Evaluation of Polyphenol Content and Antioxidant Activities of Some Selected Organic and Aqueous Extracts of Cornsilk (*Zea Mays* Hairs)

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***Abstract*—In this study, the polyphenol content and antioxidant activities of some organic and aqueous extracts of cornsilk were evaluated. Cornsilk powder was extracted with methanol, ethanol, water and ethyl acetate solvent by using soxhlet extraction method. The antioxidant activities of all cornsilk extracts were determined via β-carotene bleaching method, 2,2-diphenyl-2-picrylhydrazyl (DPPH.) radical scavenging, superoxide anion (O2-) radical scavenging and ferric reducing power activity (FRAP). The highest polyphenol content was exhibited by the methanol extract (101.99 mg GAE/g) compared to that of ethanol (93.43 mg GAE/g), water (35.34 mg GAE/g) and ethyl acetate extract (6.70 mg GAE/g). The flavanoid content of cornsilk extracts was in the range of 0.66 to 9.26 mg catechin equivalent/g extract showing the highest content found in the methanol extract. In the antioxidant assays, the methanol extract exhibited the strongest free radical scavenging and reducing activity as compared to the other extracts. In the β-carotene assay, the methanol extract (66.05%), showed highest bleaching activity compared to the ethanol (52.92%), water (38.65%) and ethyl acetate extract (26.33%). The IC_{50} values of methanol, ethanol, water and ethylacetate extract were 140.89 µg/ml, 143.55 µg/ml, 195.21 µg/ml and 411.69 µg/ml respectively. The lowest IC_{50} in the superoxide scavenging activity was exhibited by the methanol extract (261.41 µg/ml). However, the IC_{50} value of ethyl acetate extract (412.16 µg/ml) was found lower than that of ethanol (620.91 µg/m) and water extract (1174.54 µg/m). In FRAP assay, the ferric reducing activity of methanol extract reached 56.41% at 1600 µg/ml while ethanol (51.16%), water (35.01%) and ethyl acetate extract (27.21%) exhibited lower reducing activity. These results indicate that cornsilk extracts have shown strong antioxidant activities. With the highest yield of polyphenols and the strongest antioxidant capacity, the methanol extract is highly recommended to be implemented in the pharmaceutical and health related industries to treat oxidative stress related disease.**

***Index Terms*—Antioxidant activity, cornsilk, flavanoid, polyphenol**

I. Introduction

Polyphenols occurred abundantly in plants and posses of well known antioxidative properties. Polyphenol compounds are highly reactive against free radicals as hydrogen or electron donors, reducing agents and singlet oxygen quenchers due to their hydroxyl groups [1]. In living system, free radicals involved in biological processes such as phagocytosis, aging, inflammation, tissue repair and intracellular pathways. However, excessive free radicals productions from cellular metabolisms are harmful and can cause damage to biological systems [2], [3]. Flavanoid which is an important group of polyphenolic has been well documented due to its significant effects as radicals scavengers and health promoting properties [4]. The strong capacity of polyphenols in inhibiting free radicals was undeniable as many discoveries regarding the effectiveness of polyphenols in improving health, decreasing cardiovascular related problems [5] and showing strong anti-inflammatory and anticancer activity have been reported [6].

Cornsilk (*Zea mays* L.) is the byproduct of maize plant. Its soft and long stigma was found on top of female maize flowers. The light green and purplish stigma could be seen hanging like a tassle. In ancient ages, cornsilk was used in traditional medicine to treat kidney problems [7]. Presently, cornsilk is usually discarded after taking the corn for foods. This byproduct of maize actually contained various nutritional compositions including proteins, vitamins, mineral salts, carbohydrate, fibres and natural sugars [7], [8]. Cornsilk was also reported to contain phytochemicals such as alkaloids, steroids, flavanoids, anthocyanins and terpenoid class of chemicals such as α-terpineol and citronellol [9]-[14]. In biological activity investigation, cornsilk was found to reduce hyperglycemia by increasing the insulin level and recovering the beta-cells [15]. Cornsilk aqueous extract was diuretic at certain doses and exhibited promising antidepressant agent [8], [9]. However, to the best of our knowledge, the information of antioxidant activity of silks from young corns is still lacking. Thus, the study of this another plant byproduct was evaluated for the antioxidant activities by using different assays.

II. Procedure for Paper Submission

A. Plant Material

Fresh silks (10 kg) were washed with distilled water and dried in an oven (Memmert, UK) at 55˚ C. The dried cornsilk powder (CSP) was ground into powder form and stored in a screwed cap bottle at 4˚ C.

