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Robusta BP-42 coffee bean extract is a new anti-tyrosinase candidate to reduce melanogenesis activity

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ABSTRACT

Background: Skin graft is one of the best options for wound closure. This method is the gold standard for skin burns and large open wounds. Coffee, or Robusta coffes (BP-2) is believed to reduce the level of hyperpigmentation due to chlorogenic acid, a major polyphenolic found in coffee with anti-tyrosinase activity. However, the anti-tyrosinase activity of BP-42 coffee extract is unknown. This study aims to measure the chlorogenic acid content of BP-42 coffee bean extract and its anti-tyrosinase activity.
Methods: BP-42 coffee beans were extracted using ethanol by maceration technique. Then, the thin-layer chromatography (TLC) profile of the BP-42 coffee bean extract was made to determine the chlorogenic acid content. At the same time, its anti-tyrosinase activity was performed using the spectrophotometric method. The chlorogenic acid's content was determined using the TLC-densitometric method. Data were analyzed using SPSS version 20.0 for Windows.
Results: BP-42 coffee bean extract had chlorogenic acid concentrations at 12.452 mg/g extract. As expected, the extract exhibited anti-tyrosinase activity with IC_{50} at 312.213 ppm, while kojic acid showed higher bioactivity (IC_{50} = 30.696 ppm).
Conclusion: BP-42 coffee bean extract showed a notable anti-tyrosinase activity, promising to be used as the natural anti-pigmentation candidate.

Keywords: Chlorogenic acid, hyperpigmentation, IC_{50}, kojic acid, tyrosinase.
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INTRODUCTION

The skin graft is one of the best options for wound closure. This method is a gold standard for skin burn treatment.[1] Skin graft also becomes an option to wound closure for mammae areola reconstruction and vitiligo therapy because it gives an excellent appearance.[2–4] Two types of skin graft are Split Thickness Skin Graft (STSG) and Full Thickness Skin Graft (FTSG).[5] These two methods not only give patients an outstanding benefit but also provide some problems such as complications and hyperpigmentation. Some patients often feel less beautiful, and their quality-of-life decrease. Hyperpigmentation becomes a severe cosmetic issue, especially if it happens on the face and neck.[6]

Hyperpigmentation occurs due to the increase of DOPA reaction at one to three weeks after skin graft surgery. Other research shows a significant increase in melatonin enzymes such as Tyrosinase, Tyrosinase-Related Protein (TYRP1), and DOPA-chrome Tautomerase (TYRP2).[6] Those enzymes will be overexpressed for four weeks after the skin graft.[7] In addition, hyperpigmentation occurs due to increased levels and expression of α-Melanocyte-Stimulating Hormone (α-MSH) and Adrenocorticotropic Hormone (ACTH) that appear early in the skin graft healing process. The increased expression of α-MSH correlated significantly with the increase in the function of melanin transfer in melanocytes and the amount of melanin in the skin autograft pigmentation process.[8] Other studies also mention that the contraction process of the skin graft causes melanin accumulation and aggravates the degree of pigmentation. Other evidence shows that melanosomes in skin grafted larger, more pigmented, and resistant to lysosomes.[9]

Various efforts have been made to reduce the occurrence of complications of hyperpigmentation, both in the clinical and laboratory fields. Many researchers use kojic acid to reduce the effect of pigmentation. Kojic acid inhibits tyrosinase and has been extensively studied in the cosmetic industry. Kojic acid and its derivatives have radioprotective and whitening properties. Due to its tyrosinase inhibitory activity, kojic acid protects the skin from ultraviolet (UV) rays, reduces hyperpigmentation, and prevents melanogenesis. It is produced by several types of fungi and is also a byproduct of the fermentation process of certain foods, such as soy sauce and sake.[10] Although kojic acid isn't toxic in acute, chronic, reproductive, and genotoxicity studies, some reports said that some people's skin becomes more sensitive due to 2% kojic acid usage. Other reports find that kojic acid poses a risk to potential thyroid side effects.[10,11]

Coffee is very abundant in Indonesia, especially in Jember Regency. Indonesia is the third largest country in green coffee robusta production.[12] One of the best

robusta coffee strains is BP-42, provided by the Indonesian Coffee and Cocoa Research Institute, Jember Regency, East Java. BP-42 coffee has the highest quality grain and highest organoleptic score compared to other strain.[13]

Coffee is believed to reduce the amount of hyperpigmentation on the skin. Reported that coffee consumption as a diet can significantly reduce photoaging UV spots in 244 Japanese females.[14] Others report that coffee extract has anti-aging and skin-lightening effects by inhibiting elastase. The coffee extract contains caffeine and chlorogenic acid that could reduce oxidative stress and inflammation.[15,16] But the anti-tyrosinase inhibitory activity of coffee extract compared to kojic acid is not well known. This study shows a concentration comparison between kojic acid and coffee bean extract levels of anti-tyrosinase inhibitory activity.

