JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2022/27658

SHORT COMMUNICATION

Evaluation and Comparative Study of Anti-diabetic Activity of Anthocyanins Derived from Various Natural Sources

L. Veerapandi*, T. Nivetha, N. Karunyah Amirthadharshini and R. Sinthiya

Bannari Amman Institute of Technology, Erode – 638401, Tamil Nadu, India; veerapandiloganathan@gmail.com

Abstract

Diabetes is a common disorder due to metabolism, more or less people of cultural groups in the region globally. This disease is caused due to the lifelong distress and has no cure as of now. Naturopathy / Plant based chemical antibiotics are proven safe than the synthetic chemicals that are artificially synthesized. In this study, the total anthocyanin content and their antimicrobial activity was estimated for the samples. The study revealed the anti-diabetic properties of anthocyanin from several sources such as strawberries (*Fragaria ananassa*), cherries (*Prunus avium*), pomegranate (*Punica granatum),* grapes (*Vitis vinifera),* flame of forest flowers (*Canna indica*), hibiscus flowers (*Hibiscus rosa-sinensis*) and guava leaves (*Psidium guajava*). Extraction has been done by using ethanol as a base and inhibition studies have been conducted. The source '*Canna indica'* showed huge amount of anthocyanin content of 814.6 mg/100g and other sources showed varied amount of anthocyanin content. *Prunus avium* fruits showed maximum antidiabetic activity. The total phenolic and anthocyanin contents with its antidiabetic activity for different sources using acidified ethanol were studied.

Keywords: Anthocyanin, Antimicrobial Activity, Cherries, Grapes, Hibiscus Flowers, Naturopathy, Pomegranate, Strawberries

1. Introduction

Anthocyanins, a group of compounds, are one of the members of flavanoid family of polyphenol phytochemicals which are abundantly present in plant foods[1–3]. In the past decade anthocyanins have been found as health promoting ingredients in many fruits and vegetables[4]. They are water soluble pigments found in the epidermal tissues of fruits and flowers[5]. They are responsible for providing bright red - orange to blue - violet colours of many fruits and vegetables[6,7]. Plants produce anthocyanins as a defensive mechanism against environmental stress causing agents such as ultraviolet light, drought and cold temperatures[8]. Production of these anthocyanins in roots, stem and leaf tissues make them resistant to these environmental hazards[9–11].

Anthocyanin has a positive charge on the molecule which enables them to absorb light and thus produce colour. The sugar molecule (usually glucose) is esterified at the 3 position. The presence of sugar helps anthocyanin to maintain solubility in water[12]. An anthocyanidin, also termed as aglycone, does not have sugar molecule at the 3 position. All naturally occurring anthocyanins possess equilibrium between the coloured flavylium cation and colourless hydrated form[13].

1.1 Biosynthesis

Anthocyanins are synthesized via the general flavanoid pathway. These molecules are derived from three molecules of malonyl CoA from the fatty acid metabolism and one of p-coumaroyl CoA synthesized from phenyl alanine[14].

In the first step, malonyl CoA and p-coumaroyl CoA are condensed by *chalcone synthase* to produce

*Author for correspondence

Article Received on: 20.04.2021 Revised on: 30.09.2021 Accepted on: 15.11.2021

a naringenin chalcone. The B- ring naringenin flavanone is usually hydroxylated using the enzyme flavanoid 3'- hydroxylase to produce eriodictyol or the enzyme flavanoid 3'5'- hydroxylase to produce penta hydroxyl flavanone. All flavone forms are modified into dihydroflavones by the activity of flavone hydroxylase[15,16]. Dihydroflavones are further modified into leucoanthycyanidins which is then transformed to anthocyanidins in a reaction catalyzed by anthocyanidin synthase which involves oxidation and dehydration. Anthocyanidins are glycolsylated by the action of glycosyl transferase that adds glucose to the 3rd position of the C- ring of flavylium cation. Following glycosylation, anthocyanidins are modified by the addition of methyl, acyl or glycosyl groups[17,18].

1.2 Source of Anthocyanins

Distribution of Anthocyanins in nature reveal that they are a characteristic feature of angiosperms or flowering plants and are more prominent in fruits and berries than other plant parts[19]. The major food sources that contain anthocyanins belong to the families Vitaceae and Roasceae. The amount of anthocyanins produced in plant foods greatly vary because they are produced by plants in response to drought, environmental stress and ultraviolet radiation[20,21]. Other factors include climatic condition, geographical location, stage of maturity and soil characteristics[22]. For this study anthocynain from several sources such as strawberries (*Fragaria ananassa*), cherries (*Prunus avium*), pomegranate (*Punica granatum*), grapes (*Vitis vinifera*), flame of forest flowers (*Canna indica*), hibiscus flowers (*Hibiscus rosa-sinensis*) and guava leaves (*Psidium guajava*)[23–32].

