2), e29–e46.

Aziz, A., Andrianto, D., & Safithri, M. (2022). Penambatan Molekuler Senyawa Bioaktif Daun Wungu (Graptophyllum Pictum (L) Griff) sebagai Inhibitor Tirosinase. *Indonesian Journal of Pharmaceutical Science and Technology*, *9*(2), 96–107.

Choudhary, M. I., Zafar, S., Khan, N. T., Ahmad, S., Noreen, S., Marasini, B. P., Al-Khedhairy, A. A., & Atta-ur-Rahman. (2012). Biotransformation of dehydroepiandrosterone with Macrophomina phaseolina and β-glucuronidase inhibitory activity of transformed products. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *27*(3), 348–355.

Danuri, H. M., Lestari, W. A., Sugiman, U., & Faridah, D. N. (2020). In vitro α-glucosidase inhibition and antioxidant activity of mulberry (Morus alba L.) leaf ethanolic extract. *Jurnal Gizi Dan Pangan*, *15*(1), 45–52.

Davalli, P., Mitic, T., Caporali, A., Lauriola, A., & D'Arca, D. (2016). ROS, cell senescence, and novel molecular mechanisms in aging and age-related diseases. *Oxidative Medicine and Cellular Longevity*, *2016*.

Departemen Kesehatan Republik Indonesia. 2008. Farmakope Herbal Indonesia Edisi 1. Jakarta (ID) : Departemen Kesehatan Republik Indonesia.

Di Petrillo, A., González-Paramás, A. M., Era, B., Medda, R., Pintus, F., Santos-Buelga, C., & Fais, A. (2016). Tyrosinase inhibition and antioxidant properties of Asphodelus microcarpus extracts. *BMC Complementary and Alternative Medicine*, *16*(1), 1–9.

Handayani, T., & Rachma, N. (2013). Pesantren Lansia sebagai Upaya Meminimalkan Risiko Penurunan Fungsi Kognitif pada Lansia di Balai Rehabilitasi Sosial Lansia Unit II Pucang Gading Semarang. *Jurnal Keperawatan Komunitas*, *1*(1).

Harborne, J. (1987). Metode Fitokimia. Padmawinata K., Soediro I., penerjemah. *Phytochemical Methods. ITB. Bandung*.

Ikelle, I. I., Chukwuma, A., & Ivoms, S. (2014). The characterization of the heating properties of briquettes of coal and rice husk. *IOSR J Appl Chem*, *7*(5), 100–105.

Jeon, N.-J., Kim, Y.-S., Kim, E.-K., Dong, X., Lee, J.-W., Park, J.-S., Shin, W.-B., Moon, S.-H., Jeon, B.-T., & Park, P.-J. (2018). Inhibitory effect of carvacrol on melanin synthesis via suppression of tyrosinase expression. *Journal of Functional Foods*, *45*, 199–205.

Kartika, Y. (n.d.). *Aktivitas Antioksidasi Campuran Ekstrak Daun Sirih Merah dan Kulit Kayu Manis*.

Kothapalli, L., Sawant, P., AshaThomas, R. W., & Bhosale, K. (2021). Understanding the Molecular Mechanism of Phytoconstituents as Tyrosinase Inhibitors for Treatment of Hyperpigmentation. *Saudi J. Med. Pharm. Sci*, *7*, 135–144.

Kumadoh, D., Archer, M.-A., Kyene, M. O., Yeboah, G. N., Adi-Dako, O., Osei-Asare, C., Adase, E., Mintah, S. O., Amekyeh, H., & Appiah, A. A. (2022). Approaches for the elimination of microbial contaminants from lippia multiflora mold. Leaves intended for tea bagging and evaluation of formulation. *Advances in Pharmacological and Pharmaceutical Sciences*, *2022*.

Kwan, H. Y., Wu, J., Su, T., Chao, X.-J., Yu, H., Liu, B., Fu, X., Tse, A. K. W., Chan, C. L., & Fong, W. F. (2017). Schisandrin B regulates lipid metabolism in subcutaneous adipocytes. *Scientific Reports*, *7*(1), 10266.

Li, H. X., Widowati, W., Azis, R., Yang, S. Y., Kim, Y. H., & Li, W. (2019). Chemical constituents of the Piper crocatum leaves and their chemotaxonomic significance. *Biochemical Systematics and Ecology*, *86*, 103905.

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., & Bonaduce, D. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, 757–772.

Mohammed, M. S., Osman, W. J., Garelnabi, E. A., Osman, Z., Osman, B., Khalid, H. S., & Mohamed, M. A. (2014). Secondary metabolites as anti-inflammatory agents. *J Phytopharmacol*, *3*(4), 275–285.

Muzzalupo, I. (2013). *Food industry*. BoD–Books on Demand.

Puspita, P. J., Safithri, M., & Sugiharti, N. P. (2018). Antibacterial activities of sirih merah (Piper crocatum) leaf extracts. *Current Biochemistry*, *5*(3), 1–10.

Rashed, K., Medda, R., Spano, D., & Pintus, F. (2016). Evaluation of antioxidant, anti-tyrosinase potentials and phytochemical composition of four Egyptian plants. *International Food Research Journal*, *23*(1).

Safithri, M., & Kurniawati, A. (2016). Formula of Piper crocatum, Cinnamomum burmanii, and Zingiber officinale extracts as a functional beverage for diabetics. *International Food Research Journal*, *23*(3), 1123.

Safithri, M., Nur Faridah, D., Ramadani, F., & Pratama, R. (2022). Antioxidant activity of ethanol extract and fractions of Piper crocatum with Rancimat and cuprac methods. *Turkish Journal of Biochemistry*, *47*(6), 795–801.

Suganya, K. U., Govindaraju, K., Kumar, V. G., Dhas, T. S., Karthick, V., Singaravelu, G., & Elanchezhiyan, M. (2015). Blue green alga mediated synthesis of gold nanoparticles and its antibacterial efficacy against Gram positive organisms. *Materials Science and Engineering: C*, *47*, 351–356.

Tsikas, D. (2017). Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. *Analytical Biochemistry*, *524*, 13–30.

Zaelani, B. F. D. (n.d.). *Kajian In Silico dan In Vitro Piper crocatum Ruiz & Pav sebagai Inhibitor HMG-KoA Reduktase serta Aktivitas Penghambatan Terbentuknya Malondialdehida*.

Zolghadri, S., Bahrami, A., Hassan Khan, M. T., Munoz-Munoz, J., Garcia-Molina, F., Garcia-Canovas, F., & Saboury, A. A. (2019). A comprehensive review on tyrosinase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *34*(1), 279–309.

Zuo, A.-R., Dong, H.-H., Yu, Y.-Y., Shu, Q.-L., Zheng, L.-X., Yu, X.-Y., & Cao, S.-W. (2018). The antityrosinase and antioxidant activities of flavonoids dominated by the number and location of phenolic hydroxyl groups. *Chinese Medicine*, *13*, 1–12.