Antioxidative and Scavenging Properties of Polyphenolic Rich-Fraction of Cornlettes (Young Zea mays)

Wan Rosli, WI, Siti Nur Haffizah, R. Nurraihana, H.

Abstract*: *Background: Cornlettes has the potential to be used as functional pharma-nutritional ingredient in food products because of the occurrence of various bioactive compounds in it. Objective: This study was conducted to determine antioxidant and scavenging activities of phenolic rich-fractions of cornlettes extracts. Methods: DPPH radical scavenging, Ferric reducing antioxidant power and Phosphomolybdenum assay were used to determine anti-oxidative and scavenging properties of cornlettes fractions. Results and Discussion: The ethyl acetate fraction was possessed higher antioxidant activity in each antioxidant assay tested. In DPPH assay, the IC50 value of DPPH scavenging activity of the ethyl acetate, hexane, water and crude were 0.28 mg/mL, 0.26 mg/mL, 2.10 mg/mL and 1.85 mg/mL, respectively. The percentages of DPPH reduction in ethyl acetate and hexane possessed more effective anti-oxidative capacity as compared to water and crude fractions. For FRAP assay, the ethyl acetate fraction significantly exhibited the highest reducing power activity of antioxidants compared to other fractions. The IC50 value of FRAP activity of the ethyl acetate, hexane, crude and water were 0.40 mg/mL, 0.82 mg/mL, 2.24 mg/mL and 1.58 mg/mL, respectively. In addition, phosphomolybdate assay was also shown that ethyl acetate fraction had the highest total antioxidant capacity than other fractions. However, at low concentration (0.05-0.2 mg/mL), each fraction was not significant to each other. Concusion: The ethyl acetate fraction was possessed higher antioxidant and scavenging capacities followed by hexane, crude and water fractions in each antioxidant assay tested.

Index Terms*: *Antioxidant Activity, Cornlettes, Ethyl Acetate, Hexane, Phenolic Fraction.

I. INTRODUCTION

Cornlettes are one of the highly adapted crops which can be grown throughout the country. The consumption of cornlettes increases over years even though it is not as popular as sweet corn and other mature corn. Baby corn production and demands are expanding worldwide and be known to European countries, North America, Middle East and other parts of the world. Statistical information on young corn production is limited because many producing countries either neglect to make a report of its production or barely include it in sweet corn production.

Cornlettes is rarely utilized as raw food materials due to the lack of knowledge of its nutritional value and possible functional properties. Based on the report submitted to Field Fresh Food Pvt. Ltd, Thailand is one of the major exporters which are estimated to account for 80 percent of the present world's trade in cornlettes. Within Asian countries, Malaysia is the largest importing country followed by Japan, Hong Kong and Singapore. Marketing baby corn need meticulous decision for each step involves in the production including planting method, harvesting, storage and marketing technique as well as account the market value of the crop. The cornlettes suitable to be harvested manually because the mechanical corn harvesters used to strip ears of corn from their stalks are not designed to work on cornlettes. After harvesting, the baby corn ears should be placed immediately into refrigerated storage with the husks intact to conserve ear moisture and preserve quality.

The high cost of natural antioxidants has led to the use of synthetic antioxidants. However, studies conducted subsequently have demonstrated that synthetic antioxidants have toxic effects and consequently, restrictions have been imposed on their use. Cornlettes consists of antioxidants which have high phenolic substituents that enable it replaces the use of synthetic antioxidant in food products. Furthermore, the antioxidants also can be used in treatment of oxidative stress-related pathologies as a possible therapeutical strategy for the future.

Since plant substances is believed to be less toxic for medication compared to that of synthetic chemical compounds, there are many research had been done on plant extracts (Muhammad & Muhammad, 2005). Cornlettes has the potential to be used as functional pharma-nutritional ingredient in food and pharmaceutical products. This is because the fractional extract of cornlettes contained various bioactive compounds which play some potent significant roles in preventing a wide range of diseases. These bioactive compounds are beneficial to human health as they can prevent many chronic diseases such as stroke, cancer, diabetes and other illnesses (Reidah, 2013). The present study aims to investigate anti-oxidative properties of phenolic rich-fractions of cornlettes extract.