2. Materials and Methods

2.1 Chemicals and Solvents

Analytical grade chemicals were used in all experiments for the extraction, isolation of anthocyanins and media preparation for growth of bacterial cells. Pomegranate, strawberry, guava, grapes, hibiscus petals, flame of forest flowers and cherry were obtained from the local market. The extracts of the above were prepared using double distilled water and acidified ethanol.

2.2 Glassware and Apparatus

All glasswares used for the study were purchased from Borosil, India. All glasswares were immersed in 10% potassium dichromate in 25% sulphuric acid cleaning solution for an hour and washed thoroughly with tap water. Then, they were washed with diluted commercial detergent and rinsed with tap water and then with distilled water.

2.3 Sterilization

Dried glass wares were sterilized in hot air oven for 30 min at 15 lb/sq inch pressure.

2.4 Extraction of Anthocyanins from Fruit, Flower and Leaves Extracts

The respective fruits were taken and washed thoroughly with distilled water. 10g fruit was macerated with 50 ml of acidified ethanol (70% v/v acidified with 0.1 % v/v Hcl) and 50 ml of distilled water. It was stirred in dark for 4 hours. It was stored at 5^0 C for 24 hours. Samples were filtered using Whatmann 41 filter paper to get the clear extract. The extract was used immediately after the preparation (Figure 1)[22,33,34].

2.5 Antimicrobial Activity of Various Plant Sources

2.5.1 Preparation of Di-nitro Salicylic Acid Reagent

Solution 1: 37.5 g of sodium potassium tartarate was dissolved in 100 ml of distilled water.

Solution 2:125 mg of Dinitrosalicylate was dissolved in50 ml of 2N NaOH solution.

The two solutions were mixed in the ratio 5:2 and the resulting mixture was made up to 200 ml with distilled water.

2.5.2 Preparation of 1M Phosphate Buffer

Stock Solution A: 12 g of monobasic sodium/potassium phosphate was dissolved in 100 ml of distilled water.

Stock Solution B: 12 g of dibasicsodium / potassium phosphate was dissolved in 100 ml of distilled water.

According to the pH chart, 51 ml of stock solution A and 49 ml of stock solution B were mixed together and

diluted with 100 ml of distilled water to bring the pH to 6.8.

2.5.3 Preparation of Starch

1 gm of soluble starch was added to 100 ml of phosphate buffer and heated till a translucent solution was obtained to make the desired concentration of (10mg/ml).

2.5.4 Preparation of Salivary Amylase

1 ml of saliva was diluted with 10 ml distilled water (1:10).

2.5.5 Preparation of Maltose Stock Standard

500 mg Maltose was dissolved in 100ml double distilled water.

2.5.6 Preparation of Maltose Working Standard

10 ml of stock solution – made upto 100 ml with d.d H_2O.

2.6 Alpha Amylase Assay/activity of Various Plant Sources

Amylase solutions (1 mg/ml) were prepared by diluting freshly obtained human saliva in the ratio 1:10. The assay was performed in 1M phosphate buffer, pH 6.8, since determination of profile indicated that enzyme activity was maximum at neutral pH. Starch solution was added to the test tubes.

The reaction was started by incubating the phosphate buffer -starch solution mixture with 1 % sodium chloride as an activator of enzyme. The enzyme solution was added in different concentrations in the test tubes and in enzyme blank. The enzyme cocktail was mixed well and kept for 15 minutes incubation at 37 °C. After incubation the reaction mixture was arrested using 2 ml of 2 N sodium hydroxide solution and mixed well. 0.5 ml of DNS reagent was added and heated in boiling water bath for 15 minutes. The test tubes were cooled and Optical Density (OD) was measured at 540 nm. Acarbose was used as a positive control.

2.7 Standard Amylase Assay

2.7.1 Substrate Blank

2ml of phosphate buffer, 1 ml NaCl and 0.5 ml of salivary amylase was added and incubated for 10 minutes in boiling water. Then, 0.5 ml NaOH, 1ml of starch and 1 ml of DNS was added. OD was measured at 540 nm (Table 1).

