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RADICAL SCAVENGING ACTIVITY OF VARIOUS EXTRACTS AND VARIETIES OF CORN SILK

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ABSTRACT

Corn variety may result different phytochemical contents which in turn influence their antioxidant activities. In Indonesia, corn silk has not been utilized well. Therefore, we evaluated the radical scavenging activity of (1) corn silk extracts obtained from different solvents possessing different polarity, and (2) corn silk extracts from five different corn varieties. In order to perform the experiment, firstly, the selected corn silk was extracted with four solvents possessing different polarity (water, ethanol 70%, ethanol 96%, ethyl acetate). Following the extraction step, the polyphenol content in the extract was determined. From this study, the highest total phenolic content in the extract was exhibited by ethanol 70% (4.4 mg GAE/g) while it was not detected in the ethyl acetate-based extract. Additional test was performed to determine the total flavonoid content. Similar to previous finding, ethanol 70% performed the best solvent with total flavonoids reached 695 mg RE/g. Secondly, ethanol 70% was then employed to extract phenolic content in corn silk from five different varieties (P11, P21, P27, DK85, DK88). The extracts were subjected to determine their total phenolic content and radical scavenging activity. The results show P11 exhibited the highest total phenolic content (1.58 mg GAE/g) while other varieties shown similar content (0.8-1 mg GAE/g). Accordingly, the corn silk extract of P11 performed the highest radical scavenging activity with % inhibition of 78. It can be concluded that corn variety contributes significantly to the phytochemical contents that influences their ability to neutralize free radical compounds, and it is beneficial for further development of functional food.

Keywords: corn silk, variety, antioxidant, radical scavenging activity.

INTRODUCTION

Several synthetic antioxidants such as α-tocopheryl succinate and vitamin E derivated with 4-substituted resorcinol moiety possesses anti inflammation and anti tyrosin property, respectively (Shimizu *et al.*, 2001; Weber *et al.*, 2002); however, they have proven to be highly toxic. The use of food preservatives such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) also has to be limited due to their side effects. For this reason, in recent years, there is a tremendous interest to explore natural antioxidants to be used as supplements to human health (El-Ghorab *et al.*, 2007; Tachakittirungrod *et al.*, 2007; Fu *et al.*, 2011; Liu *et al.*, 2011; Vinholes *et al.*, 2011; Guo *et al.*, 2012; Guo *et al.*, 2016). Phenolic compounds bounded in plants have been claimed to act as reduction agent, hydrogen donor and singlet oxygen neutralizer against free radical compounds (Ebrahimzadeh *et al.*, 2008; Liu *et al.*, 2011).

The presence of phenolic compounds has been detected in fruits (Sánchez-Moreno *et al.*, 2005), vegetables (Islam *et al.*, 2016), leaves (Ammar *et al.*, 2009), roots (Chen *et al.*, 2008) and agricultural by-products such as corn silk (Liu *et al.*, 2011), fruit peels (Irawaty *et al.*, 2014) and seeds (Soong and Barlow, 2004). Corn silk is a bundle of silky, long and yellownish parts that elongated beyond the cob covering the edible part. Generally, corn silk is predominantly discarded due to lack of effective utilization. However, corn silk has been considered as one of important parts in traditional Chinese medicine to treat edema, gout, rheumatism, arthritic and kidney. Scientifically, corn silk has been reported to exhibit optimistic activities such as diuresis effect (Velazquez *et al.*, 2005), hyperglycemia reduction (Guo *et al.*, 2009), anti depressant activity (Ebrahimzadeh *et al.*, 2009), anti fatigue action (Hu *et al.*, 2010), anti hyperlipidemic effect (Kaupa *et al.*, 2011), antidiabetic effect (Zhao *et al.*, 2012), nephrotoxicity reduction (Sepehri *et al.*, 2011), anti inflammantory activity (Habtemariam, 1998) and neuroprotective effect (Choi *et al.*, 2014). Thus, corn silk is prominently shown to be a potent natural antioxidant. The great performances of corn silk reported earlier have been claimed due to the presence of phytochemicals such as polyphenols (Rahman and Rosli, 2014), flavone glycosides (Liu *et al.*, 2011) and polysaccharide (Zhao *et al.*, 2012).