MATERIAL AND METHODS

Coffee Extract Solution

Ground coffee beans strain BP-42 provided by the Indonesian Coffee and Cocoa Research Institute. The inclusion criteria of this coffee bean are homogenous and passed the quality control by the Indonesian Coffee and Cocoa Research Institute. We used all the extract; no randomization was needed because it was already homogenous. We prepared six concentrations: 52 ppm, 102 ppm, 156 ppm, 204 ppm, 260 ppm, and 306 ppm. We begin with making the primary solution 520 ppm and 1020 ppm. To make a 520 ppm solution, 1% DMSO (0.1 ml) was added to 5.2 mg of grounded coffee beans. After that, it dissolved with phosphate buffer pH 6.5 to 10 ml. To make a 1020 ppm primary solution, 1% DMSO (0.1 ml) was added to 10.2 mg of grounded coffee beans. After that, it dissolved with phosphate buffer pH 6.5 to 10 ml. Furthermore, the primary solution that has been made is diluted with phosphate buffer pH 5.6 to make each concentration of 52 ppm, 102 ppm, 156 ppm, 204 ppm, 260 ppm, and 306 ppm.

High-Performance Thin Layer Chromatography (HPTLC)

Spotting was carried out on the silica gel F245 TLC plate. Made a ratio of toluene eluent: ethyl acetate: water: formic acid (1.5: 9: 0.5: 0.5). Added 1.5 ml of toluene in a 25 ml glass beaker and homogenized. Then the eluent was put in the chamber until the elution process marked by the eluent reached the upper limit on the filter paper. In addition to the elution process with ascending expansion, 2 spots of 1 mM standard solution were applied (1420 ng, 2130 ng) and 3 spots (2 µl) of the sample. Next, the plate is inserted into the TLC chamber saturated with the eluent and allowed to elude to the mark. The plates were dried, and the stains formed were observed in light UV 365 nm.

Kojic Acid Solution

Kojic acid solution was prepared in 6 concentrations: 0.05 mM, 0.1 mM, 0.15 mM, 0.2 mM, 0.25 mM, and 0.3 mM (equivalent to 7, 14, 21, 28, 35, and 42 ppm). The preparation begins with 1 mM kojic acid primary solution by dissolving 1.4 mg of kojic acid into 10 ml phosphate buffer pH 5.6. After that, the primary solution that has been made is diluted with phosphate buffer pH 5.6 to obtain solutions with concentrations of 0.05 mM, 0.1 mM, 0.15 mM, 0.2 mM, 0.25 mM, and 0.3 mM.

L-Tyrosinase Substrate Solution

8.119 mg of L-Tyrosine was dissolved in 10 ml phosphate buffer pH 6.5 to obtain 10 mM L-Tyrosine. Then the solution was diluted by pipetting 1 ml of the primary solution and then stirred with phosphate buffer pH 6.5 to a volume of 10 ml to obtain a 1 mM L-Tyrosine solution.

Enzyme Dilution

We dissolved the enzyme in 10 ml phosphate buffer pH 6.5 to obtain a concentration of 2,499.882 units/ml. After that, the tyrosinase solution was diluted with phosphate buffer pH 6.5 to 10 ml to obtain a concentration of 500 units/ml. Next, a second derivative was made by diluting it with 10 ml of Ph 6.5 phosphate buffer to obtain a concentration of 350 units/ml.

Inhibition of Tyrosinase Activity

In each well of the 96-well plate, 70 µL of coffee extract or kojic acid standard was added, 40 µL of tyrosinase enzyme (350 units/mL) was added and then incubated for 5 minutes at room temperature. After incubation, 110 µL L-tyrosine 1 mM was added to each hole and incubated for 45 minutes at room temperature. This incubation time is based on the kinetic experiments performed. Next, sample absorbance was observed with a microplate reader at a wavelength of 510 nm to determine the percent inhibition and the value of the 50% inhibitory concentration (IC50).

Statistical Analysis

Tyrosinase inhibitory activity data were expressed as mean±SD. Significant differences between groups were assessed by one-way ANOVA followed by group-to-control comparisons by Tukey's test; $p < 0.005$ was considered significant. Data were analyzed using SPSS version 20.0 for Windows.