2.7.2 Test Blank

2 ml of phosphate buffer, 1 ml starch, 1 ml NaCl, and 0.5 ml of salivary amylase was added and incubated for 10 minutes and 0.5 ml of NaOH and 1 ml of DNS was added. OD was measured.

Table 1. Estimation and standardization of blank

Contents	Test blank(ml)	Enzyme blank(ml)	Substrate blank(ml)	Reagent blank(ml)
Buffer	2	2	2	2
Starch	1	1	-	-
Amylase	0.5	-	.5	-
Nacl	1	1	1	1
Incubated in boiling water bath for 10 minutes				
NaOH	0.5	0.5	0.5	0.5
Starch	-	-	1	-
Amylase	-	0.5	-	-
DNS	1	1	1	1
OD at 540 Nm	0.14	0.11	0.09	0.05

2.7.3 Enzyme Blank

2 ml of phosphate buffer, 1 ml of NaCl and 1 ml of starch was added and incubated for 10 minutes in boiling water. Then, 0.5 ml NaOH, 0.5 ml of salivary amylase and 1 ml of DNS was added. OD was measured at 540 nm.

2.7.4 Reagent Blank

2ml of phosphate buffer and 1 ml of NaCl was incubated for 10 minutes in boiling water. Then, 0.5 ml NaOH and 1 ml of DNS was added. OD was measured at 540 nm.

2.8 Estimation and Standardization of Maltose

2.8.1 Maltose Standardization

A series of labeled test tubes were taken and maltose standard was pipette out in these test tubes. The maltose standard was in the range of 0.5 ml to 2.5 ml of concentration 1 mg/ml. After maltose is added, 0.5 ml DNS and 2 ml 2N NaOH was added to the test tubes. Total volume was made up to 7 ml with distilled water. Contents of the tube are mixed well and treated with boiling water bath for 10 minutes (Table 2).

2.9 Estimation and Standardization of Acarbose

2.9.1 Test

Two test tubes were added with 2 ml of phosphate buffer, 0.5 ml of starch, 1ml of NaCl, 0.5 ml of salivary amylase, 1 ml of acarbose and 2 ml of acarbose and incubated for 10 minutes in boiling water. Then, 0.5 ml NaOH and 1 ml of DNS was added. OD was measured at 540 nm.

2.9.2 Blank

Two test tubes were added with 2 ml of phosphate buffer, 0.5 ml of starch, 1 ml of NaCl, 1 ml of acarbose and 2 ml of acarbose and incubated for 10 minutes in boiling water. Then, 0.5 ml NaOH, 0.5 ml of salivary amylase and 1 ml of DNS was added. OD was measured at 540 nm.

2.9.3 Estimation and standardization of α-amylase from various plant sources

2 ml of phosphate buffer, 1 ml of starch, 1 ml of NaCl, 0.5 ml salivary amylase was added to the test tubes containing 1 ml of the sample and incubated for 10 minutes in boiling water. 0.5 ml of NaOH and 1 ml of DNS was added. OD was measured at 540 nm.

3. Results and Discussion

3.1 Extraction of Various Plant Extracts

Figure 1. Preparation of extracts.

Table 2. Estimation and standardization of Maltose

Contents	Blank(ml)	S_1(ml)	S_2(ml)	S_3(ml)	S_4(ml)	S_5(ml)
Volume of working standard (ml)	-	1	2	3	4	5
Volume of distilled water (ml)	7	6	5	4	3	2
Volume of DNSA (ml)	1	1	1	1	1	1
Volume of NaOH (ml)	0.5	0.5	0.5	0.5	0.5	0.5
Incubated in boiling water bath for 10 minutes						
Optical density at 540 nm	0	0.05	0.14	0.23	0.30	0.4

3.2 Antibacterial Activity of Various Plant Extracts

3.2.1 Estimation and standardization of maltose

The intensity of orange yellow colour developed was observed at 540 nm[10] (Figures 2 and 3).

3.2.2 Estimation and Standardization of Acarbose

Acarbose is an anti-diabetic drug used to treat type 2 diabetes. It was procured from local drug store, Chennai; manufactured at Bayer Pharmaceuticals, India. Acarbose blocks starch and inhibits the alpha glucosidase from carbohydrates. That is, digestion is prevented (Figure 4).

