Sains Malaysiana 41(10)(2012): 1217–1221

Total Polyphenol Content and Free Radical Scavenging Activity of Cornsilk (*Zea mays* hairs)

(Kandungan Polifenol Jumlah dan Aktiviti Perencatan Radikal Bebas dalam Rerambut Jagung Muda (*Zea mays Hairs)*

A.R. Nurhanan, W.I. Wan Rosli* & S.S.J. Mohsin

ABSTRACT

*Total polyphenol content and free radical scavenging activity of cornsilk (*Zea mays *hairs) extracts were determined. Our result showed the total polyphenol content of methanolic and water extracts were 272.81 mgGAE/100 g and 256.36 mgGAE/100 mg dry plant, respectively. The total flavonoid content of methanolic extract (38.01 mg catechin equivalent/100 g) was significantly higher ($p \leq 0.05$) than the water extract (4.11 mg catechin equivalent/100 g). In radical scavenging activity, the activity of methanolic extract was significantly higher ($p \leq 0.05$) than the water extract. The methanolic extract scavenged at 81.7% of free radicals at 1000 µg/mL while the water extract showed 63.5% of inhibition. In conclusion, cornsilk contained high polyphenol components with strong free radical scavenging activity thus could be considered as potential source of natural antioxidant.*

*Keywords: Antioxidant assay; cornsilk (*Zea mays *hairs); radical scavenging activity; total polyphenol*

ABSTRAK

*Kandungan jumlah polifenol dan aktiviti perencatan radikal bebas dalam ekstrak rerambut jagung muda (*Zea mays *hairs) telah ditentukan. Hasil kajian mendapati jumlah kandungan polifenol dalam ekstrak metanol and berair masing-masing adalah 272.81 mgGAE/100 g dan 256.36 mgGAE/100 mg (asas kering). Kandungan flavonoid bagi ekstrak metanolik (38.01 mg catechin/100 g) adalah lebih tinggi secara signifikan ($p \leq 0.05$) daripada ekstrak berair (4.11 mg catechin/100 g). Dalam aktiviti perencatan radikal bebas, peratus perencatan ekstrak metanol adalah lebih tinggi secara signifikan ($p \leq 0.05$) berbanding dengan ekstrak berair. Ekstrak metanol merencat sebanyak 81.7% pada 1000 µg/mL sementara ekstrak berair menunjukkan peratus perencatan sebanyak 63.5%. Kesimpulannya, sutera jagung mengandungi komponen polifenol yang tinggi dengan aktiviti perencatan radikal bebas yang kuat. Hal ini mencadangkan sutera jagung dipertimbangkan sebagai sumber anti-pengoksida semula jadi yang berpotensi.*

Kata kunci: Aktiviti perencatan radikal bebas; polifenol jumlah; sutera jagung; ujian antioksida

Introduction

Zea mays or the corn plant is grown in many parts of the world for their economic importance. The plants are cultivated in almost all region of the world including Africa, America, Australia and Asia. In Malaysia, specifically, in Kelantan which is located in northern east of Malaysia, the corns are cultivated 4 to 5 times per year mainly for the sweet corns. Apart from sweet corn which is the main product of the corn plantation industry, about 25% of young corns or baby corns are produced from the corn plantation (survey done earlier-unpublished observation). Usually the mature corns are harvested after 65-75 days of planting while the baby corns could be collected earlier that is after 45-55 days of planting. Both the mature corns and the baby corns are used to prepare varieties of food. The utilization of mature corns in the food industry is broad. Corn is favoured for its sweet taste and that it provides significant nutritional elements essential for human health. It also contributes to many new scientific findings where research on corn and its uses are still being actively conducted and may involve many areas of studies (Dermastia et al. 2009; Hassanein et al. 2009; Krakowsky et al. 2006; Malumba et al. 2008; Sanchez et al. 2002).

Young *Zea mays* corn fruit or baby corn present with long and yellowish stigma or cornsilk as part of the fruit. The 10-20 cm long silks are found inside the husks and could be seen as a tassel of the fruits. While the baby corns are utilized as a vegetable, the cornsilk are usually discarded as waste. It is estimated that about 50 000 tonnes of cornsilk are being thrown away in Malaysia alone each year (estimation is calculated based on the fact that the total cornsilk weight is usually equal to the weight of the young baby corn and the number of small holders planting corns as a source of baby corns in the country).

