Thai J. Pharm. Sci. Vol. 38 (Suppl.) 2013

EFFECT OF ETHANOL CONCENTRATION ON CYANIDIN-3-GLUCOSIDE, TOTAL MONOMERIC ANTHOCYANINS, TOTAL PHENOLIC CONTENT AND RADICAL SCAVENGING PROPERTIES IN PURPLE CORN (*Zea mays L.*) SEED AND COB.

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KEYWORDS: Purple corn, Anthocyanins, Solvent extraction

INTRODUCTION

Purple corn (*Zea mays* L.) has been extensively grown in South America, mainly in Peru and Bolivia, and used to prepare drinks and desserts for centuries due to its high pigment content. Purple corn seed and cob contains high concentration of anthocyanins which are an important group of flavonoid compounds. Anthocyanins in purple corn have been used as a source of colors and phytonutrients over the last years. Many health benefits have been associated with purple corn, including reduction of oxidation stress, prevention of coronary heart disease, obesity and diabetics, and anticancer activity. Due to its health benefits, we will study the preparation process of anthocyanin-rich extract as the raw material for medical and health supplement purpose.

The aim of this study is to investigate the effect of ethanol concentration on the cyanindin-3-glucoside content (C3G), total anthocyanins content (TAC), total phenolic contents (TPC) and free radical scavenging (DPPH (1,1-diphenyl-2-picrylhydrazyl) method) of purple corn (*Zea mays L.*) seed and cob. The ethanol concentration has been used as 90%, 70%, 50%, 30% and 0% v/v ethanol.

MATERIALS AND METHODS

Plant material Purple corn seed (PCS) and cob (PCC) were obtained from Nakhonsawan Province, Thailand. The seeds and cob were separated,and then washed, dried and ground into coarse particles.

Anthocyanin-rich extract preparation The powder of purple corn seed and cob (25,10 g.) were extracted by 75 and 70 ml of aqueous ethanol by using sonicator for 10 min. The extraction were studied at the concentration of 90%, 70%, 50%, 30%, 0%, v/v ethanol. The supernatants were filtered through a Whatman No.1. and concentrated by using a rotary evaporator at 40 ºC under vacuum condition.

Preparation of the sample The anthocyanin extracts were dissolved in 0.01%-HCl-acidified-water and partitioned with chloroform in separatory funnel. The upper aqueous layer, containing the anthocyanins, was collected while the lower which is the chloroform layer was carefully discarded. Residual chloroform was removed from the anthocyanin extract by using a rotary evaporator at 40 ºC under vacuum condition. Extracts were taken to 50 ml ~~in~~ volumetric flask adjust with 0.01%-HCl-acidified-water and kept at −80 ºC until analysis.

Determination of cyanindin-3-glucoside content in purple corn seed and cob extracts by HPLC method Cyanindin-3-glucoside content was determinate on Waters Alliance System e2695 LC system equipped with Waters model 2998 a photodiode-array detector and an Empower software (Waters Corporation, USA). HPLC separation was conducted using a reversed phase 5 μm Symmetry C18 column (3.9 × 150 mm, Waters Corp.) coupled with a guard column of the same stationary phase (Waters Corp.). The mobile phase used were A, 1% phosphoric acid/10% acetic acid/5% acetonitrile in water, and B, 100% acetonitrile. Anthocyanins were separated by using a linear gradient from 0% to 80% A in 25 min. An injection volume of 20 μLwith a 1mL/min of flow rate was used. Spectral information at the wavelength of 520 nm was collected. Measurements were carried out in triplicate with the calculations being based on a standard curve and data were presented as mg /100g dry weight. This method was modified from the procedure described by Jing and Giusti (2009).

Determination of polyphenol and total monomeric anthocyanins content and antioxidant activity Total anthocyanins content (TAC) was evaluated by pH-differential method (Giusti and Wrolstad, 2001). Data were presented as mg/100g dry weight.

Total phenolics content (TPC) was determined using Folin–Ciocalteu reagent (Singleton and Rossi,1965). Measurements were carried out with the calculations being based on a standard curve based upon gallic acid. Results were expressed as mg gallic acid equivalents (GAE)/100g dry weight.

The free radical scavenging activity of the extracts was perform by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay according to the procedure described by Brand-Williams et al. The results are expressed as milligrams of Trolox equivalents (TE) per 100 g of dry sample (mgTE/100 g dry weight).

Statistical analysis The results are expressed as means ± standard error of mean (S.E.M.). Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA) followed by a *post-hoc* Duncan's Multiple Range test for multiple comparisons. Statistical significance was assessed as $p<0.05$.