B. Chemicals and Reagents

Gallic acid, (+)-catechin, BHT, β-carotene, linoleic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitro blue tetrazolium (NBT) and X-phosphate were purchased from Sigma Co. (St. Louis, MO,USA). Folin-Ciocalteau reagent was obtained from Merck Co. (Darmstadt, German), xanthine and xanthine oxidase dismutase (XOD) from Fisher Co. (Fair Lawn, USA). All the other chemicals and solvents were of analytical grade standards.

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C. Extraction Procedure

CSP (20 g) was extracted with ethyl acetate, ethanol, methanol and water using soxhlet extraction method. The extract was then vacuumed evaporated (50˚ C) by using rotary evaporator (Heidolph, Germany).

D. Determination of Phenolics Content

The phenolics content of each extract was determined by using Folin-Ciocalteau method. One milliliter of the extract (1000 µg/ml) and 0.5 ml of Folin-Ciocalteau reagent (1:1) were added into a 10 ml volumetric flask. The solution was swirled and added with 1.5 ml of sodium bicarbonate (20% w/v) and raised with distilled water. The solution was left to stand at room temperature for 2 hours in the dark. The absorbance was recorded at 765 nm by using UV-Vis spectrophotometer (Varians, USA) against blank. The phenolics content was compared with gallic acid standard curve and expressed as (mg GAE/g crude extract).

E. Determination of Flavanoid Content

In this test, 0.25 ml of the extract (1000 µg/ml) was added into a bottle followed by addition of 75 µl of sodium nitrate (5% w/v). The mixture was reacted for 6 minutes afterwhich 150 µl of aluminum chloride (10% w/v) was added. The mixture was left to react for another 5 minutes before added with 0.5 ml of NaOH (1 M). The solution was raised to 2 ml with distilled water. The absorbance of the sample was measured at 510 nm by using UV-Vis spectrophotometer (Varians, USA). Catechin was used as a standard and the flavanoid content was expressed as mg CAE/g crude extract.

F. Antioxidant Activity Determined by β-Carotene Bleaching Method

Beta-carotene (2 mg) was dissolved in 10 ml chloroform. The solution (1 ml) was transferred into a 50 ml round bottom flask and mixed with linoleic acid (0.02 ml) and Tween 20 (0.2 ml). Chloroform was removed under vacuum at 40˚ C by using rotary evaporator (Heidolph, Germany) and the flask was added with 100 ml of distilled water. The mixture was shaken vigorously and the emulsion (2 ml) was filled in a test tube which was priorily added with 200 µl of the plant extract (500 µg/ml). For the control sample, 200 µl of methanol solvent (70% v/v) was added with 2 ml of the emulsion. BHT was used as a positive control. The samples were incubated at 50˚ C for 120 minutes. The absorbance was recorded at every 20 minutes intervals at wavelength 470 nm by using UV-VIS spectrophotometer (Varians, USA). The bleaching inhibition (%) of the beta carotene was compared to the control and was calculated as follow:

$$\text{Inhibition}(\%)=\frac{[R_{\text{control}}-R_{\text{sample}}]}{R_{\text{control}}}\times 100$$

The bleaching rates, $R=\ln\left(\frac{A_{t=0}}{A_{t=t}}\right)\times\frac{1}{t}$

G. DPPH Free Radical Scavenging Activity

The free radical scavenging activity of the extract was performed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable free radicals. Plant extract (4 ml) (50 µg/ml to 800 µg/ml) was mixed with 1 ml of DPPH solution (1mM). For the negative control, 4 ml of methanol and 1 ml of DPPH solution were used while BHT was used as the positive control. The reaction mixture was left in the dark for 30 minutes at room temperature before the absorbance was recorded at 520 nm by using UV-Vis spectrophotometer (Varians, USA). The free radical scavenging activity of the sample of each concentration was expressed by percentage of inhibition and was calculated as follow:

$$\text{Scavenging activity}(\%)=\frac{[\text{Abs}_{(\text{negative control})}-\text{Abs}_{(\text{sample})}]}{\text{Abs}_{(\text{negative control})}}\times 100$$