Cornsilk have been used in ancient times as traditional remedy. The silks were used in various regions to treat urological disorders and as a mild diuretic agent (Cáceres et al. 1987). Kidney stones, nephritis, cystisis are amongst the condition mentioned (Maksimović et al. 2005). Other than kidney problems, cornsilk were reported to treat

prostate problem, edema and gout (Maksimović et al. 2005). Cornsilk contains protein, vitamins, carbohydrates, Ca^{2+}, K^{+}, Mg^{2+} and Na^{+} salts, fixed and volatile oils, steroids (sitosterol and stigmasterol), alkaloids, saponins, tannins and flavonoid (Velazquez et al. 2005). Cornsilk also contains phytochemical components which exhibited antioxidant activities. The *Maydis stigma*'s potential antioxidant activities in terms of reducing ferric capacity, scavenging free radicals, chelating catalytic metal ions and inhibition of lipid peroxidation has been proven by many authors (Ebrahimzadeh et al. 2008; Maksimović et al. 2005). The silks were observed to contain significant amount of polyphenol compounds. Polyphenols are known for its antioxidant characteristics and has been found to be rich in vegetables, fruits, herbs and other plant sources. The capabilities of polyphenols as free radical scavengers, complexers of pro-oxidant metals, reducing agents, quenchers and oxygen singlet formation are undeniable. Free radicals have been claimed to affect human health by causing diseases such as cancer, hypertension, heart attack and diabetes (Prasad et al. 2009). Many polyphenolics containing plant sources were reported to exhibit antiradicals, antimutagenic, antibacterial and cytotoxic effects (de Mejía et al. 1999; Kilani et al. 2008; Pereira et al. 2007) and therefore dietary intake rich in polyphenols may reduce the risk associated with many health related diseases. Reports on the antioxidant activity of cornsilks are still lacking. Therefore the aims of this study were to determine the total polyphenol and total flavonoid content of cornsilk extracts. The antioxidant activity of the cornsilk extracts is also determined through the free radical scavenging analysis.

Materials and Methods

Plant Material

The young fruits of *Zea mays* were purchased (3 kg) from local wet market in Kota Bharu, Kelantan. The baby corn was dehusked and fresh cornsilk were detached from the fruit. Fresh cornsilk were dried in an oven (Mermet, USA) at 55°C until they turned brown in colour. The dried cornsilk were ground into a powder form, sieved and kept in airtight container at 4°C until used.

Chemicals and Reagents

Folin-Ciocalteau reagent, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), ascorbic acid, gallic acid and (+)-catechin were purchased from Sigma Chemical Co. (St. Louis, MO). Butylated hydroxytoluene (BHT) was purchased from Merck and all other chemicals were of analytical grade.

Sample Preparation

Methanolic extract of dried cornsilks were prepared by extracting 25 g of dried cornsilk in 100 mL methanol/water (80% v/v) solvent. The dried cornsilk was homogenized (Ika, Germany) in the solvent for 15 min. The water extract was prepared by adding 10 g of dried cornsilk into 100 mL of hot water for 15 min with constant stirring. Both methanolic and water extracts were vacuumed-filter twice, centrifuged (3,000 rpm, 10 min) and kept in wrapped bottle at 4°C until used.

Total Polyphenol Content

The total phenolic content of aqueous methanol and water extracts were determined by using the Folin-Ciocalteau calorimetric method. Briefly, 1 mL of sample was added into a 25 mL volumetric flask followed by the addition of 1 mL Folin-Ciocalteau reagent (1N). The mixture was shaken slowly and left to react at room temperature for 5 min. After 5 min, 10 mL of sodium bicarbonate (7% w/v) was added into the mixture. The flask was filled with distilled water and left to stand at room temperature in the dark for 40 min. Distilled water was used as blank. Samples absorbances were recorded at 750 nm against the blank. The total polyphenol content was compared to that of gallic acid standard curve previously prepared covering the concentration of 20 to 100 μg/mL. Samples were measured in triplicate analysis.

Total Flavonoid Content

Total flavonoid content was determined according to the methods described by Ozsoy et al. (2008). About 1.25 mL of distilled water was added to the sample (0.25 mL) after which 75 μL of sodium nitrate (5% w/v) was added. The mixture was left to stand for 6 min before being added with 150 μL of 10% (w/v) aluminium chloride. The mixture was further left to react for 5 min before 0.5 mL of NaOH (1 M) solution was added. The mixture was made up to 2.5 mL with distilled water. The absorbance of the samples was read at 510 nm by using UV-Vis spectrophotometer (Varians, USA). The total flavonoid content was calculated by referring to the catechin standard curve previously prepared in the ranged of 0 to 25 μg/mL.