Plant phytochemicals is affected by several factors including genetic intrinsic characters and environmental conditions (Iqbal and Bhanger, 2006; Gündüz and Ozdemir, 2014), production season (Iqbal and Bhanger, 2006), and pre- and pros-harvest processing (Sánchez-Moreno *et al.*, 2005; Parra *et al.*, 2007; Huang *et al.*, 2008). A study of phytochemicals content in corn silk showed how the variety influences the composition of phenolics, flavonoids and anthocyanin contents and thus, their antioxidant activities were much different (Sarepoua *et al.*, 2015). Similarly, the effect of variety on phytochemicals content was also reported on pears (Guan *et al.*, 2014), strawberry (Wang and Zheng, 2001), bean (Fan *et al.*, 2016) and grape (Margraf *et al.*, 2016).

The information regarding to the influence of corn variety on the ability of corn silk, obtained from local corn planted in East Java (Indonesia), extract to scavenge free radical compounds is still lacking. Therefore, in this study, the influence of corn variety on the amounts of phenolic and flavonoid contents in corn silk extracts has

been determined, and their extract abilities to neutralize the selected radical compound have been evaluated.

MATERIAL AND METHODS

Plant material preparation

Fresh corns were collected from local farmers around Krian and Mojokerto, East Java, Indonesia. Corn silk was removed subsequently and dried in an oven set at 40°C for 48 h until its moisture content is around 10% $^{w}/_{w}$, finely powdered to obtain the size of -100/+120 mesh, and kept in a closed container at a temperature of ± 5°C for further use.

Chemicals

2,2-Diphenyl-1-picrylhdrazyl (DPPH, Aldrich), ethanol, ethyl acetate (≥ 99,5%, Merck), gallic acid (≥ 99%, Sigma®), rutin (≥ 94%, Sigma®), Folin-Ciocalteu reagent (Merck), sodium carbonate (≥ 99%, Merck), aluminum chloride (99%, Ferak) were purchased and used without further purification.

Procedures

A pre-determined corn silk was mixed with solvents possessing different polarity (water, ethanol 70%, ethanol 96%, ethyl acetate) with a ratio of 1:20 $^{w}/_{v}$ at room temperature. The filtrate was then separated by filtering the solution through a Whatman No. 1 filter paper. The extract was further subjected for analyses to determine total phenolics content, total flavonoids content, and its antioxidant capacity by using DPPH free radical scavenging activity assay. Whilst total phenolics content (TPC) is expressed as gallic acid equivalent (GAE), total flavonoids content (TFC) is presented as rutin equivalent (RE). The antioxidant capacity of the extracts is reported as % inhibition which indicates the amount of DPPH free radical compound that can be neutralize by the extract.

Total phenolic content analysis

Total phenolic content was determined according to the Folin-Ciocalteu colorimetric method (Anagnostopoulou *et al.*, 2006) with slight modification. Briefly, 1 mL of sample was added to 5 mL of Folin-Ciocalteu's reagent (1:1) and 4 mL of Na_2CO_3 solution (7.5%). The mixture was then incubated at room temperature for 30 min in dark condition. The absorbance was subsequently measured at 730 nm using a spectrophotometer (Shimadzu, UVmini-1240). Total phenolic content was expressed as Gallic Acid Equivalent (GAE)/mg sample.

Total flavonoid content analysis

Total flavonoid content was determined by using aluminum chloride colorimetric method (Wu *et al.*, 2009) with modification. Approximately, 1.5 mL of sample was mixed with 1.5 mL of aluminum chloride solution. After 30 min of incubation period in dark condition, the absorbance was measured at 431 nm against a blank of methanol using a spectrophotometer (Shimadzu, UVmini-1240). Total flavonoid content was expressed as Rutin Equivalent (RE)/mg sample

DPPH free-radical scavenging assay

DPPH radical scavenging activity was determined according to (Liu *et al.*, 2011) with modification. 1 mL of sample and 1.25 mL of DPPH solution (3 mM) were mixed in a reaction tube and incubated at room temperature in dark conditions. The absorbance was then measured 30 min later using a spectrophotometer (Shimadzu, UVmini-1240) at 520 nm. The assay was repeated but sample was replaced with ethanol for control. The radical scavenging activity was measured as a decrease in the absorbance and was calculated by using the following equation:

$$\text{Percentage inhibition} = [(1 - A_S/A_C)] \times 100 \quad (1)$$

where A_S and A_C are the absorbances of sample and the control, respectively.