RESULTS AND DISCUSSION

Effect of ethanol concentration on cyanindin-3-glucoside content in purple corn (Zea mays L.) seed and cob. Cyanidin-3-glucoside content (C3G) in purple corn (*Zea mays L.*) seed and cob were quantified using HPLC method. The optimum HPLC system was comprised of a C18 reverse phase column (Xterra column, 5µm, 3.9 x 150 mm), gradient elution with 1% phosphoric acid/10% acetic acid/5% acetonitrile in water (A) and acetonitrile (B) as mobile phase and UV detection at 520 nm. C3G content were determined by comparison to caribration curve (Figure 1) which derived from separated injections of five concentrations of C3G versus the peak area. Linearity was found in the concentration range between 10-50 ppm with correlation coefficient (r^2) of 0.9997. The C3G retention time of reference standard and samples was the same at 4.88 minute (Figure 2).

The effect of ethanol concentration on C3G was investigated over the range of 0-90% v/v ethanol. The content of the C3G in PCS and PCC extracts increased with ethanol concentration up to a maximum at 70% v/v and then decreased at 90% v/v (Figure 3). The optimal ethanol concentration for PCS and PCC extracts were 70% v/v ethanol ($p<0.05$) that showed the highest C3G content (2.62 ± 0.00 and 28.38 ± 0.50 mg/ 100 g dry weight). The result of this study revealed that C3G content in PCC extract was more than PCS extract.

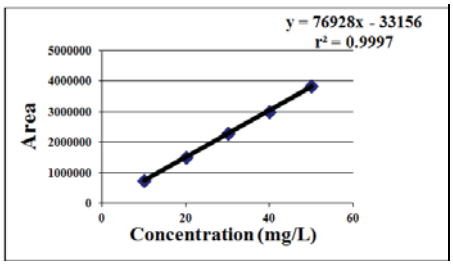

Figure 1 Calibration curve of cyaniding-3-glucoside (y=76928x-33156, r^2= 0.9997)

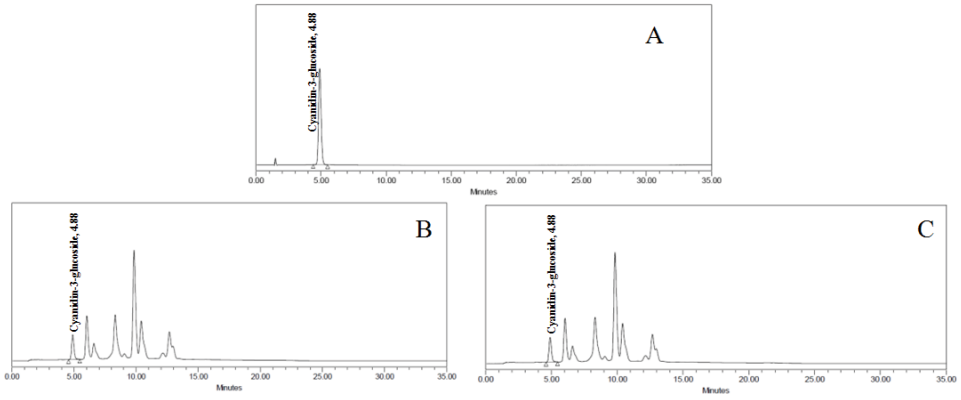

Figure 2 HPLC Chromatograms analysis of the anthocyanins (A) Referense standard of cyanidin-3-glucoside (B) PCS extracted with 70% ethanol (C) PCC extracted with 70% ethanol Column: Xterra C_{18} 3.9 x 150 mm Mobile phase: 1% phosphoric acid/10% acetic acid/5% acetonitrile in water (A) and acetonitrile (B), (gradient elution)

Effect of ethanol concentration on anthocyanins content in purple corn (Zea mays L.) seed and cob.
The total anthocyanin content in PCC and PCS extract could be determined using visible spectrophotometry. Figure 4 showed anthocyanin contents in 5 ethanol concentrations varied from 0.95 ± 0.00-491.22±5.73 mg/100g dry weight. The TAC of 70% v/v ethanol extracts from PCS and PCC showed the highest as 79.65 ± 0.26 and 491.22 ± 5.73 mg/ 100 g dry weight. The increase of the ethanol concentration in the extraction solution up to 70% resulted to increase TAC. The TAC of extracts significantly decrease at 90% v/v ethanol. In previous research in *Benitaka cultivar grapes* study, it has similarly results which showed solvents containing 70% ethanol in water leads to extract the higher content of total anthocyanins when comparing with 60% and 80% v/v of ethanol (Vanini, L.S. et al., 2009)

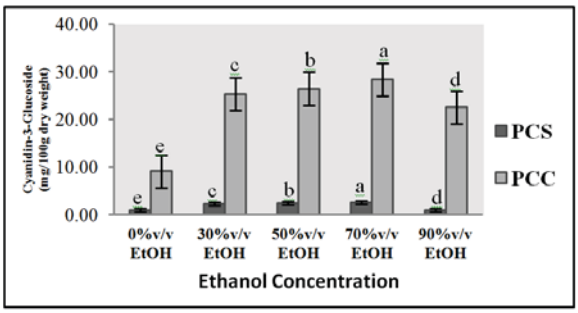

Figure 3 Cyanidin-3-glucoside content of PCC and PCS extract. Data are presented as mean ±SEM. [a-e] Point with different letters in each figure means significant difference ($p \leq 0.05$).

