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Original Article

Formulation of Traditional Mask Powder Containing the Mixture of *Coffea robusta, Angelica keiskei* and *Oryzae sativa*, and its Activity as Tyrosinase Enzyme Inhibitor

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ABSTRACT

Objective: Formulate a traditional mask powder with the mixture of *Coffea robusta* (CR) green bean, *Angelica keiskei* (AK) leaf and *Oryzae sativa* (OS) and evaluate its activity as an inhibitor of the enzyme tyrosinase. **Methods:** The traditional mask powder was formulated by mixing homogeneously CR bean, AK leaf and OS, which is sieved by 125-µm sieve and sealed by foil aluminium. Subsequently, the traditional mask powder was evaluated including organoleptic, pH and *microbial contamination test.* The total phenolic content of CR bean, AK leaf and OS was determined by Folin-Ciocalteu test, and the inhibitory activities of the tyrosinase enzyme was determined using L-Tyrosine as substrate. **Results:** The physical stability of the traditional mask powder containing CR bean, AK leaf and OS was stable after 7 days of storage. The total phenolic content of CR bean, AK leaf and OS were 9.51, 2.80 and 2.2 gGAE/100g, respectively. The CR bean, AK leaf and OS had tyrosinase enzyme inhibitor activity with IC_{50} value 321.52, 930.10 and 339.55 µg/mL, respectively, while the IC_{50} of traditional mask powder was 127.60 µg/mL, and the IC_{50} kojic acid used as a positive control was 573.10 µg/mL. **Conclusion:** This study demonstrated that the traditional mask powder made the mixture of the CR bean, AK leaf and OS has a potential as a skin lightening agent.

Key words: *Angelica keiskei*, *Coffea robusta*, *Oryzae sativa*, Traditional mask powder, Tyrosinase enzyme inhibitor.

INTRODUCTION

Skin aging and loss of tensile strength can cause wrinkles, roughness, and dryness.[1-2] Furthermore, hyperpigmentation occurs due to the overproduction of melanin[3], and the accumulation and overproduction of melanin can lead to a serious aesthetic problem due to age spots or melasma.[4-5] The enzyme tyrosinase is the key factor in the synthesis of melanin.[3] Therefore, the inhibition of the activities of the tyrosinase enzyme would be an important step in preventing hyperpigmentation disorder.[4-5] The use of natural sources as an inhibitor of the enzyme tyrosinase can be used as an alternative material due to the few harmful side effects.

Coffea robusta L. (CR) is a natural source used in the inhibition of the tyrosinase enzyme. Caffeine and chlorogenic acid contained in CR Bean is a possible source as an antioxidant, due to its synergistic antioxidant, promotes tyrosinase inhibitory activity.[7-8] The previous study showed that tyrosinase enzyme inhibitor activity of CR bean extract was observed similar to that of kojic acid used as a positive control.[9]

Angelica keiskei (AK) recognized as a medicinally important herb in Asia is also a plant that has a potential source as an antioxidant and tyrosinase enzyme inhibitor.[6,10] Chalcones, which has two aromatic rings with an unsaturated chain, is one of the popular groups of compounds in AK that can prevent overproduction of melanin by tyrosinase enzyme inhibition.[11] The previous studies reported that the isoprenoid-substituted moiety content in AK leaf extract increased the flavonoid's ability to inhibit melanin biosynthesis.[12] The xanthoangelol and 4-hydroxyderricin from AK extract can also inhibit tyrosinase enzyme with IC_{50} values of 15.87 ± 1.21 µM and 60.14 ± 2.29 µM, respectively.[13]

Oryzae sativa (OS) has many bioactive functions, including immunomodulating agents, angiotensin-I-converting enzyme (ACE) inhibitors, antibiotics, antioxidants, and opioids.[14] In addition, the protein content in OS showed strong inhibitory activities against ACE, α-amylase, and β-glucosidase. The previous study reported that peptide CT-2 (Leu–Gln–Pro–Ser–His–Tyr) isolated from rice bran efficiently inhibit melanogenesis in mouse, indicating that rice bran is a potential source as an agent for melanin-related skin disorder treatment.[15]

Despite the inhibitory activity of the tyrosinase enzyme of each material, the activity of the mixture was not examined. In this study, the traditional mask powder from the mixture of CR bean, AK leaf and OS was formulated, and evaluated its activity as an inhibitor of tyrosinase enzyme. In addition, the effect of CR bean, AK leaf and OS, alone, on tyrosinase enzyme activity was examined, and that the determined phenolic content was assumed to have a potential as tyrosinase enzyme inhibitory agent.