RESULTS AND DISCUSSIONS

Solvent polarity

Different solvents such as water, ethanol 70%, ethanol 96% and ethyl acetate have been employed to extract phenolic compounds from corn silk. Figure-1 shows the amount of total phenolics compounds observed in corn silk extracts.

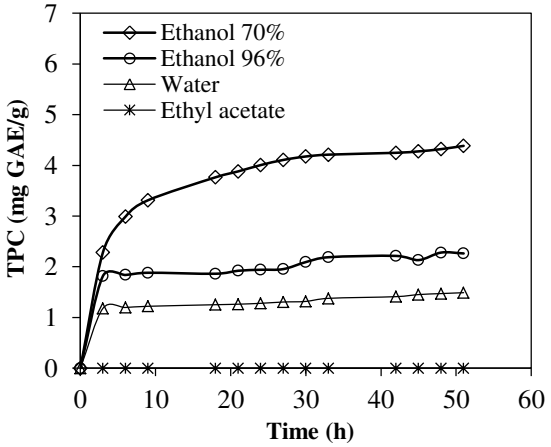

Figure-1. Effect of solvent polarity on TPC as a function of extraction period.

As seen, type of solvent exhibited different amount of phenolics can be extracted from corn silk. The employment of ethanol 70% was found to be the best solvent with phenolics compounds observed in the extract was 4.4 mg GAE/g of dry corn silk at the extraction time of 51 min. Following ethanol 70%, ethanol 96% and water gave the TPC value of 2.3 and 1.5 mg GAE/g, respectively on the same extraction period. On the other hand, ethyl acetate did not exhibit was an inefficient solvent for phenolic contents extraction. The results suggest ethanol 70% is more appropriate to be employed for extracting

phenolic compounds from corn silk. Moreover, ethanol is acceptable for human consumption and compatible with food systems, allowing its future application as the solvent in antioxidant extraction processes.

Closer inspection to the results displayed in Figure-1, the extraction of phenolic compounds from corn silk is greatly influenced by polarity of the extracting solvents. Water is a strong-polar solvent (index polarity of 9) while ethanol is a relative low-polar solvent (index polarity of 5.2), and they can be mixed with each other in any proportion. With the addition of water to ethanol, polarity of the mixture solvent will increase continuously and thus, more polar phenolic compounds can be well extracted. However, the types of phenolics present in corn silk have not been detailed reported in literature and thus, more studies are required to solve this ambiguity. There is no general information of ethanol concentration required to extract all phenolic compounds (Sultana *et al.*, 2009; Dent *et al.*, 2013). However, it is emphasized that non-polar property of ethanol is important to break down the cell wall that aids in the release of phenolics compounds to the solvent (Lapornik *et al.*, 2005). It also reveals in Figure-1, the use of pure water as the extraction solvent inhibits the extraction process which is shown by the decrease of total phenolic compounds extracted by a factor of 0.66 compared to ethanol 70%. This may be explained by either the necessity of ethanol in the system to assist the extraction process as mentioned previously or limited phenolics compounds solubility in water. In the case of ethyl acetate, the lowest polarity level (index polarity of 4.4) of ethyl acetate among all solvents investigated in the present work may not facilitate the release of the phenolic compounds into the solvent medium. Furthermore, the diverse structure of phenolic compounds possessed by plant materials (Liu *et al.*, 2011) may have built the complexity of the extraction process because of its specific solubility and polarity.

The finding of the present work that ethanol/water mixture is a good solvent for phenolic extraction from corn silk is in agreement with previous investigations. For example, Sultana et al. (2009) revealed that the addition of 20% water in ethanol has increased the extraction of total phenolic compounds of medicinal plant materials up to 54% (Sultana *et al.*, 2009). In addition, Thoo*et al*. (2013) found the amount of total phenolic and flavonoid compounds extracted from *Andrographispaniculata* powder was observed higher when a mixture of ethanol and water used as the extracting solvent rather than single solvent of pure ethanol or water (Thoo *et al.*, 2013). A similar finding was reported by other group (Chan *et al.*, 2009; Dent *et al.*, 2013) for phenolic compound extraction from citrus and Dalmatian wild sage, respectively.

Figure-2 shows the profiles of total flavonoids detected in corn silk extracts. Among four solvents with different polarity employed, the highest flavonoids compounds was observed in ethanol 70%, followed by water and ethanol 96% with the TFC values were 695, 484 and 172 mg RE/g. From this study, we found that the amount of flavonoids extracted in water was higher than in ethanol 96%, indicating the flavonoid compounds are favored in water solvent. Ethyl acetate was the inefficient solvent for flavonoids extraction, similar to the case of phenolic compounds mentioned previously.