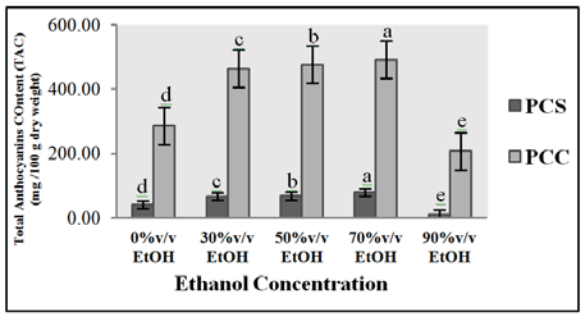

Figure 4 Total anthocyanins content (TAC) (mg/100 g dry weight) of PCC and PCS extract. Data are presented as mean±SEM. [a-e] Point with different letters in each figure means significant difference ($p \leq 0.05$).

Effect of ethanol concentration on total phenolics content in purple corn (Zea mays L.) Seed And Cob.
Measurement of the TPC in PCS and PCC extracts showed that the increase of ethanol in the extraction solution (from 0% to 70%) resulted to increase TPC of the extracts but TPC of the extracts decrease at 90% of ethanol (Figure 5). The TPC of PCS and PCC at 70% ethanol PCS was 345.07 ± 5.07 mg GAE/100g dry weight , 1227.02 ± 5.89 mg GAE/100g dry weight ,respectively.
From the literature review, the red grape pomace study show that the solvents containing 70% and 50% ethanol in water leads to extracting of higher content of total phenolics content, as compared to 10% and 30% ethanol, and to methanolic solutions (Monrad et, 2010).
Effect of ethanol concentration on radical scavenging properties in purple corn (Zea mays L.) seed and cob. The DPPH radical assay has been widely used to evaluate the antioxidant activity of fruit and vegetable extract. The method is based on the the reaction that hydrogen-donating antioxidants reduce violet DPPH free radical to yellow DPHH-H, a non-radical form (Kumaran and karunakaran, 2005) The reduce amount of DPPH absorption at 517 nm indicated the radical-scavenging ability of antioxidants.
Figure 6 displays DPPH radical-scavenging activity of the anthocyanins from of PCS and PCC extract. The results show that the radical-scavenging activities of antioxidants increased with the increment of ethanol concentrations (from 0% to 70%v/v ethanol) but decrease at 90% of ethanol. The free radical scavenging activities of PCS and PCC at 70%v/v ethanol PCS were 567.62 ± 1.38 and 2539.02 ± 23.29 mg TE/100 g dry weight, respectively.

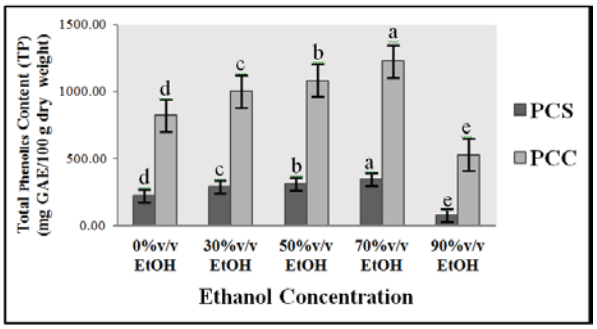

Figure 5 Total phenolics content (TPC) (mg gallic acid equivalents (GAE)/100g dry weight.) of PCS and PCC extract. Data are presented as mean±SEM. [a-e] Point with different letters in each figure means significant difference ($p \leq 0.05$).

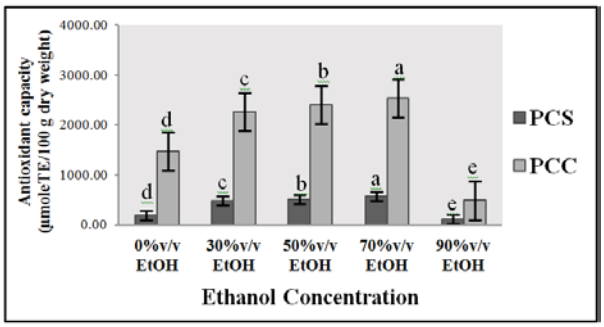

Figure 6 The free radical scavenging activity (milligrams of Trolox equivalents (TE) per 100 g of dry sample (mgTE/100 g dry weight) of PCC and PCS extract was extracted Data are presented as mean ±SEM. a-e Point with different letters in each figure means significant difference ($p \leq 0.05$).

CONCLUSION

From this study, 70% ethanol extraction was found to be the most effective concentration on cyaniding-3-glucoside, anthocyanins, total phenolics content and antioxidant activity.

ACKNOWLEDGMENTS

This study was supported by Thailand Instituted of Scientific and Technological Research (TISTR), Pathumthani 12120. We thank all TISTR colleagues who in one way or another help this project to succeed.

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