H. Xanthine Oxidase Dismutase Scavenging Activity

In this assay, the NBT solution was prepared by mixing Tris-HCl (3.15 g), $MgCl_2$ (0.1 g), X-phosphate (0.015 g) and NBT (0.034 g) in 100 ml distilled water. X-phosphate and NBT were priorily dissolved in 21.5 µl and 48.5 µl dimethyl formamide respectively and combined with the distilled water. In a separate bottle, the reaction mixture containing Na_2CO_3 (0.53 g), EDTA (0.004 g) and xanthine (0.05 g) were dissolved in 90 ml distilled water. NBT solution (10 ml) was then added into the reaction mixture. To determine the scavenging activity, 799 µl of reaction mixture and 200 µl of plant extract (50 µg/ml to 800 µg/ml) was filled in a cuvette. After that, 1 µl XOD enzyme was added and the absorbance was quickly measured at 560 nm. BHT was used as the positive control. In the negative control, the sample was replaced with distilled water and contained 799 µl of reaction mixture and 1 µl of XOD. The absorbance of the negative control was maintained at 0.4 at 80 seconds. The percentage of scavenging activity was calculated as:

$$\text{Scavenging activity}(\%)=\frac{[\text{Abs}_{(\text{-ve control})}-\text{Abs}_{\text{samlpe}}]}{\text{Abs}_{\text{-ve control}}}\times 100$$

I. Reducing Power

One milliliter of the extract (200 µg/ml to 1000 µg/ml) or BHT (50 µg/ml to 400 µg/ml) was filled into separate test tubes. Phosphate buffer (0.2 M, pH 6.6) (2.5 ml) and potassium ferricyanide (1% (w/v) K_3FeCN_6) (2.5 ml) were then added into the tube. The mixture was incubated at 50º C in a waterbath for 20 minutes. After that 2.5 ml of trichloroacetic acid (TCA) (10% w/v) was added and the mixture was centrifuged at 3000 rpm for 10 minutes. The upper layer (2.5 ml) of the solution was pipetted out and mixed with 2.5 ml of distilled water in another tube. Subsequently, 0.5 ml of iron (III) chloride (0.1% w/v) was added into the TCA solution and left to react for 10 minutes. The colour changes of the mixture were measured spectrophotometrically at 700 nm (Varian, USA). The antioxidant activity was expressed as percent and was calculated using formula $[1-(1-A_{\text{sample}}/A_{\text{control}})]\times 100$ [18].

J. Statistical Analysis

All experiments were conducted in triplicate and data expressed as mean $\pm$ SD. The analysis of variance (ANOVA) and Tukey's multiple comparison were considered significant at $p\leq 0.05$ (SPSS Version 18, SPSS Inc. Chicago).

III. Results and Discussion

A. Yield of Extract, Phenolic and Flavanoid Content

The yield of extracts obtained varied from 3.27% to 33.10%

and was different significantly depending on the solvent used (Table I).

TABLE I: YIELD OF EXTRACT (%), POLYPHENOL AND FLAVANOID CONTENT OF CORNSILK EXTRACTS

Cornsilk extract	Yield (%)	Polyphenol content (mg GAE/g)	Flavanoid content (mg CE/g)
Methanol	33.10 + 0.93 a	101.99 ± 8.05 a	9.26 ± 1.23 a
Ethanol	27.73 + 0.40 b	93.43 ± 2.26 a	7.55 ± 0.37 a
Water	14.63 + 0.36 c	35.34 ± 2.17 b	8.40 ± 0.48 a
Ethyl acetate	3.27 + 0.08 d	6.70 ± 0.51 c	0.66 ± 0.02 b

a-dThe superscript with different letter within column is different statistically at p≤0.05

The polyphenol content of CSP varies from 6.70 to 101.99 mg GAE/g crude and different significantly among all extracts (Table 1). The total phenolic content was in the following order: methanol>ethanol>water>ethyl acetate. On the other antioxidant compound, the flavanoid content of CSP extracts ranging from 0.66 to 9.26 mg CE/g crude extract. Polar solvents have frequently been given a priority due to its potent extracting capacity mainly of polyphenol compounds [16]. The different polarity of solvents was enabled to diffuse different constituents from plant material and consequently affects the yield of the extract [17].

B. β-Carotene Antioxidant Activity

In this assay, hydroperoxides produced by the linoleic acid will oxidize β-carotene and degrade the orange colour of β-carotene. Thus the presence of an antioxidant in the system enables to neutralize the radicals and minimize the bleaching action of β-carotene. The bleaching activity among all extracts was found different significantly (p≤0.05) showing the highest β-carotene bleaching activity of BHT (81.30%, Fig 1). The methanol extract of CSP exhibited the highest activity (66.04%) followed by the ethanol (52.92%), water (38.65%) and ethyl acetate extract (26.33%).

C. DPPH Free Radical Scavenging Activity

DPPH scavenging activity (%) had increased with the increased concentration of the extract in all samples (Fig 2). The stable DPPH· free radical accepts an electron or hydrogen radical from donors to form a stable molecule which could be seen as colour reduction. Methanol extract was the strongest electron or hydrogen donor due to the highest DPPH scavenging activity. The IC_{50} of DPPH scavenging activity of the methanol, ethanol, water and ethyl acetate extracts were 140.89 µg/ml, 143.55 µg/ml, 195.21 µg/ml and 411.69 µg/ml respectively. BHT however showed the highest value (59.08 µg/ml).