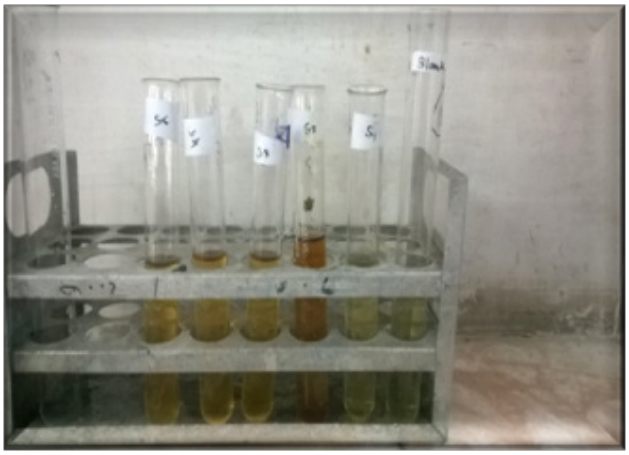

Figure 2. Maltose Test.

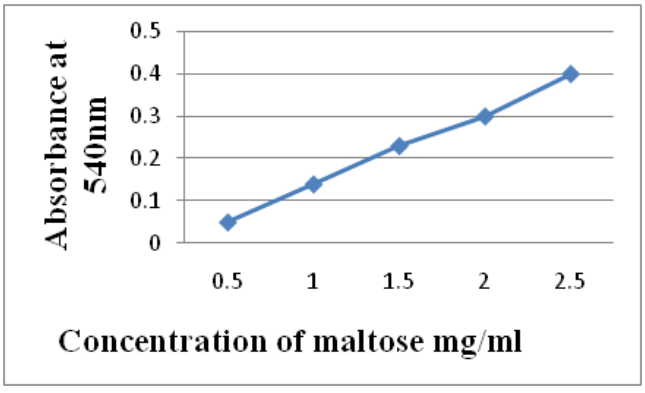

Figure 3. Calibration curve of maltose.

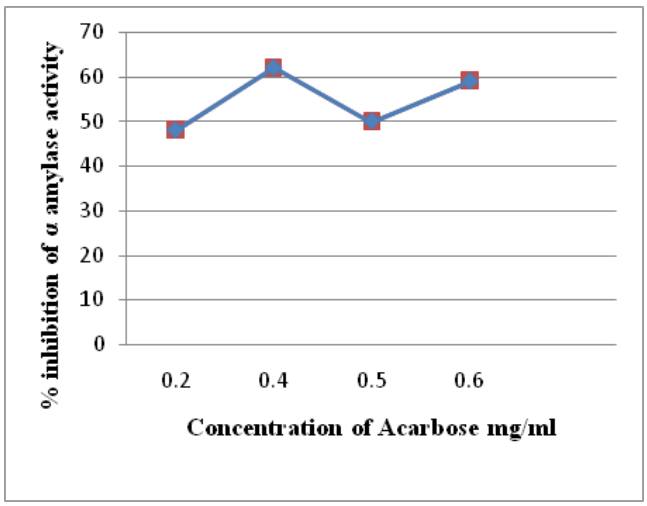

Figure 4. Inhibition activity of Acarbose.

Figure 5. Amylase activity test.

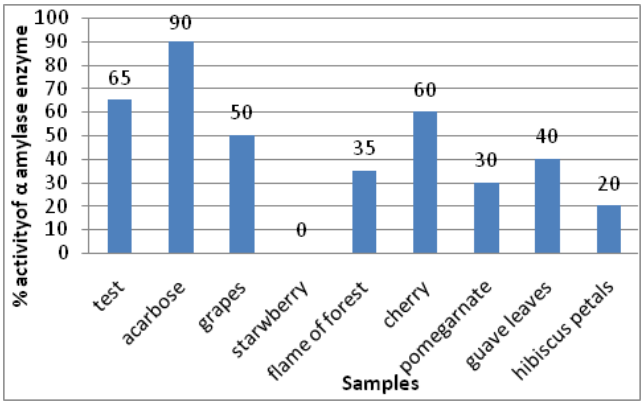

Figure 6. Effects of Anthocyanins on α-amylase activity.

3.3 Alpha Amylase Inhibition

The alpha amylase inhibitors interfere with enzymatic action which prevents the liberation of α-D glucose from oligosaccharides and disaccharides resulting in delayed glucose absorption and decrease in postprandial glucose levels.

Samples were extracted using water aqueous extracts of cherry, strawberry, pomegranate, grapes, guava leaves, hibiscus petals, flame of forest flowers were found to inhibit α-amylase among the samples. Interesting observation was that, out of all extracts lipid fraction of Strawberry have been found to give promising inhibitory activity on amylase (Figures 5 and 6).