MATERIALS AND METHODS

Plant material

The AK leaf was collected from Lombok, West Nusa Tenggara, while CR green bean and OS were collected from Arjasari, Bandung, West Java,

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Indonesia. All materials were approved by the School of Life Sciences and Technology, Bandung Institute of Technology (ITB) No. 401/I1.CO2.2/PL/2019 and No. 700/I1.CO2.2/PL/2019.

Chemicals

The tyrosinase enzyme, L-Tyrosine, Gallic acid and Quercetin were purchased from Sigma Aldrich, while kojic acid dipalmitate was purchased from Thornhill Advanced Research Inc**.** All other chemicals were of pharmaceutical and technical grades.

Phytochemical screening

Phytochemical screening was conducted to observe the presence of secondary metabolites in CR bean, AK leaf and OS, including flavonoids, alkaloids, quinones and phenols.[17-18]

Determination of total phenolic content

The total phenol content of all materials was determined using the Folin-Ciocalteu test. A total of 0.5 mL of each sample was dissolved in methanol (CR bean was 1000 ppm, AK leaf was 700 ppm and OS was 4000 ppm) and mixed with 5 mL Folin-Ciocalteu reagent diluted with distilled water (1:10) and 4 mL Na_2CO_3 (1 M). The mixture was incubated for 15 minutes and then determined spectrophotometrically at a wavelength of 765 nm. *The total phenol content was calculated in gallic acid equivalent (GAE), which was used as a general reference compound.*[19-20]

Tyrosinase Inhibitory Activity

The sample was dissolved in phosphate buffer consisting of 50 mM, pH 6.8, and 1% dimethyl sulfoxide to achieve the concentrations of 500 µg/mL. 70 µL of the sample solution, which was added to 30 µL of the tyrosinase enzyme (Sigma, 333 units/mL in phosphate buffer) placed in the microwell plates. Furthermore, the plates were incubated for 5 min at room temperature, then approximately 110 µL of the substrate in 1 mM L-tyrosine was added and incubated at room temperature for 30 min. The sample solution was measured using a microwell plate reader at a wavelength of 492 nm to determine the 50% inhibitory (IC_{50}) concentration value. The percentage of tyrosinase enzyme activity inhibition was determined as follows:

$$\text{inhibition (\%)} = [(A-B)/A] \times 100,$$

A is the absorbance of kojic acid used as a reference at 492 nm, and B is the absorbance of each sample at 492 nm.[5]

Formulation of traditional mask powder

The traditional mask powder was formulated by preparing the physical mixture from all materials. The CR bean, AK leaf and OS with weight ratio of 1:1:2 were ground by mortar and pestle, and sieved using a 125-µm sieve. All materials were mixed to obtain a physical mixture and sealed using foil aluminium. Its physical stability was evaluated including organoleptic, pH and *microbial contamination test after 7 day-storage.*

RESULT AND DISCUSSION

Phytochemical screening result of CR bean, AK leaf and OS are shown on table 1.

The phenols were observed in all raw materials of traditional mask powder, indicating that CR bean, AK leaf and OS was predicted as a potential for tyrosinase enzyme inhibitory agent. To confirm the present of phenols in the raw materials, the total phenolic content of each material was determined using the Folin-Ciocalteu test. The result showed that the total phenolic content of CR bean, AK leaf and OS in terms of gallic acid equivalent were 9.51, 2.80 and 2.2 gGAE/100g, respectively.