Effect of extraction time

Extraction time is important parameter in optimizing the extraction of phenolics and/or flavonoids compounds. From literature, the extraction time varies between 17 min to 60 min depending on the materials being extracted and extraction technique (Dent *et al.*, 2013; Maran *et al.*, 2013;Thoo *et al.*, 2013). In the present work, TPC observed in the extracts of ethanol 70% were increased gradually with increasing of extraction period up to 51 h (Figure-1). However, for ethanol 96%, 1.8 mg GAE/g was extracted after 3 h and after 33 h, the total phenolic compounds observed in the extract was around 2.2 mg GAE/g (20%). Prolonged the extraction time over 33 h did not increase the amount of phenolics in the extract. For water solvent, approximately 1.2 mg GAE/g was observed in the extract after 3 h-extraction time. Increasing the extraction time for another 48 h increased the amount of phenolic compounds extracted by 25%. It is obvious that the extraction time had an important influence on the phenolics extracted from corn silk. However, no increase was observed in the corn silk/ethyl acetate system which may be due to solubility factor.

Figure-2 shows the effect of extraction time on the total flavonoids detected in the extracts. As seen, the amount of total flavonoids compounds extracted from corn silk is gradually increased along with extraction period for the three solvents. For extraction time of 3 h, approximately 381, 263 and 114 RE/g was detected in the extract of ethanol 70%, water and ethanol 96%, respectively. Prolonged the extraction time to 51 h, the release of total flavonoids into the solvents was observed increasead by 82, 84 and 51 % for the subsequent solvents. Compared to the amount of phenolics extracted for the same extraction time, the results suggest the specific compounds, either phenolics or flavonoids, requires its specific time to get release into the extraction solvent.

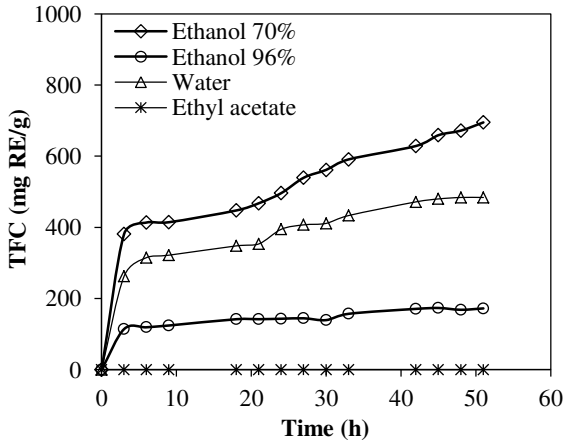

Figure-2. Effect of solvent polarity on TFC as a function of extraction period.

Effect of corn variety

TPC detected in the five different corn silk extracts are shown in Figure-3. The amount of TPC was found 1.58±0.07, 0.78±0.06, 1.09±0.04, 0.73±0.06, and 0.88±0.07 mg GAE/g of dry corn silk for local corn varieties of P11, P21, P27, DK85, and DK88, respectively. The results showed that phenolic compounds in corn silk are closely related to variety which influences the growth process and thus, its phenolic and mineral contents. This finding is supported by other studies that phytochemical content of corn silk is greatly affected by corn variety, plant maturity, and growing conditions (Gray *et al.*, 2003; Sarepoua *et al.*, 2015). Moreover, different cultivar area and harvest time were also reported to have influence on the phenolic content of natural plants. On average across the five varieties, corn silk from P11 exhibited the highest for total phenolic content, providing capability to neutralize free radical compounds.

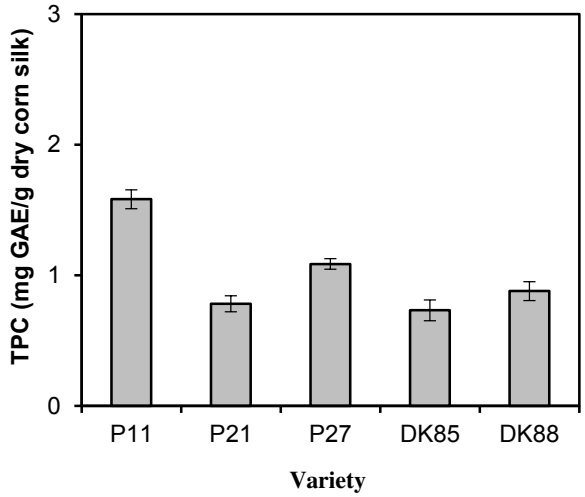

Figure-3. Effect of variety on TPC detected in corn silk extracts.