3.4 % of Alpha Amylase Activity

Inhibition without any extracts	= 65%
Acarbose	=90%
Amylase activity in grapes	= 50%
Amylase activity in strawberry	= 0%
Amylase activity in cherry	= 60%
Amylase activity in pomegranate	= 30%
Amylase activity in guava leaves	=40%
Amylase activity in flame of forest flowers	= 35%
Amylase activity in hibiscus petals	= 20%

4. Conclusion

The results of the above study indicated that properties of anthocyanins from Hibiscus, Guava leaves, Strawberries, Cherries, Grapes, Pomegranate and Flame of Forest flowers showed maximum amount of amylase inhibitory activity. *Prunus avium* fruits were showed maximum antidiabetic activity. This inference paved for using anthocyanins extracts as potential antibacterial agents

5. References

1. Anderson OM. Anthocyanin occurrences and analysis. Proceedings of the International Workshop on Anthocyanins: Research and Development of Anthocyanins, Adelaide, South Australia; 2002.
2. Kahkonen M, Meier C, Hopia A, Oksman-Caldentey KM. Antimicrobial properties of phenolic compounds from berries. J. Appl. Microbiol. 2001:494–507. https://doi.org/10.1046/j.1365-2672.2001.01271.x. PMid:11309059
3. Sama K, Sivaraj R, Rajiv P. In vitro antidiabetic activity of anthocyanin extract of *Asystasia gangetica* (Chinese violet) flower. 2013; 3:88–92.
4. Ioanaignat, Volf I, Popa VI. A crictical review of methods for characterization of polyphenolic compounds in fruits and vegetables. Food Chem. 2010:126–32. https://doi.org/10.1016/j.foodchem.2010.12.026. PMid:25213963
5. Diana A, Garban G, Garban Z. The anthocyanins biologically-active substances of food and pharmaceutic interest. Ann. Univ. Dunarea Jos Galati Fascicle VI: Food Technol. 2003; VI:106–15.
6. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. J. Agric. Food. Chem. 2006; 54:4069–75. https://doi.org/10.1021/jf060300l. PMid:16719536
7. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Concentrations of Anthocyanins in common foods in the United States and Estimation of Normal Consumption. 2006; 54:4069–75. https://doi.org/10.1021/jf060300l. PMid:16719536
8. Castaneda-ovando A. Structural identification of anthocyanins' Food Chem. 2009; 113:859–71. https://doi.org/10.1016/j.foodchem.2008.09.001
9. Chalker-Scott L. Environmental significance of anthocyanins in plant-stress responses. Photochem. Photobiol. 1999; 70:1–9. https://doi.org/10.1111/j.1751-1097.1999.tb01944.x
10. Howell AB, Vorsa N, Marderosian AD, Foo LY. Inhibition of the adherence of P-ambriated *Escherichia coli* to uro- epithelial surfaces by proanthycyanidin extracts from cranberries. N Engl J Med. 1998; 339:1085–96. https://doi.org/10.1056/NEJM199810083391516. PMid:9767006
11. Li R, Wang P, Guo Q, Wang Z. Anthocyanin composition and content of the *Vaccinium uliginosum* berry. Food