The organoleptic characteristic of traditional mask powder from the mixture of CR bean, AK leaf and OS, did not change significantly after 7 days of storage. In addition, the pH of traditional mask powder from the mixture CR bean, AK leaf and OS after dispersed into water is shown in the figure 1.

The result showed that the pH from the mixture of CR bean, AK leaf and OS were similar after 7 days of storage and remained within the acceptable pH for mask powder, which is between 4.5 – 6.5, according to Indonesia National Standards (SNI).[21] The value of significance was lower than 0.05, indicating that the difference of pH after 7 days of storage was not significant.

The result of *microbial contamination test of* traditional mask powder from the mixture of CR bean, AK leaf and OS is shown in table 3.

The result showed that the number of bacteria of traditional mask powder from the mixture of CR bean, AK leaf and OS sativa remained within the acceptable number of bacteria, according to the Indonesia National Standards (SNI). The specify limits on total yeast and mold counts was 1 x 10^4 CFU/gram, while for bacteria was 1 x 10^6 CFU/gram.[21] These findings indicated that traditional mask powder from the mixture of CR bean, AK leaf and OS was safe to be used as a topical preparation, especially as a skin lightening agent.

Tyrosinase enzyme inhibitory activity

The tyrosinase enzyme inhibitory activity from CR bean, AK leaf, OS and traditional mask powder were determined by measuring the amount of dopachrome from L-tyrosine, as shown in Table-3.

The result showed that the IC_{50} values of CR bean and OS were lower than kojic acid, indicating that the activity in tyrosinase enzyme inhibition was stronger than kojic acid. The high amounts of total phenolic compounds contained in coffee and its synergistic antioxidant lead to a good tyrosinase enzyme inhibition.[22-23] The previous study reported that feruloyl sucrose ester isolated from OS can inhibit tyrosinase enzyme.[24] In addition, AK leaf was higher than kojic acid, indicating that tyrosinase enzyme inhibitory activity in the AK leaf was weaker than kojic acid. This data was in line with the previous study, which reported that chalcones content in AK leaf showed an inhibitory activity of the enzyme tyrosinase.[12]

Table 1: Phytochemical screening result of CR bean, AK leaf and OS.

Plant species	Flavonoids	Alkaloids,	Quinones	Phenols
CR bean	+	+	+	+
AK leaf	+	+	+	+
OS	-	-	+	+

Table 2: The result of microbial contamination test of traditional mask powder from the mixture of CR bean, AK leaf and OS.

Days	The number of bacteria (CFU/gram)
0	7.65 x 10^2
3	3.10 x 10^1
5	3.50 x 10^1
7	5.71 x 10^1

Table 3: The tyrosinase enzyme inhibitory activity of traditional mask powder from the mixture of CR bean, AK leaf and OS.

Sample	IC_{50} (µg/mL)
CR bean	321.52
AK leaf	930.10
OS	339.55
Kojic acid	573.10
Traditional mask powder	127.60

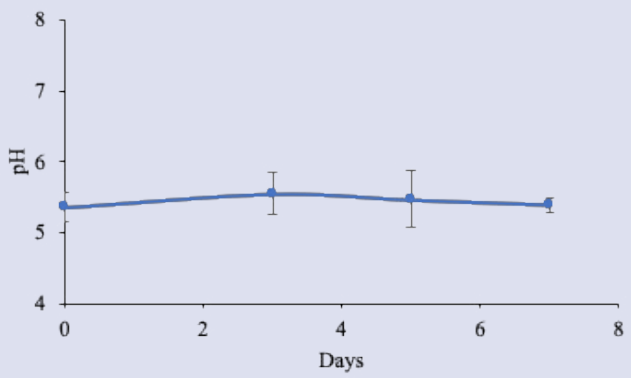

Figure 1: pH of traditional mask powder from the mixture CR bean, AK leaf and OS after dispersed into water.