RESULTS

The elution of the TLC plate is seen on 365 nm UV light in Figure 1, while Table 1 shows that all samples have damped stains. Coffee extracts have a similar Rf value to the chlorogenic acid standard, as shown by the arrow in Figure 1. The results of the spectral assessment of standard chlorogenic acid in Figure 1 showed that the spectra have a maximum wavelength of (λ) 328 and an average absorbance value of 456 AU. Then, the wavelength is used to analyze the levels of chlorogenic acid in the extract. Purity is seen based on the value of r(m,e), which indicates a correlation between the spectra at the peak position (m) and the end of the peak (e). Table 1 shows that the correlation value of chlorogenic r(m,e) acid is more than 0.99. We reckoned that the chromatogram spot/peak is pure.

The purity and identity test results showed a correlation between the spectra of the standard line and the sample. Both have the same concentration. The correlation value is more than 0.99. This value means that coffee extract is identical to the chlorogenic acid standard. Figure 2 shows the coffee spectra wave represented by light green. Dark green and orange coincide and have the same peak as the standard defined by dark purple and light purple. We reckoned that the coffee extract

Table 1. The identity and purity of chlorogenic acid spots in the TLC profile of Robusta BP-42 Coffee extract.

Track	Rf	Assigned Substance	Maximum Signal	r(s,m)	r(m.e)
1	0.19	Chlorogenic Acid	432 AU @ 327 nm	0.999176	0.999871
2	0.18	Chlorogenic Acid	546 AU @ 328 nm	0.999525	0.999354
3	0.18	Chlorogenic Acid	432 AU @ 328 nm	0.999181	0.999664
4	0.18	Chlorogenic Acid	448 AU @ 329 nm	0.999517	0.999697
5	0.19	Chlorogenic Acid	432 AU @ 328 nm	0.999594	0.999754

Rf: Retention factor; AU: Absorbance Units; e: peak end position; m: peak apex position

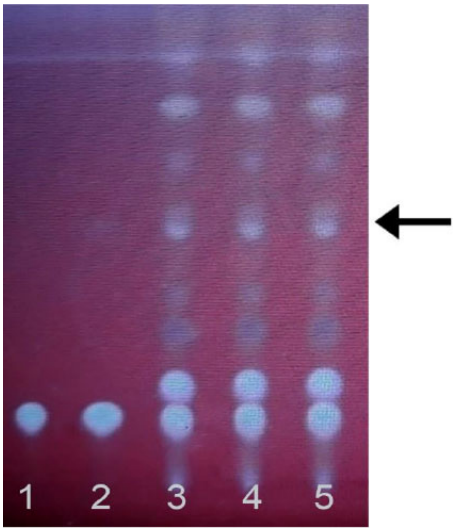

Figure 1. The TLC profile of Robusta BP-42 coffee extract under UV 365 nm light. (1) 1420 ng chlorogenic standard; (2) 2130 ng chlorogenic standard; and (3-5) 2 µl of the sample BP-42 coffee extract.

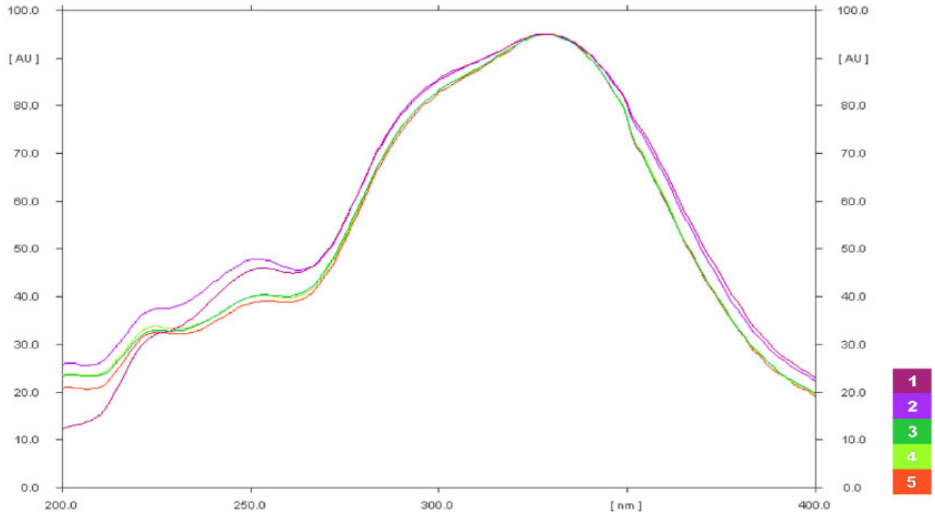

Figure 2. The spectra of thin layer chromatography profile. (1) 1420 ng chlorogenic acid; (2) 2130 ng chlorogenic acid; and (3-5) Various concentrations of chlorogenic acid found in the BP-42 coffee extracts.

compound detected is identical to the chlorogenic acid. We also calculated the chlorogenic acid content at BP-42 coffee bean extract from high-performance thin-layer chromatography results. We have generated that the chlorogenic acid content of our coffee bean extract was 12.452 mg/g.