DPPH Scavenging Activity

The scavenging activity was carried out by using free radicals DPPH colouring method (Pereira et al. 2007). Briefly, 4 mL of sample was added into a bottle followed by 1 mL of DPPH solution (1 mM). In the 5 mL reaction mixture the sample concentration tested were 200 μg/mL to 1000 μg/mL. The mixture was left in the dark for 30 min. Absorbances of samples were recorded at 520 nm by using UV-Vis spectrophotometer (Varians, USA). Ascorbic acid and BHT were used as positive control. The radical scavenging activity was expressed by percentage of inhibition and was calculated as follow:

$$\%\ \text{DDPH radical scavenging} = (1 - As/Ac) \times 100$$

where, Ac is the absorbance of control, As is the absorbance of sample solution and control is the DPPH solution only (without sample)

Statistical Analysis

Data was expressed as means ± standard deviation of triplicate analysis. The t-test analysis was performed to determine the differences among the means by using SPSS V.12.

Results and Discussion

The estimation of total polyphenol content was carried by using the Folin-Ciocalteau reagent. The yellow colour of phosphotungstic acid reagent (Folin reagent) was reduced to blue colour in alkaline solution. The intensity of blue coloured complex increased with the increasing number of hydrogen donating groups in the phenolic compounds thus indicates higher total phenol content (Kaur et al. 2008). The total polyphenol content was expressed in mg GAE/100 g dry plant. From the gallic acid standard curve, the aqueous methanolic extract was observed to be higher in polyphenol than that of the water extract. The methanolic extract was estimated to contain 272.81 mg GAE/100 g dry plant while the water extract contained 256.36 mg GAE/100 mg dry plant. The total polyphenol content of the cornsilk extract in our study was found to be lower than the cornsilk extracts reported by other researchers (Ebrahimzadeh et al. 2008; Maksimović et al. 2005). Although the total polyphenol content was found to be lower, the content of plant components may be different depending on its origin (Ebrahimzadeh et al. 2008). The total flavonoid content of cornsilk extract was described in terms of catechin equivalent. By using catechin standard curve, the aqueous methanolic extract contained 38.01 mg catechin/100 g dry plant which was higher than the water extract (4.1 mg catechin/100 g dry plant). Antioxidant activity of cornsilk extracts was conducted by using the DPPH free radical method. In this assay, the ability of cornsilk extracts to donate hydrogen atom or electron to the unpaired DPPH radical was determined by the reduction of DPPH radical into the reduced form DPPH-H. The DPPH solution which could be seen as purple in colour would change to pale purple or yellowish when reacted with radical scavenger.

The scavenging activities of the extracts are shown in Figure 1. The scavenging activity of methanolic extract was significantly higher (p<0.05) than the water extract of all concentration tested. The aqueous methanolic extract exhibited higher level of scavenging activity compared to the water extract. In the aqueous methanolic extract, the percentage of inhibition was 81.7% at 1000 µg/mL and the water extract scavenged 63.5% at the same concentration. At the lowest concentration tested which was at 200 µg/mL, the scavenging activity of the aqueous methanolic extract and water extract were 71.5% and 59.4%, respectively. The percentage of inhibition of ascorbic acid and BHT however were higher compared to the DPPH analysis for both extracts (Figure 2). At the lowest concentration (20 µg/mL) the ascorbic acid and BHT scavenged at 95% and 82.2% of radicals respectively. By comparing with other cornsilk extracts the scavenging activity of our cornsilk extracts were found to be lower than Iranian cornsilk which scavenged at 91% at 800 µg/mL (Ebrahimzadeh et al. 2008).