Revised Manuscript Received on December 30, 2018.

Wan Rosli, WI, Nutrition and Dietetics Program, School of Health Sciences, Universiti Sains Malaysia,

Siti Nur Haffizah R., Nutrition and Dietetics Program, School of Health Sciences, Universiti Sains Malaysia.

Nurraihana H., Nutrition and Dietetics Program, School of Health Sciences, Universiti Sains Malaysia.

II. Materials and Methods

Sample Preparation

Fresh young cornlettes was purchased from local wet markets in Kota Bharu town, Kelantan state which is located at Northern East of Peninsular Malaysia. Upon arrival in the laboratory, the cornlettes was detached from the fruit stalks and cut along its diameter vertically as ease for grinding. Then, the fresh cornlettes was dried in oven at 50°C for two days. The dried cornlettes was ground into powdered form by using a universal blender (National; MX-895) and then stored in the air tight plastic bag before proceeding with further analyses.

Extraction of Crude Cornlettes

Extraction was done according to the methods described by Chan et al. (2009) and Hismath et al. (2011) with some adaptations. Approximately 4 g of dried cornlettes sample was extracted with 40 ml of 60% methanol in 250 mL beaker. The beaker was covered with aluminium foil to prevent light exposure and the mixture was shaken at a constant rate for 30 min using a water bath shaker at 60°C. After the extraction, the cornlettes extract was cooled to room temperature and filtered through nylon filter mesh and a Whatman No. 1 filter paper into air tight Schott bottle. The filtrate was then concentrated to dryness using rotary evaporator at 50°C and lyophilized in freeze-dryer.

Preparation of Cornlettes's Fractions

5 g of crude extract was dissolved in 100 mL distilled water, (1:20) and sequentially extracted thrice using 100 mL hexane and ethyl acetate solvents respectively. The solvent extracted fractions were then evaporated using rotary evaporator to dryness to obtain residues. The residues were then reconstituted using double distilled water to obtain stock solution of the aqueous extracts having concentration of 10 mg/mL. The stock solutions were stored at -10°C in air tight container.

DPPH Radical Scavenging Assay

The antioxidant activity of phenolic fractions of cornlettes was measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical according to the method described by Brand-Williams et al., (1995) with slight modifications. Exactly, 0.2 mL of the extracts at different concentrations was added 1 mL of 0.1 mM methanol solution of DPPH. The solutions were stand at room temperature for 30 minutes in the dark area. The absorbance was measured at 517 nm using UV-vis spectrophotometer. BHT was used as the reference standard. Samples were prepared and measured in triplicates. The result was expressed in percentage of inhibition using the following equation:

$$\text{Percentage of inhibition} = (A_{control} - A_{sample}) \times 100$$

The radical scavenging activity was expressed as IC_{50}. The IC_{50} values of the phenolic fractions were compared with synthetic standard, BHT.

Phosphomolybdenum Reduction Assay

The total antioxidant capacity of the phenolic fractions of cornlettes was evaluated using the phosphomolybdenum reduction assay method according to the procedure described by Prieto et al. (1999). Exactly, 0.3 mL extract was combined with 3 mL reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 90 minutes. The absorbance of the solution was measured at 695 nm using UV-vis spectrophotometer.

Ferric Reducing Antioxidant Power Assay

The reducing power assay of phenolic fractions of cornlettes was determined according to the method established by Oyaizu (1986). Exactly, 0.5 mL of each extracts was mixed with 0.5 mL of phosphate buffer (0.2 M, pH 6.6) and 0.5 mL of 1 % potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes, and then 0.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 650 rpm for 10 min. The supernatant (0.5 mL) of the solution was mixed with 0.5 mL of distilled water and 0.1 mL of 0.1% ferric chloride. After 10 minutes, the absorbance was measured at 700 nm using UV-vis spectrophotometer. BHT was used as the standard reference. The antioxidant activity was expressed as percent and was calculated using formula [1-(1-Asample/Acontrol)] x 100 (Kaur et al. 2008).

