TOTAL PHENOLIC AND FLAVONOID CONTENT, FREE RADICAL SCAVENGING ACTIVITY AND TYROSINASE INHIBITION OF CORN COB (ZEA MAYS) EXTRACT

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INTRODUCTION

Indonesia is an agrarian country which is abundant in the availability of crop resources. Corn is one of the major crop in Indonesia. Based on Statistic Indonesia the corn yields in 2015 valued 19,612,435 tons and has increased 604,009 thousand tons compared to the yields in 2014 [1]. Part of the corn used as food source is its kernels, and the consumption of corn resulted large number of waste of corn cobs. Based on data, there is about 13 million tons of corn cob waste which is produced in Indonesia [2]. Meanwhile, the previous result proved that corn cob waste had flavonoids content [3]. Antioxidant is proven to have correlation with flavonoids content [4]. Although corn cob is a waste, yet it is proven to have some benefits in the previous researches. Some of the benefits are as anti-tumor [5], anti-fungus [6], and antioxidant [7].

An antioxidant can inhibit reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) as well as free radicals [8]. Antioxidant can be obtained from natural and synthetic sources [9]. The synthetic antioxidants included BHA (Butylated Hydroxy Anisole), BHT (Butylated Hydroxy Toluene), PG (Propyl Gallate), and TBHQ (Tertiary Butyl Hydro Quinone). Synthetic antioxidants are more effective, but the recent studies show that some synthetic antioxidants can cause toxic and mutagenic effects [10]. Therefore, it is necessary to perform research on natural antioxidant. The phenolics content in corn cob was reported to have good antioxidants in preventing free radicals [3, 11].

Flavonoids compound is reported to have activity as tyrosinase enzyme inhibitor [12]. Skin has melanin, which is black pigments that protect human’s skin from excessive Sun’s ultraviolet exposure, while tyrosinase is an enzyme which plays an important role in melanin production [13]. Whitening agent works in the melanin production stage with a mechanism to inhibit tyrosinase enzyme maturation or inhibit granule pigment (melanosomes) from melanocytes to keratinocytes around them. Whitening agent mechanism works by controlling exosomes secretion which is secreted from melanocytes [14]. Therefore, phytochemicals compound which can inhibit tyrosinase enzyme as melanin producer is needed.

The antioxidant activity of corn cob fractions has previously reported using DPPH radical scavenging method. The result showed that ethyl acetate fraction has highest DPPH radical scavenging, compared to butanol, ethanol, petroleum ether and water fractions [15]. The previous study also reported that corn cob extracts of 50% ethanol extract, 80% methanol extract, 50% methanol extract, 80% methanol and ethyl acetate extract exhibited antioxidant activities using DPPH, ABTS (acid 2,2-Azino-bis-3-etilbenzatiazolin-6-sulfonat) and FRAP (Ferric reducing-antioxidant power) methods [3]. However, the activities of corn cob extract toward tyrosinase enzyme inhibitor and DPPH assay method have been not reported yet. Therefore, this research was aimed to evaluate the activity of tyrosinase enzyme inhibition and to evaluate antioxidant activity using DPPH method.

MATERIALS AND METHODS

Material Sample

Corn cobs are obtained from local farmers in Klaten, Central Java, Indonesia. The plant determination was conducted at the Jamu Department Laboratory, Poltekkes Kemenkes Surakarta, Indonesia with the certificate number was LB.02.06/11.1.1/420/2020.

Other materials used in this study included 2,2-diphenyl-1-picrylhydrazyl (DPPH), from Sigma-Aldrich), quercetine (p. a., Sigma-Aldrich), gallate acid (p. a., Sigma-Aldrich), L-3,4-dihydroxystyryl-Alanine (L-DOPA, p. a. Sigma-Aldrich), methanol (E. Merck, Darmstat Germany), dymethil sulfoxide (DMSO from Sigma-Aldrich), quercetine (p. a., Sigma-Aldrich), gallate acid (p. a., Sigma-Aldrich), chlororm (p. a., Merck) and Folin-Gocalteu phenol LP.

Extraction the corn cobs

The corn cobs then dried to get Simplícia with simplícia quality parameter. Next, the simplícia is changed into powdered form with specific refined degree according to Mesh measurement standard. Corn cob simplícia powder is extracted using 70% ethanol applying maceration method for 5 d. Every 100 g of sample is extracted with 1 L 70% ethanol. The liquid extraction result from the maceration is then evaporated using rotary evaporator water bath so that thick extract of corn cob is formed [15].

