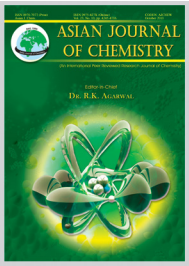
Asian Journal of Chemistry; Vol. 24, No. 6 (2012), 2829-2830

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



NOTE

Determination of Antioxidant Activity and Total Anthocyanin Contents of Extracts from Pulp and Peel of *Malus domestica*

HAJI MUHAMMAD SHOAIB KHAN* and NAVEED AKHTAR

Department of Pharmacy, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur, Bahawalpur, Pakistan

*Corresponding author: Fax: +92 62 9255243; Tel: +92 33 46483010; E-mail: shoaib.khan@iub.edu.pk

(*Received*: 6 August 2011; *Accepted*: 22 January 2012) AJC-11013

Concentration of polyphenolic compounds, such as anthocyanins and the antioxidants in apples (*Malus domestica* kala kolo) seems to be different with environmental conditions and part of the fruit. Main objective of this study was to quantify the anthocyanin contents and to determine antioxidant activity in peel as well as pulp of apple (*Malus domestica* kala kolo) which is the best variety found in Pakistan. In this work, antioxidant activity and total anthocyanin contents of apple (*Malus domestica* kala kolo) pulp and peel extracts were investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as stable free radical while anthocyanin contents were measured by pH differential method. The result showed that peel has higher scavenging ability on DPPH. At the concentration of 0.10 mg/mL. The DPPH scavenging rates of peel were about 92 % of vitamin C while pulp were about 42 % of it. The results demonstrated different anthocyanin contents in peel and pulp of fruit. The total anthocyanin contents were 95 % and 0.003 % of peel and pulp respectively in comparison to the standard. Anthocyanins have significant contribution to the total antioxidant activity, which varies considerably depending on the part of fruit.

Key Words: Anthocyanin, Antioxidant activity, Apple.

Antioxidant compounds have been investigated in plant materials commonly in vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs and crude plant drugs[1]. Antioxidants compounds have a great deal with oxidative stress responsible for free radical damage[2]. These compounds are reflection to be involved in skirmishing oxidative stress and therefore, can be helpful in treating and preventing atherosclerosis, cardiovascular, neurodegenerative diseases and cancer like oxidation related diseases[3]. Scientific reports have confirmed that fruits and vegetables have a capability to provide protection against chronic diseases related oxidative stress. Anthocyanins are most important constituents of fruits and vegetables[4]. Anthocyanins are group of antioxidants distributed in leaves, fruits and flowers and responsible for pigmentation[5]. Delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin are the most abundant anthocyanins in plants[6].

Another significant property of anthocyanins is their antioxidant activity, which plays a vital role in the prevention of neuronal and cardiovascular illnesses, cancer and diabetes, among others. There are several reports focused on the effect of anthocyanins in cancer treatments human nutrition and its biological activity.

However, it is reported that apples has different classes of bioactive compounds, which exhibits antioxidant activity due to phenolic compounds. Major groups of phenolic compounds are hydroxycinnamic acids, flavan-3-ol, flavonol, dihydrochalcones and anthocyanins[7]. The amount of anthocyanin in fruits is considerable important[8]. The present study was undertaken for determination of antioxidant activity of anthocyanins and total anythocyanins content found in extracts of *Malus domestica* kala kolo (pulp and peel).

All solvents for extraction and separation were of analytical grade, purchased from merck, Germany. Potassium chloride and sodium acetate trihydrate were obtained from Vetec (Rio de Janeiro, Brazil), 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (Germany). Fresh fruits (*Malus domestica* kala kolo) with similar colour (dark red) were picked for extraction of anthocyanins from local market.

Extraction of apple anthocyanin: Anthocyanins were extracted from crushed fresh pulp tissues and air dried powdered peel (20 g) of apple (*Domestica malus* kala kolo), according to the modified method of Zheng *et al.*[9]. Extracting was performed twice at 25 ºC for 0.5 h in a homogenizer (Euro-Star, IKA D 230, Germany) twice. 100 mL of methanol/acetone/water (3.5:3.5:3, v/v/v) containing 1 % formic acid was

used as extracting solvent. All extracts were combined and filtered. The collected filtrates were kept for overnight. The collected filtrate was centrifuge for 15 min at 6000 g. The supernatant was collected and evaporated under vacuum at 35-40 ºC to remove methanol and acetone. Lipophilic pigments were then eliminated from the aqueous phase by two successive extractions in a separating funnel with a twofold volume of petroleum ether. The aqueous phase was collected and further extracted three times by ethyl acetate (ethyl acetate: aqueous phase = 1:1, v/v) in the separatory funnel. Three ethyl acetate phases were collected, evaporated and dried under vacuum at 35 ºC. All samples were stored at -4 ºC prior to further investigations.