The total flavonoid content (TFC) of the different corn varieties are shown in Figure-4. As seen, on average the TFC values can be definitely separated into two different categories, i.e. the high group consists of P11, P21 and DK85 with TFC values in the range of 2.2 to 2.3 mg RE/g dry corn silk. On the other hand, the low group exhibited by both P27 and DK88 show the TFC values are 1.6 and 1.14 mg RE/g dry corn silk, respectively.

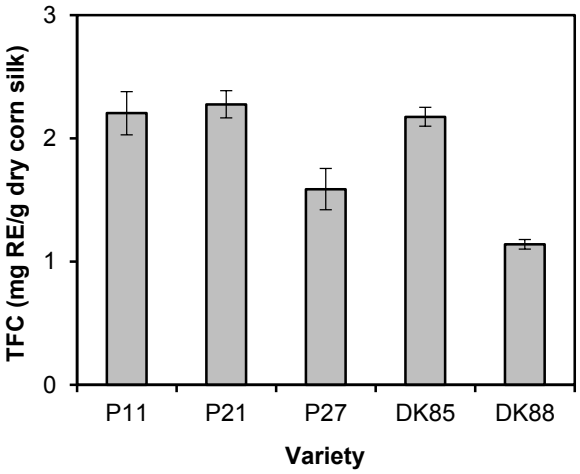

Figure-4. Effect of variety on TFC detected in corn silk extracts.

The radical DPPH scavenging activity, presented as % inhibition, of corn silk extracts prepared from five different varieties are shown in Figure-5. Overall, all corn silk varieties revealed a dose-dependent response when exposed to different concentration of extract (data not shown). As shown in the figure, the scavenging activity of corn silk extract of P11 exhibited the highest scavenging activity that the extract is able to neutralize 78% of DPPH free radical compound available in the system, followed by DK85, P27, DK88 and P21 with % inhibition of 44, 35, 34 and 28, respectively.

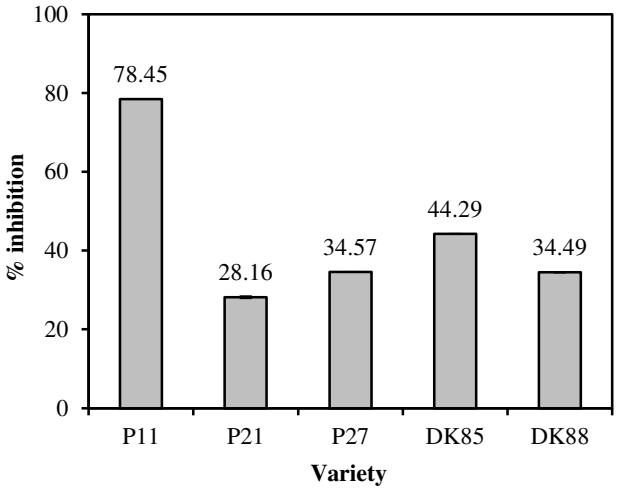

Figure-5. Effect of variety on DPPH free radical scavenging activity detected in corn silk extracts.

Positive correlation between total phenolic/flavonoid contents and DPPH scavenging activity is reported in literature (Ardestani and Yazdanparast, 2007; Sarepoua *et al.*, 2015). The highest ability of corn silk extract from P11 to neutralize DPPH free radical (Figure-5) maybe due to the highest phenolic content in the extract (Figure-3) as well as its flavonoid content (Figure-4).

CONCLUSIONS

The study showed that plant variety influenced the amount of phenolics and flavonoids and thus, possesses antioxidant activity to certain extents. Present study also demonstrated that corn silk extract of P11 had the most promising antioxidant source, even its activity was observed lower than ascorbic acid which is widely accepted as antioxidant agent. Further study on the isolation of individual compounds and the effect of corn silk maturity level on its antioxidant activity is necessary for the development of functional food and natural additive. Moreover, the molecular mechanisms involved in antioxidant activity as well as the efficacy by *in vivo* study and the safety matter require more attention.

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