Statistical Analysis

All the determinations were conducted at least three times (n=3). Statistical analysis was performed using IBM SPSS Statistics 22. Experimental data were reported as mean ± standard deviation (SD) which subjected to ANOVA. For multiple comparison of means, post-hoc Tukey test was applied and significant difference was determined at $p<0.05$.

III. Results and Discussion

DPPH Radical Scavenging Assay

The percentage reduction of DPPH radical is depending to the concentration of the obtained extracts. All the four phenolic rich fractions of cornlettes studied have shown an increment in scavenging activity percentage of scavenging activity for all concentrations (0.05-1.0 mg/mL) (Figure 1). Synthetic standard, BHT showed the highest percentage of scavenging activity for all the concentrations. At 0.05 mg/mL, hexane fraction showed the highest percentage of scavenging activity of (28.22±1.86%) followed by ethyl acetate (13.52±1.30%), water (8.63±1.14%) and crude (7.98±0.13).

Among all fractions, ethyl acetate fraction exhibits the most potent scavenging activity (76.92±1.52%) at 0.5 mg/mL concentration. In comparison with other fractions, hexane has exhibited the second most potent (61.21±1.77%) in percent of inhibition at 0.5 mg/mL concentration. Similar percentage of inhibition (approximate 55%) was observed at 0.35 mg/mL concentration for both hexane and ethyl acetate fractions. As shown on Figure 1, the percentages of DPPH reduction in ethyl acetate and hexane possessed more effective anti-oxidative capacity as compared to water and crude fractions.

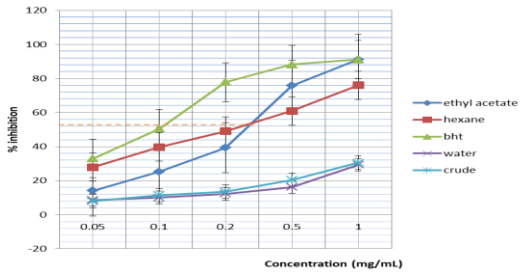

Figure 1. DPPH radical scavenging activity of cornlettes fractions

The IC_{50} value of DPPH scavenging activity of the ethyl acetate, hexane, water and crude were 0.28 mg/mL, 0.26 mg/mL, 2.10 mg/mL and 1.85 mg/mL, respectively. Based on IC_{50} values, both hexane and ethyl acetate fractions show significantly difference between each other as well as both water and crude fractions. However, scavenging activities of two pairs of fractions (ethyl acetate-hexane and water-crude) were not significantly difference to each other. In DPPH assay, the ethyl acetate fractions noticeable showed higher activity than the hexane, which in turn was more active than water and crude fractions. This is due to the high amount of polyphenolic constituents present in the fraction (Ayaz et al., 2014). The antioxidant activity increased proportionally with the polyphenol content.

Ferric Reducing Antioxidant Power Assay

In this assay, the presence of reducers (antioxidants) causes the conversion of the Fe^{3+}/ferricyanide complex to ferrous form, Fe^{2+} (Duh, Tu & Yen, 1999). The Fe^{3+} reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action and can also be strongly correlated with other antioxidant properties (Manach et al., 2004). Generally, the FRAP activity was increase parallelly with the increment concentration of fractions. BHT as standard sample showed the highest reduction of antioxidant activity for all the concentrations (0.05-1.0 mg/mL). Each fraction showed significantly increment of reducing activity along with the increment of fraction concentrations (Figure 2).