Chem. 2011; 125:116–20. https://doi.org/10.1016/j.foodchem.2010.08.046
12. Puupponen-Pimia R, Nohynek L, Meier C, Kahkonen M, Heinonen M, Hopia A, Oksman-Caldentey KM. Antimicrobial properties of phenolic compounds from berries. Journal of Applied Microbiology. 2001; 90:494–507. https://doi.org/10.1046/j.1365-2672.2001.01271.x. PMid:11309059
13. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents. 2005; 26:343–56. https://doi.org/10.1016/j.ijantimicag.2005.09.002. PMid:16323269. PMCid:PMC7127073
14. Tanaka Y, Sasaki N, Ohmiya A. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J. 2008; 54:733–49. https://doi.org/10.1111/j.1365-313X.2008.03447.x. PMid:18476875
15. Gao H, Huang YN, Gao B, Xu PY, Inagaki C, Kawabata J. 'α-Glucosidase inhibitory effect by the flower buds of *Tussilago farfara*' L. Food Chem. 2008; 106:1195–201. https://doi.org/10.1016/j.foodchem.2007.07.064
16. Griffin PJ, Fogarty WM. Preliminary observation of starch degrading system elaborated by Bacillus polymyxa. Biochem. Soc. Trans. 1973; 1:397–400. https://doi.org/10.1042/bst0010397
17. Jakobek L, Seruga M, Medvidovi M, Novak I. Anthocyanin content and antioxidant activity of various fruit juices. Deutsche Lebensmittel-Rundschau: Zeitschrift für Lebensmittelkunde und Lebensmittelrecht. 2007; 103:456–72.
18. Myrbrack K., Neumuller, G. Chemistry and mechanism of action 1st ed., Vol.1. Academic. 1950; 226–28.
19. Vankar PS, Srivastava J. Comparative study of antioxidant activity, phenol and flavonoid contents in red, yellow varieties of *Canna indica* and *Hibiscus rosasinensis*, prospective natural food dyes. Int. J. Food Eng. 2008; 4:1–15. https://doi.org/10.2202/1556-3758.1232
20. Philip T. An anthocyanin recovery system from grape wastes. J. Food Sci. 1974; 39:859. https://doi.org/10.1111/j.1365-2621.1974.tb17999.x
21. Tamimi M, Santiago M, Blancojerez L. Comparative study of anthocyanins. JOMS. 2007:223–9.
22. Bernfeld P. [17] Amylases, α and β. Method. Enzymol. 1955; 1:149–58. https://doi.org/10.1016/0076-6879(55)01021-5
23. Bridle P, Garcia-Viguera C. Analysis of anthocyanins in strawberries and elderberries A comparison of capillary zone electrophoresis and HPLC. Food Chem. 1997; 59:299–304. https://doi.org/10.1016/S0308-8146(96)00176-8
24. Cacace JE, Mazza G. Optimization of extraction of anthocyanins from black currants with aqueous ethanol. J. Food Sci. 2003; 68:240–8. https://doi.org/10.1111/j.1365-2621.2003.tb14146.x
25. Da Silva, Lopes F, *et al*. Anthocyanin pigments in strawberry. LWT LWT-Food Sci Technol. 2007; 40:374–82. https://doi.org/10.1016/j.lwt.2005.09.018
26. Filimon RV, Beceanu M, Niculaua, Cristina. Arion *Cercetari agronomice*. Moldova. 2011; 44:81–91. https://doi.org/10.2478/v10298-012-0027-4
27. Fischer UA, Carle R, Kammerer DR. Identification and quantification of phenolic compounds from pomegranate (*Punica granatum* L.) peel, mesocarp, aril and differently produced juices by HPLC-DAD-ESI/MSn. Food Chem. 2011; 127:807–21. https://doi.org/10.1016/j.foodchem.2010.12.156. PMid:23140740
28. Garcia-Viguera C, Zafrilla P, Tomas-Barberan FT. The use of acetone as an extraction solvent for anthocyanins from strawberry fruits. Phytochem Anal. 1998; 9:274–7. https://doi.org/10.1002/(SICI)1099-1565(199811/12)9:6<274::AID-PCA416>3.0.CO;2-G
29. Miguel G, Fontes C, Antunes D, Neves A, Martins D. 'Anthocyanin concentration of *Assaria pomegranate* fruits during different cold storage conditions. J. Biomed. Biotech. 2004; 5:338–42. https://doi.org/10.1155/S1110724304403076. PMid:15577199. PMCid:PMC1082890
30. Wang Hui, Yang-Ji Du, Song H. α-Glucosidase and α-amylase inhibitory activities of guava leaves. Food Chem. 2010; 123:6–13. https://doi.org/10.1016/j.foodchem.2010.03.088
31. Zhao X, Yuan Z, Fang Y. Characterization and evaluation of major anthocyanins in pomegranate (*Punica granatum* L.) peel of different cultivars and their development phases. European Food Research and Technology. 2012:198–295
32. Yoshida Y, Koyama N, Tamura H. Color and anthocyanin composition of strawberry fruit changes during fruit development and differences among cultivars, with special reference to the occurrence of pelargonidin 3-malonylglucoside. J. Jpn. Soc. Hortic. Sci. 2002; 71:355–61. https://doi.org/10.2503/jjshs.71.355
33. Merken HM, Beecher GR. Liquid chromatographic method for separation and quantification of anthocyanins. J. Agric Food Chem. 2000; 48:577–99. https://doi.org/10.1021/jf990872o. PMid:10725120
34. Mozetič Branka, Trebše P. Identification of sweet cherry anthocyanins and hydroxycinnamic acids using HPLC coupled with DAD and MS detector' Acta Chim. Slov. 2004; 51:151–8.