Kojic acid has a higher inhibition rate compared to BP-42 coffee bean extract. At every concentration. kojic acid shows a higher inhibition rate than coffee extract. But at higher concentrations. BP-42 coffee bean extract shows much difference to kojic acid than at lower concentrations, as shown in Figure 3. The IC_{50} value indicates the inhibitory activity of the tyrosinase enzyme. The concept of concentration value at 50% inhibition (IC_{50}) is widely used in pharmaceuticals to measure effectiveness in inhibiting biological or biochemical functions. IC_{50} is a concentration that can inhibit 50% (half) of enzyme activity. The IC_{50} value is essential to determine the inhibitor's potential in inhibiting enzymatic reactions. The result showed that the IC_{50} of the BP-42 coffee bean extract and kojic acid were 312.213 ppm and 30.696 ppm. Respectively as seen in Figure 4.

DISCUSSION

Our study proved that BP-42 coffee extract contains chlorogenic acid at 12.452 mg/g. This concentration is relatively high compared to other strains of coffee. The chlorogenic acid content from Africa robusta green coffee extract is 11.3 mg/g.[17] Robusta coffee generally has higher chlorogenic acid than arabica coffee. Arabica coffee only has chlorogenic acid at 0.543 mg/g; it's subpar than any robusta.[18] Jember as a place to grow BP-42 coffee has met the criteria to make an excellent coffee. Requirements for robusta are grown at lower elevations (<1400 m). Roughly 90% of Jember's area is below 1000m; it became very suitable for robusta coffee growth. The high yearly rain in the Jember area also supports excellent coffee growth. Indonesian soil is also essential because it's primarily volcanic and has plenty of nutrients and compounds to support coffee growth. Coffee from Indonesia, especially Jember, is one of the best in the world.[19–23]

Chlorogenic acid is well known for its anti-inflammatory effect. In-silico studies by other Indonesian researchers suggest chlorogenic acid can be an anti-hyperpigmentation agent by inhibiting the tyrosinase enzyme.[24,25] Other investigations in Thailand revealed that a combination of chlorogenic acid and caffeine possesses excellent biological activity to reduce tyrosinase activity and has a good potential for further development for cosmetics and anti-aging products.[26,27]

BP-42 coffee extract had an IC_{50} of tyrosinase activity at 312.213 ppm compared to kojic acid at 30.969 ppm. Our result showed that robusta coffee extract from Jember could be a candidate for anti-tyrosinase. However, it was lower than kojic acid. one of the standard anti-

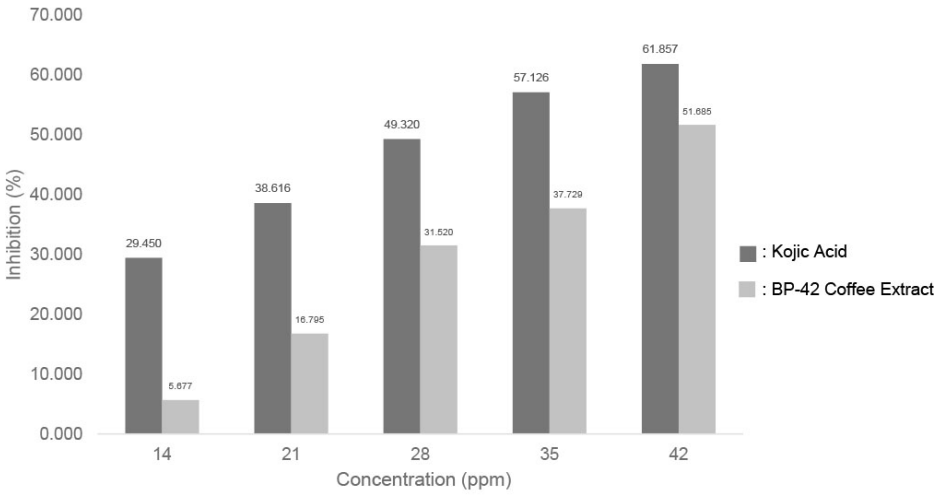

Figure 3. Inhibition rate of tyrosinase activity between Kojic Acid and BP-42 Coffee Extract.