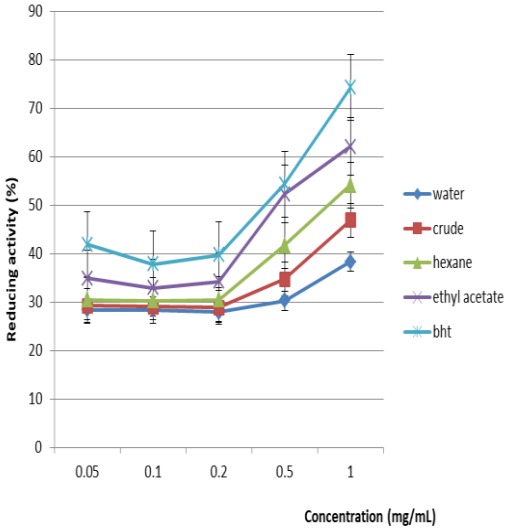

Figure 2. FRAP assay of phenolic fractions of cornlettes extract

Among all fractions, ethyl acetate fraction exhibits the most potent reducing activity for all concentrations as compared to other fractions. At the highest concentration used, the ethyl acetate fraction showed the highest reducing power activity (54.35±0.63%) compared to the hexane (39.69±3.58%) and crude fraction (37.84±5.82%). Meanwhile, the lowest activity was showed by the water fraction (41.82±4.97%). The ethyl acetate fraction had higher electron donating ability compared to other fractions as it showed the highest reducing activity (Nurhanan & Wan Rosli, 2012). The IC_{50} value of FRAP activity of the ethyl acetate, hexane, crude and water were 0.40 mg/mL, 0.82 mg/mL, 2.24 mg/mL and 1.58 mg/mL, respectively. BHT showed the highest IC50 value (0.18 mg/mL). Lower IC50 value indicates a higher antioxidant activity. Based on IC_{50} values, both crude and water fraction show significant difference to each other and hexane fraction show low significant IC_{50} value to ethyl acetate fraction. Many studies demonstrated that the plant extract possess a strong reducing capacity. The compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, thus they can act as primary and secondary antioxidants (Ravisankar et al., 2014).

Phosphomolybdenum reduction assay

The total antioxidant capacity of cornlettes extracts was also determined by using phosphomolybdenum assay. The assay is based on the fact that molybdenum (VI) is reduced to molybdenum (V) in the presence of a reducing agent (antioxidant), forming a green phosphomolybdate (V) complex, which can be evaluated spectrophotometrically at 695 nm (Prieto et al., 1999). Increase in absorbance of the reaction mixture indicated the increase in total antioxidant capacity of the extract was shown in Figure 3.

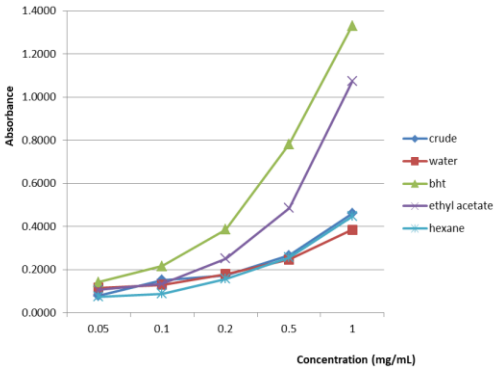

Figure 3. Phosphomolybdenum reduction assay of cornlettes fractions

At 0.05 mg/mL, water fraction showed the highest antioxidant activity (0.1164) followed by ethyl acetate (0.1060), crude (0.0794) and hexane fractions (0.0750). Beginning from 0.2 mg/mL, ethyl acetate fraction exhibited the highest antioxidant activity (0.2518) and greater mean difference from other fractions.

Similar trend of increase in total antioxidant capacity was observed at concentration 0.5 mg/mL and 1.0 mg/mL for crude, hexane and water fraction respectively. Generally, ethyl acetate fraction exhibits the highest total antioxidant capacity especially at concentrations of 0.2, 0.5 and 1.0 mg/ml in the phosphomolybdenum assay as compared to other fractions (Figure 3). At concentration 0.05 mg/mL to 0.2 mg/mL, each fraction was not significant to each other ($p>0.05$). Crude, hexane and water fraction exhibited low significant to each other at concentration 0.5 mg/mL and 1.0 mg/mL.