Total flavonoid determination

The determination of total flavonoids was performed according to Farmakope Herbal Indonesia [16]. Weigh carefully about 100 mg of...
extract, put into an Erlenmeyer flask, add 25 ml of methanol P, extract for 1 hour with a magnetic stirrer. Filter into a 25 ml flask, add methanol P through the filter to the mark. A-10 mg of gallic acid was put in a 25 ml flask, dissolve with methanol P, add methanol P to the mark. Make a series of dilution of the pure compound solution into the levels of 100, 70, 50, 30, 15, and 5 μg/ml respectively. For each 1 ml of the test solution and each series of the pure compound solution into the appropriate container, add 5.0 ml of retail Folin-Gocaltiu LP (7.5% in water). Let stand for 8 min, add 4.0 ml of 1% NaOH, incubate for 1 hour. Measure the absorbance of each solution at a maximum absorption wavelength of approximately 730 nm. Measure the blank in the same way, without adding test solutions. Make a calibration curve. The total phenolic content is stated as gram equivalent gallic acid every 100 g subfraction dry weight (% b/b EAG).

**Total phenolic determination**

Total phenolic determination in following with Farmakope Herbal Indonesia II edition. Weigh carefully about 50 mg of extract of corn cob, put it into an Erlenmeyer flask, add 25 ml ethanol P, extract for 1 hour with a magnetic stirrer. Filter into a 25 ml flask, rinse the filter paper with 70% LP ethanol and add 70% LP ethanol to the mark. Weigh carefully about 10 mg of quercetin into a 25 ml volumetric flask, dissolve it and add P ethanol to the mark. Make a series of dilution of the comparison solution with the levels of 100, 75, 50 and 25 μg/ml, respectively. Pipette separately 0.5 ml test solutions and and each series pure solutions into suitable containers, add 1.5 ml of P ethanol, 0.1 ml of 10% aluminum chloride P, 0.1 ml respectively 1M sodium acetate and 2.8 ml of water. Shake and let stand for 30 min at room temperature. Measure the absorbance at the maximum absorption wavelength. Measure the blank in the same way, without the addition of aluminum chloride. Make a calibration curve. The total flavonoids content is stated as gram equivalent quercetin every 100 g subfraction (% b/b EK).

**Antioxidant activity determination**

Antioxidant activity determination using DPPH method [17]. Each 50 μl corn cob extract with varied concentration is added by putting into 1.0 ml of the test solution and each series of the pure compound solution into the appropriate container, add 5.0 ml of retail Folin-Gocaltiu LP (7.5% in water). Let stand for 8 min, add 4.0 ml of 1% NaOH, incubate for 1 hour. Measure the absorbance of each solution at a maximum absorption wavelength of approximately 730 nm. Measure the blank in the same way, without adding test solutions. Make a calibration curve. The total flavonoids content is stated as gram equivalent quercetin every 100 g subfraction (% b/b EK).

**Tyrosinase inhibition**

In vitro tyrosinase inhibition test using spectrophotometri UV-Vis. L-DOPA exactly weighed 4.96 mg, then dissolved with phosphate buffered solution (pH=6.8) in a volumetric flask until it reached 10 ml volume. Prevent this solution from light [18]. Tyrosinase exactly weighed 1 mg, then dissolved with phosphate buffered solution (pH=6.8) in a volumetric flask until it reached 10 ml volume. Dissolved tyrosinase has 496 unit/ml activity. The solution is kept in low temperature (2-8°C) [18].

20 mg kojic acid powder is exactly weighed, then dissolved with phosphate buffered solution until it reached 10 ml volume (2000 μg/ml) in a volumetric flask. Do the dilution process until varied concentration of kojic acid 1500; 1000; 500; 250; 125 and 62.5 μg/ml are obtained [19]. 20 mg of fraction/extract is exactly weighed, then dissolved with 1 ml of DMSO and fulfilled with 10 ml (2000 μg/ml) of phosphate buffered solution in a volumetric flask. Do the dilution process until varied concentration of kojic acid 1500; 1000; 500 and 50 μg/ml are obtained [19]. Prepare 4 test tubes (A,B,C,D) then pipette 110 μl solution of L-DOPA 2.5 mmol and 3 ml of phosphate buffered solution pH 6.8 into each tube incubated for 10 min. After being incubated, add to each tube:

- Tube A: 0.13 ml of phosphate buffered solution and 70 μl of tyrosinase enzyme
- Tube B: 0.2 ml of phosphate buffered solution
- Tube C: 0.13 ml of sample solution and 70 μl tyrosinase enzyme solution
- Tube D: 0.1 phosphate buffered solution and 0.1 sample solution

The tubes are incubated for 25 min and are read their absorbance levels at 475 nm wave length.