The presence of hydroxyl groups in the cornsilk extracts may contribute to the radical scavenging activity. Plant phenolics and flavonoids in the extract reduced DPPH radicals by their ability to donate hydrogen. Plant flavonoid is believed to reside in their free radical-scavenging capacity and their antioxidant activity increases with an increase in the number of hydroxyl groups that they bear and a decrease in their glycosylation (Rice-Evans et al. 1996). It has been suggested that higher level of flavonoid content in aqueous methanol extract contributed

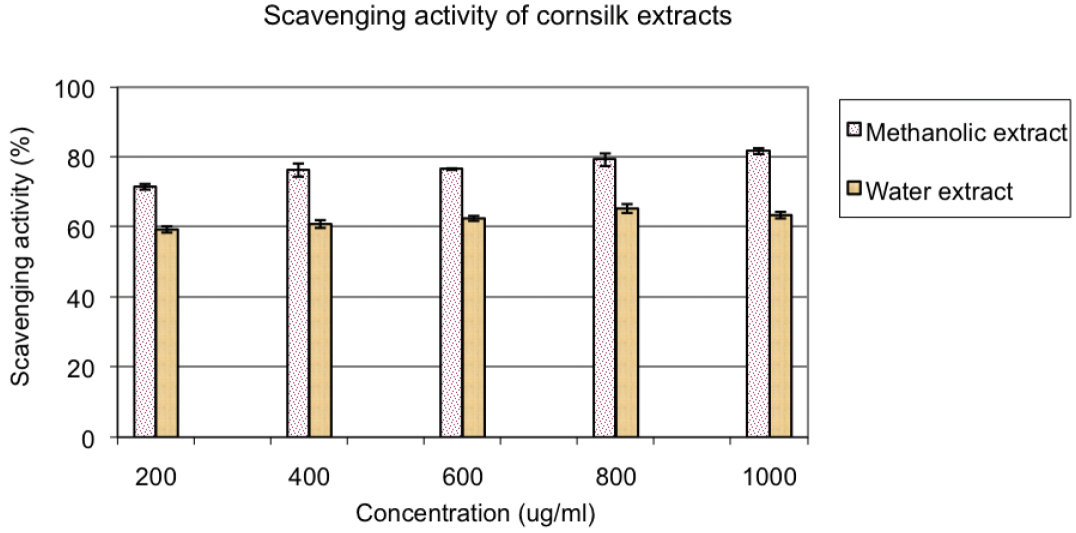

FIGURE 1. DPPH radical scavenging activity of cornsilk extracts. Values are means ± sd (n=3)

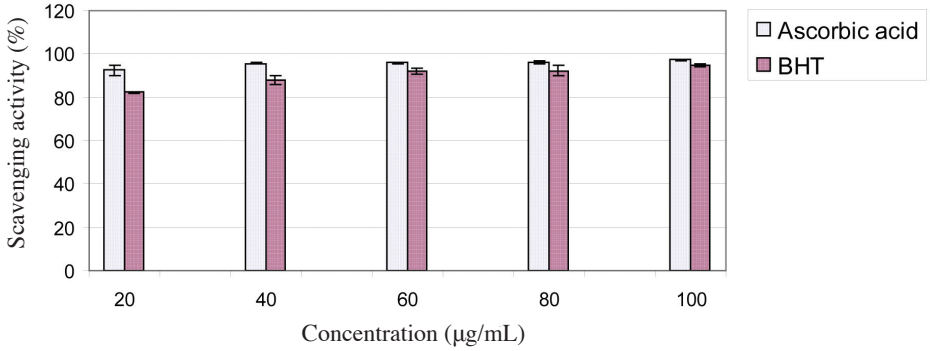

FIGURE 2. DPPH radical scavenging activity of ascorbic acid and BHT used as reference. Values are means ± sd (n=3)

to the strong scavenging activity. The high flavonoid content is thought to give higher antioxidant activity. This phenomenon is also observed in other plant.

CONCLUSION

The present study showed that the aqueous methanol extract of cornsilk contained high level of total polyphenol. The flavonoid content was also high in the extract therefore contributing to the strong DPPH scavenging activity. The water extract which showed a lower level of total polyphenol and flavonoid content exhibited strong scavenging activity. Free radical scavenging activity is one of the most important characteristics used to determine antioxidant activity. With its high scavenging activity, this plant waste product may benefit the health and food industries.

ACKNOWLEDGEMENTS

We thank USM grant (304/PPSK/61310056), USM fellowship fund (1001/PPSK/8143005) and School of Health Sciences, USM for this research undertaking.

REFERENCES

Cáceres, A., Girón, L.M. & Martinez, A.M. 1987. Diuretic activity of plants used for treatment of urinary ailments in Guatemala, *Journal of Ethnopharmacology* 19: 233-243.

de Mejía, E.G., Castaño-Tastado, E. & Loarca-Piña, G. 1999. Antimutagenic effects of natural phenolic compounds in beans. *Mutation Research* 441: 1-9.