IV. CONCLUSION

In DPPH essay, ethyl acetate fractions showed a gradually higher activity than the hexane, water and crude fractions. The percentages of DPPH reduction in ethyl acetate and hexane fractions possessed more effective anti-oxidative capacity as compared to water and crude fractions. In FRAP assay, ethyl acetate fraction exhibited the highest reduction of antioxidant activity for all concentrations than other fractions. The IC_{50} values of FRAP activity of the ethyl acetate, hexane, crude and water were 0.40 mg/mL, 0.82 mg/mL, 2.24 mg/mL and 1.58 mg/mL, respectively. In Phosphomolybdenum reduction assay, ethyl acetate fraction also recorded the highest total antioxidant capacity than other fractions. In conclusion, the ethyl acetate fraction was possessed higher antioxidant activity followed by hexane, crude and water fractions in each antioxidant assay tested. Further analyses are planned to investigate the efficacy of ethyl acetate fraction towards nutritive values and pharmacological properties in both *in vitro* and *in vivo* models.

V. ACKNOWLEDGEMENTS

We would like to thank Ministry of Higer Education (MOHE) of Malaysia for FRGS funding (203.PPSK.6171190) which facillitae the smooth running of this study. The authors also acknowledge the Forensic Laboratories of The School of Health Sciences, USM Health Campus in allowing the used of equipments and anonymous reviewers who gave constructive recommendations that enhanced the value of this manuscript.

REFERENCES

1. Ayaz, M., Junaid, M., Ahmed, J., Ullah, F., Sadiq, A., Ahmad, S. & Imran, M. (2014). Phenolic contents, antioxidant and anticholinesterase potentials of crude extract, subsequent fractions and crude saponins from Polygonum hydropiper L. *BMC Complementary and Alternative Medicine*. **14(1)**: 145-148.
2. Brand-Williams, W., Cuvelier, M.E. & Berset, C. (1995) Use of a free radical method to evaluate antioxidant activity. *Food Sci Technol*. **28**: 25–30.
3. Chan, S.W., C.Y. Lee, C.F. Yap, W.M. Wan Aida, C.W. & Ho, C. (2009). Optimisation of extraction conditions for phenolic compounds from limau purut (*Citrus hystrix*) peels. *International Food Research Journal.* **16(2)**: 203-213.
4. Deciga-Campos, M., Rivero-Cruz, I., Arriaga-Alba, M., Castaneda-Corral, G., Angeles-Lopez, G.E., Navarrete, A. & Mata, R. (2007). Acute toxicity and mutagenic activity of Mexican plants used in traditional. *Journal of Ethnopharmacology*, **110**: 334-342
5. Duh, P.D., Tu, Y.Y. & Yen, G.C. (1999). Antioxidant activity of the aqueous extract of harn jyur (*Chrysanthemum morifolium Ramat*). *Lebensm. Wiss Technol*. **32**: 269-277
6. Hismath, I., Wan Aida, W.M. & Ho, C.W. (2011). Optimization of extraction conditions for phenolic compounds from neem (*Azadirachta indica*) leaves. *International Food Research Journal.* **18(3)**: 931-939
7. Kaur, R., Arora, S. & Singh, B. (2008). Antioxidant activity of the phenol rich fractions of leaves of Chukrasia tabularis A. *Juss. Bioresour. Technol*. **99(16)**: 7692-7698.
8. Manach, C., Scalbert, A., Morand, C., Remes, C. & Jimenez, L. (2004). Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition*. **79**: 727-747
9. Muhammad, H.S. and Muhammad, S. (2005). The use of Lawsonia inermis linn. (henna) in the management of burn wound infections. *African Journal of Biotechnology*. **4(9)**: 934-937.
10. Nurhanan, A.R., Wan Rosli, W.I & Mohsin S.S.J (2012). Total polyphenol content and free radical scavenging activity of cornsilk (Zea mays hairs).Sains Malaysiana 41(10): 1217–1221
11. Oyaizu M. (1986). Studies on products of browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. Jpn J Nutr. 44: 307–315.
12. Prieto, P., Pineda, M. & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal Biochem*. **269(2)**: 337-41
13. Ravisankar, N., Sivaraj, C., Seeni, S., Joseph, J. & Raaman, N. (2014). Antioxidant activites and phytochemical analysis of methanol extract of leaves of *Hypericum hookerianum*. *Int J Pharm Pharm Sci*. **6(4)**: 456-460
14. Reidah, A.I.M.M. (2013). Characterization of phenolic compounds in highly-consumed vegetable matrices by using advanced analytical techniques.(PhD thesis, University of Granada).