D. Xanthine Oxidase Dismutase Scavenging Activity

In this antioxidant assay, the xanthine/xanthine oxidase system generated the superoxide radicals which then reacted with 2,4-iodiphenyl-3,4-nitrophenyl-5-phenyltetrazolium chloride (NBT) to form a blue formazan. Antioxidant compounds scavenged the superoxide radicals and reduced the colour of formazan. The superoxide scavenging activity of the extracts increased parallel with the increment of concentration tested (Fig 3). On the other result, methanolic extract exhibited the strongest superoxide scavenging activity followed by ethyl acetate, ethanol and water extract. The IC_{50} value of the scavenging activity of methanol, ethyl acetate, ethanol and aqueous extracts were 261.41 µg/ml, 412.16 µg/ml, 620.91 µg/ml and 1174.54 µg/ml respectively.

E. Ferric Reducing Power Activity

The reducing power activity of cornsilk extracts was significantly lower than BHT (p<0.05, Fig 4). At the highest concentration used, the methanol extract of CSP showed the highest reducing power activity (56.41%) compared to the ethanol (51.16%) and water extract (35.01%). Meanwhile, the lowest activity was showed by the ethyl acetate extract (27.21%). Methanol extract had higher electron donating ability compared to other extracts as it showed the highest reducing activity.

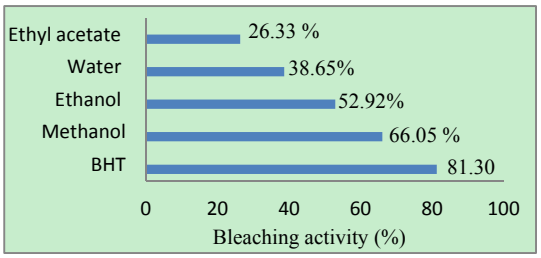

Fig. 1. The β-carotene bleaching activity of cornsilk extracts at 0.5 mg/ml after 2 hours incubation at 50˚C

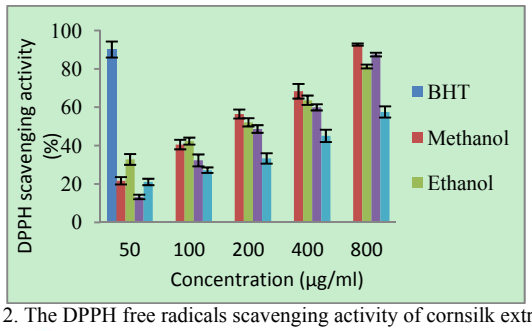

Fig. 2. The DPPH free radicals scavenging activity of cornsilk extracts

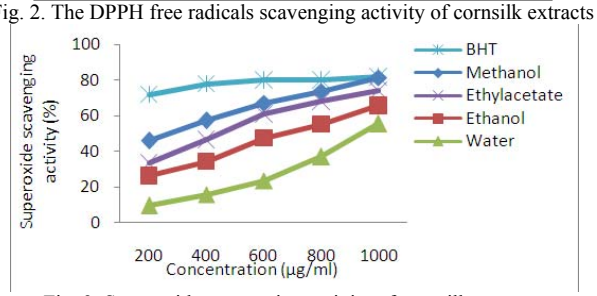

Fig. 3. Superoxide scavenging activity of cornsilk extracts

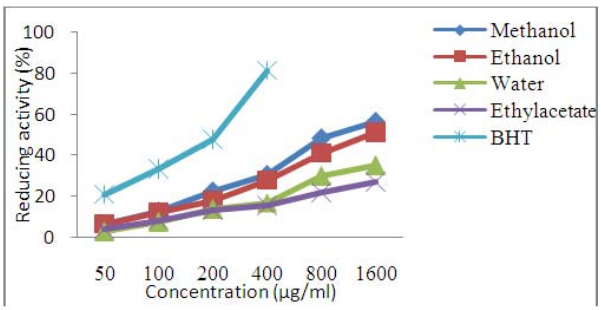

Fig. 4. Ferric reducing activity of cornsilk extracts

IV. CONCLUSION

In conclusion, cornsilk has shown good antioxidant activities with the methanolic extract showing excellent antioxidant properties in all free radical scavenging and reducing activities. The antioxidant activity of cornsilk thus could be highly attributed by the significant amount of

polyphenols and flavanoids present in this plant byproduct. Therefore, cornsilk from young corns could be considered as a potential source of antioxidant to benefit the food and health industries.

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