Dermastia, M., Kladnik, A., Koce, J.D. & Chourey, P.S. 2009. A cellular study of teosinte *Zea mays* subsp. *parviglumis* (Poaceae) caryopsis development showing several processes conserved in maize. *American Journal of Botany* 96: 1798-1807.

Ebrahimzadeh, M.A., Pourmorad, F. & Hafezi, S. 2008. Antioxidant activities of Iranian corn silk. *Turkish Journal of Biology* 32: 43-49.

Francisco, M. L. & Resurreccion, A.V.A. 2009. Total phenolics and antioxidant capacity of heat-treated peanut skins. *Journal of Food Composition Analysis* 22: 16-24.

Hassanein, R.A., Bassuony, F. M., Baraka, D. M. & Khalil, R. R. 2009. Physiological effects of nicotinamide and ascorbic acid on *Zea mays* plant grown under salinity stress: Change in growth, some relevant metabolic activities and oxidative defence systems, *Research Journal of Agriculture & Biology Science* 1: 72-81.

Kaur, R., Arora, S. & Singh, B. 2008. Antioxidant activity of the phenol rich fractions of leaves of *Chukrasia tabularis* A. Juss. *Bioresource Technology* 99: 7692-7698.

Kilani, S., Sghaier, M. B., Kimem, I., Bouhlel, I., Boubaker, J., Bhouri, W., Skandrani, I, Neffatti, A., Ammar, R. B., Dijoux-Franca, M. G., Ghedira, K., & Chekir-Ghedira, L. 2008. *In vitro* evaluation of antibacterial, antioxidant, cytotoxic and apoptotic activities of the tubers infusion and extracts of *Cyperus rotundus*. *Bioresource Technology* 99: 9004-9008.

Krakowsky, M.D., Lee, M. & Coors, J.G. 2006. Quantitative trait loci for cell wall components in recombinant inbred lines of maize (*Zea mays* L.) II: leaf sheath tissue. *Theoretical and Applied Genetic* 112: 717-726.

Maksimović, Z., Malenčić, D. & Kovačević, N. 2005. Polyphenol contents and antioxidant activity of *Maydis stigma* extracts. *Bioresource Technology* 96: 873-877.

Malumba, P., Vanderghem, C., Deroanne, C. & Béra, F. 2008. Influence of drying temperature on the solubility, the purity of isolates and the electrophoretic patterns of corn proteins. *Food Chemistry* 111: 564-572.

Ozsoy, N., Can, A., Yanardag, R. & Akev, N. 2008. Antioxidant activity of *Smilax excelsa* L. leaf extracts. *Food Chemistry* 110: 571-583.

Pereira, J.A., Oleiveira, I., Sousa, A., Valentão, P., Andrade, P.B., Ferreira, I.C., Ferreres, F., Bento, A., Seabra, R. & Estevinho, L. 2007. Walnut (Juglans regia L.) leaves: Phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. *Food and Chemical Toxicology* 45: 2287-2295.

Prasad, K.N., Yang, B., Dong, X., Jiang, G., Zhang, H., Xie, H. & Jiang, Y. 2009. Flavonoid contents and antioxidant activities from *Cinnamomum* species. *Innovative Food Science and Emerging Technologies* 10: 627-632.

Rice-Evans, C.A., Miller, N.J. & Panganga, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids: A review. *Free Radical Biology and Medicine* 20: 933-956.

Sanchez, J.E., Paul, E.A., Willson, T.C., Smeenk, J. & Harwood, R.R. 2002. Corn root effects on the nitrogen-supplying capacity of a conditioned soil. *Agronomy Journal* 94: 391-396.

Velazquez, D.V.O., Xavier, H.S., Batista, J.E.M. & de Castro-Chaves, C. 2005. *Zea mays* L. Extracts modify glomerular function and potassium urinary excretion in conscious rats. *Journal of Phytomedicine* 12: 363-369.

W.I. Wan Rosli*
Nutrition Program, School of Health Sciences
Universiti Sains Malaysia Health Campus
16150 Kubang Kerian
Kelantan, Malaysia

A.R. Nurhanan & S.S.J. Mohsin
School of Health Sciences
Universiti Sains Malaysia Health Campus
16150 Kubang Kerian
Kelantan, Malaysia

*Corresponding author; email: wrosli@kck.usm.my

Received: 27 July 2011
Accepted: 21 May 2012