Inhibition percentage of tyrosinase was calculated with Equation 2 below:

\[\%\text{Inhibition} = \left(\frac{(A - B) - (C - D)}{(A - B)}\right) \times 100\% \]  

With A: Absorbance blank solution is negative with enzyme
B: Absorbance blank solution is negative without enzyme
C: Absorbance sample solution with enzyme
D: Absorbance sample solution without enzyme

**RESULTS**

**Total flavonoid and phenolic content**

Based on the previous researches, it is proven that the compounds which take responsibility for antioxidant activity are phenolic and flavonoids, so the antioxidant activity from natural ingredients is correlated with phenolic and flavonoids compounds [20-22]. The result of phenolic and flavonoids content measurement can be seen in table 1.

| Content         | Concentration |
|-----------------|---------------|
| Phenolic        | 1.76% b/b EAG |
| Flavonoids      | 0.42 % b/b EK |

Table 1 shows that extract ethanol has phenolic content valued 1.76 % b/b EAG higher than flavonoids content valued 0.42%.

**Antioxidant activity**

Quantitative antioxidant activity was performed using UV-Vis spectrophotometri with DPPH method. The DPPH method principle of measurement is based on compound's ability to experience DPPH radical color intensity decrease by counting its absorbance level at 517 nm wave length [23]. The DPPH color degradation process is directly proportional to the concentration of the test material added [24].

The parameter to interpret that a compound has antioxidant activity ability is the IC50 value, mean is the concentration from a substrate which cause 50% of DPPH activity [25]. The smaller IC50 value shows that the compound is more active as an antioxidant. The result of the IC50 value antioxidant activity of corn cob fraction is shown in table 2.

The result in table 2 shows that extract ethanol has IC50 value parameter 38.57 μg/ml. Meanwhile vitamin C as positive control have bigger activity antioxidant with IC50 value is 3.55 μg/ml. Based on this results so it can be concluded that extract ethanol responsible for antioxidant activity alongside with flavonoids and total phenolic content. Flavonoids compound can reduce free radicals oxidation by donating hydrogen atom so that it can act as antioxidant [26].

**Tyrosinase enzyme inhibition**

The purpose of tyrosinase enzyme inhibition testing is to know the ability of corn cob fraction in inhibiting tyrosinase in forming melanin. The enzyme used in this test is mushroom tyrosinase with dengar substrate L-DOPA and kojic acid as positive control. It is
known that kojic acid is a tyrosinase inhibitor which is clinically used to overcome skin hyperpigmentation [27]. The result of IC\textsubscript{50} corn cob fraction tyrosinase enzyme inhibition can be seen on Table 3.

Table 2: IC\textsubscript{50} value antioxidant activity of corn cob extract

| Sample      | IC\textsubscript{50} (µg/ml) |
|-------------|-------------------------------|
| Extract ethanol | 38.57±13.78                 |
| Vitamin C   | 3.55±0.04                     |

The data were given in mean±SD; n = 3

Table 3: IC\textsubscript{50} value tyrosinase enzyme inhibitor corn cob extract

| Sample    | IC\textsubscript{50} (µg/ml) |
|-----------|-----------------------------|
| Extract ethanol | 919.78                     |
| Kojic acid | 150.79                      |

Table 3 shows the highest activity of inhibiting tyrosinase enzyme is kojic acid as positive control with parameter smallest IC\textsubscript{50} value 150.79 µg/ml, while extract ethanol has higher value of IC\textsubscript{50} is 919.789 µg/ml. It is proven that corn cob Extract ethanol also has weak ability in inhibiting tyrosinase enzyme.

Based on research tyrosinase inhibitory activity of the ethyl acetate fraction of C. fistula leaf that concluded highest polyphenol content, there may have been compounds that interfered with the activity of polyphenols in inhibiting tyrosinase so that the ethyl acetate fraction was less significant in inhibiting tyrosinase [20].

CONCLUSION

The results of tyrosinase inhibition of corn cob extract is values IC\textsubscript{50} 38.57 µg/ml. Total phenolic content of the corn cob extract is 1.76 %b/b EAG, while the total of flavonoids content is 0.42%b/b EK. The conclusion is corn cob extract have antioxidant activity and tyrosinase inhibition.

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AUTHORS CONTRIBUTIONS

The author confirms equal responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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