Interestingly, the IC_{50} values of the traditional mask powder was higher than other sample (CR bean, AK leaf, OS and kojic acid), indicating that its enzyme tyrosinase inhibitory activity was the strongest. It was speculated that the combination of CR bean, AK leaf, OS showed strong synergistic activity in tyrosinase enzyme inhibitory. However, the mechanism of synergistic activity needs further investigated, especially the relationship between the compound contained in traditional mask powder with tyrosinase enzyme inhibitory activity. In order to fully understand its potentials as a skin lightening agent. This study was the initial research regarding the synergistic activity of CR bean, AK leaf, OS on tyrosinase enzyme inhibitory.

CONCLUSION

In this study, tyrosinase enzyme inhibitory of the traditional mask powder from the mixture of CR bean, AK leaf, OS was investigated. The mixture of CR bean, AK leaf, OS was successfully formulated into the traditional mask powder, the physical stability was acceptable according to the Indonesia National Standards (SNI). An in vitro study showed that the IC_{50} of traditional mask powder was lower than every of its component, indicating that synergistic activity from the combination of CR bean, AK leaf, OS in tyrosinase enzyme inhibitory was observed. However, further research on its synergistic activity in relation to the inhibitory activities of the enzyme tyrosinase is needed to fully understand its potentials as a skin lightening agent. This study provides the first research results on the synergistic activity of the plant material mixture on inhibitors of the enzyme tyrosinase, which was safe as a topical preparation, especially as a skin lightening agent.

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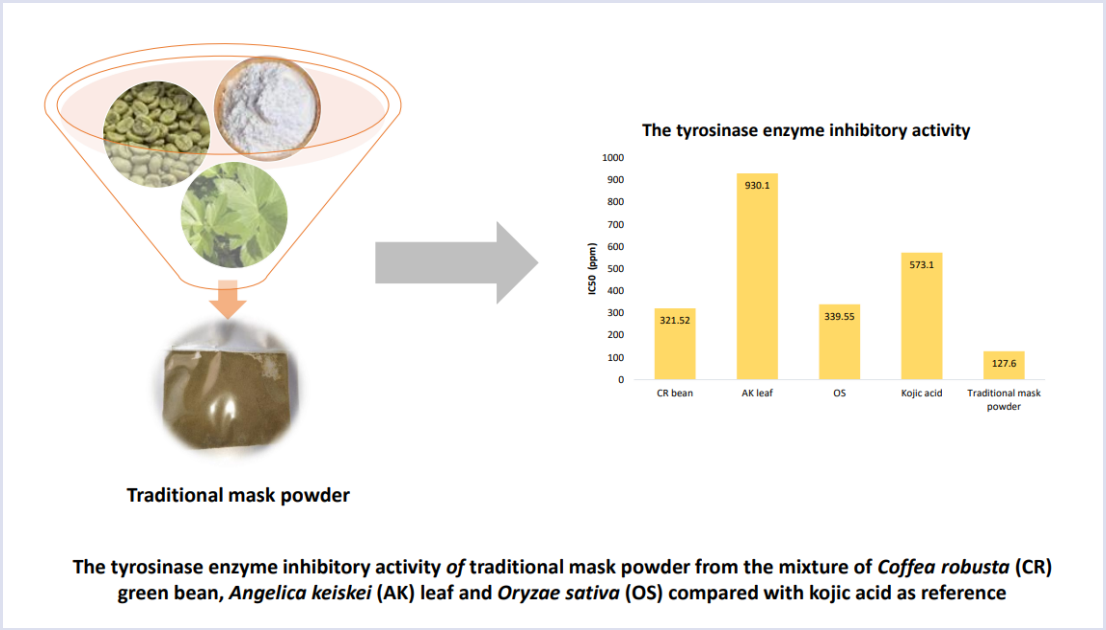
GRAPHICAL ABSTRACT



The tyrosinase enzyme inhibitory activity *of* traditional mask powder from the mixture of *Coffea robusta* (CR) green bean, *Angelica keiskei* (AK) leaf and *Oryzae sativa* (OS) compared with kojic acid as reference

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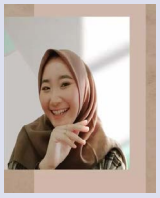

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