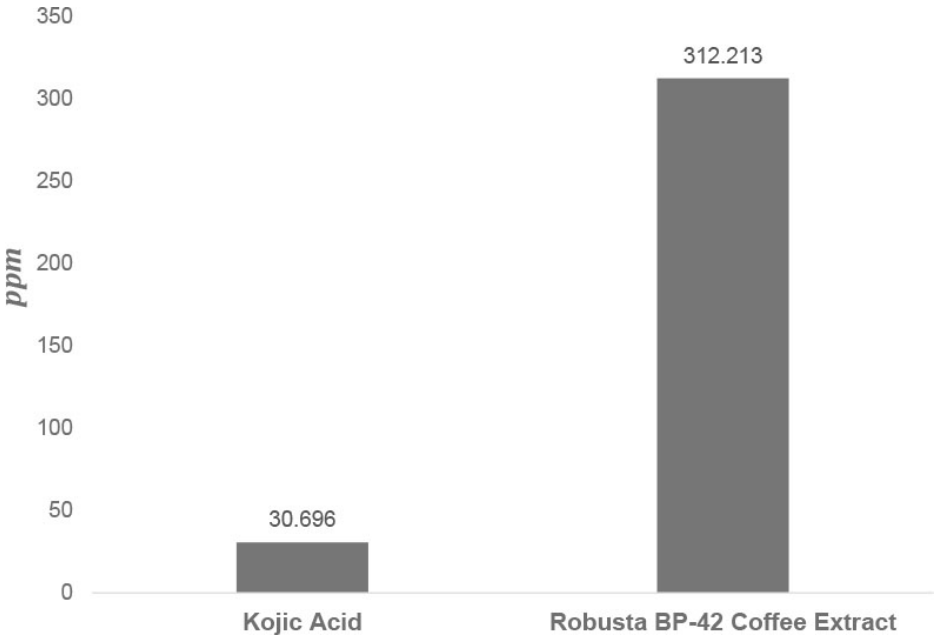

Figure 4. The IC_{50} value of kojic acid and robusta BP-42 coffee extract.

tyrosinase. This positive result proves that Robusta coffee extract has the potential to continue to be developed as a skin-lightening ingredient. Robusta coffee's anti-inflammatory, antioxidant, and antibacterial abilities make it possible to reduce unwanted effects compared to other anti-tyrosinase standards, such as hydroquinone and kojic acid.[8,16] Those compounds have side effects that are pretty severe on the skin and can even cause malignancy.[28] The tyrosinase activity was reduced by BP-42 coffee extract primarily because of chlorogenic acid. Chlorogenic acid is an essential isomer exhibiting antioxidant activities and DNA damage protective effects to various extents.[29,30] But, it has a consideration about the roasting of coffee beans. The highest concentration achieves when coffee isn't roasted. The concentration of chlorogenic acid in coffee beans would decrease if they were roasted at high heat. The longer and higher temperature coffee are roasted, the more the chlorogenic acid will be reduced.[26,31,32]

In this study, we used a raw extract of BP-42 coffee beans. We didn't use any specific active compound of BP-42 coffee beans. Further research should isolate every specific active substance of BP-42 coffee beans. Then, compare every pure isolate to see its anti-tyrosinase activities.

CONCLUSION

We conclude that BP-42 coffee bean extract has 12.452 mg/g chlorogenic acid. Our extract can inhibit tyrosinase activity with IC_{50} at 312.213 ppm. This IC_{50} result is lower than kojic acid at 30.696 ppm. However, our coffee extract still has an excellent potential to become a candidate for an anti-hyperpigmentation agent because of other benefits that others don't.

CONFLICT OF INTEREST

The Ethical Committee of Medical Research Faculty of Dentistry. Universitas Jember, Indonesia, approved this study, with reference number 1657/UN25.8/KEPK/DL/2022.

ETHICAL CONSIDERATIONS

The authors declare that there is no conflict of interest.

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AUTHOR CONTRIBUTION

In this article, Ulfa Elfiah acted as the main conceptor, organized the research method, wrote the main draft and evaluated it. David Sontani Perdanakusuma supported and developed the research methodology. Iswinarno Doso Saputro supported the research method and edited the draft. Misnawi supported the research by giving the BP-42 coffee bean and the method to extract the coffee.

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