Evaluation of antioxidant activity: The DPPH (1,1-diphenyl-2-picrylhydrazyl) stable free radical was used for the determination of free radical scavenging activity of extracts. A solution of DPPH was mixed in 10 µL of plant extract for final volume upto 100 µL in 96 well plates. Mixed contents were incubated at 37 ºC for 0.5 h. Optical density was analyzed at 517 nm using ascorbic acid as standard. Due to potent antioxidant activity of ascorbic acid, it is referred as standard to investigate the antioxidant activity of extracts. Experiment was done in triplicates. Results were taken as mean and standard error of mean of three independent experiments.

$$\% \text{ DPPH scavenging activity} = [100-(\text{OD of test sample}/\text{OD of controlled}) \times 100]$$

Total anthocyanin content: Monomeric anthocyanin content of the apple peels and pulp was quantified by using a spectophotometric pH differential protocol[10]. The anthocyanin content of the pulp and peel were analyzed. The apple pulp and peel extracts were mixed thoroughly with 0.025 M potassium chloride pH 1 buffer in 1:10 ratio of extract to buffer. The absorbance of the mixture was then measured at 510 and 700 nm using Elisa plate reader (BioteK Synergy HT, USA). The apple pulp and peel extracts were then combined similarly with sodium acetate buffer pH 4.5 and the absorbance of these solutions was measured at the same wavelengths. The anthocyanin content was calculated as follows:

$$\text{Total monomeric anthocyanins (mg/100 g of fresh peel)} = A \times \text{m.w.} \times 1000/ (e \times C)$$

where, A is absorbance = (A515-A700) pH 1.0-(A515-A700) pH 4.5; m.w. is molecular weight for cyanidin 3-glucoside = 449.2; is the molar absorptivity of cyanidin 3-glucoside = 26900; and C is the concentration of the buffer in mg/mL.

In the present study, total anthocyanin content of extracts (by methanol/acetone/water/formic acid) from apple Peel was superior to that from pulp of fruit. The total anthocyanin contents of peel extracts determined by pH differential method were 95 % while the pulp extract contained limited amount of anthocyanin contents *i.e.* 0.003 %. It was demonstrated from the results that pulp and peel extract showed significant antioxidant activity 42 and 92 %, respectively resulted by DPPH method.

The total anthocyanin content and antioxidant activity vary considerably depending on the part of the fruit (*Malus domestica* var kala kolo) analyzed.

Apple peel possesses high contents of anthocyanins compared to fresh pulp. It has also been reported from previous studies that apple pulp has no anthocyanin[11]. Present results show resemblance with this fact and it proves that apple (*Malus demestica* kala kolo) has negligible anthocyanins in pulp when extracted. The nature and distribution of these anthocyanins between the pulp and the peel of the apple is also different. However, pulp contributes catechins, procyanidins, phloridzin, phloretin glycosides, caffeic acid and chlorogenic acid. The peel possesses all of these compounds and has additional phenolics not found in the pulp, such as anthocyanins and quercetin glycosides[11].

In addition there was a significant positive correlation between total anthocyanin content and antioxidant activity in pulp and peel, suggesting that peel removal may induce a more significant anthocyanin loses which has a strong contributing in preventing of chronic disease.

Conclusion

There was a significant positive correlation between anthocyanins and antioxidant activity. In the production of processed apples product, apple peals are often discarded, but clearly they posses high level of antioxidants which can be used for various s purpose in food, pharmaceutical and cosmetic industry. The regular consumption of peel must be recommended to maximize the dietary intake of compounds that may have health benefits for consumer such as reduced risk of cancer and cardiovascular disease.

ACKNOWLEDGEMENTS

The authors thank the Chairman, Department of Pharmacy and The Islamia University of Bahawalpur for financial support.

REFERENCES

1. M.P. Kähkönen, A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja and T.S. Kujala, *J. Agric. Food Chem.*, **47**, 10 (1999).
2. S. Ali, N. Kasoju, A. Luthra, A. Singh, H. Sharanabasava and A. Sahu, *Food Res. Int.,* **4**, 1 (2008).
3. J. Sun, J. Yao, S. Huang, X. Long, J. Wang and E. García-García, *Food Chem.*, **117**, 2 (2009).
4. L.S. Wang and G.D. Stoner, *Cancer Lett.*, **269**, 2 (2008).
5. Q. Du, J. Zheng and Y. Xu, *J. Food Compos. Anal.*, **21**, 390 (2008).
6. S. Pascual-Teresa and M.T. Sanchez-Ballesta, *Phytochem. Rev.*, **7**, 2 (2007).
7. B. Lata, A. Trampczynska and J. Paczesna, *Sci. Horticult.*, **121**, 2 (2009).
8. A.E. Solovchenko, O.B. Chivkunova, M.N. Merzlyak and I.V. Reshetnikova, *Russ. J. Plant Physiol.*, **48**, 801 (2001).
9. Y.H. Zheng, C.Y. Wang, S.Y. Wang and W. Zheng, *J. Agric. Food Chem.*, **51** (2003).
10. M.M. Giusti and R.E. Wrolstad, Anthocyanins: Characterization and Measurement with UV-Visible Spectroscopy, New York: John Wiley & Sons, pp. 1-13 (2001).
11. F.G.K. Vieira, G.S.C. Campelo, C. Copetti, L.V. Gonzaga and E.C. Nunes, *Arch. Latinoam. Nutr.*, **59**, 101 (2009).