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Anthocyanin Compositions in Different Colored *Gladiolus Species*: A Source of Natural Food Colorants

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Abstract The anthocyanin compositions in the flowers of different colored gladiolus genotypes (Red Flair, Violetta, Pink Event, Ice Cap and Green Star) were assessed for promoting new uses as natural food colorants. Wide variation was observedabout their total and individual anthocyanin constituents. There was a significant difference ($p < 0.01$) in the total anthocyanin pigments of the flowers of different gladiolus varieties. The Red Flower had the highest (9.05 color value per gram dry weight) anthocyaninpigments, followed by Pink Event (3.37 color value per gram dry weight). High-performance liquid chromatography (HPLC) was used for the assessment of individual anthocyanin pigments. Four common anthocyanins, namely, delphinidin, pelargonidin, cyanidin, and malvidinwere used as standards, and the contents of the samples were compared with these commercial standards. Red Flair, Violetta, and Pink Event happen to contain delphinidin and pelargonidin. Red Flair appears to contain all the four anthocyanidins. The occurrence of the markers was not detected in Ice Cap and Green Star varieties. The indication of this study on anthocyanins in various gladiolus flower is valuable to practice as natural food colorants in foodstuff and interrelated industries.

Keywords: *anthocyanin, cyanidin, delphinidin, malvidin, pelargonidin*

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1. Introduction

Anthocyanin is a natural food colorant whose pigment ranges from reddish purple to intense blue. Anthocyanin is found majorly in berries, cherry, eggplant skin, black rice, black carrot, grapes, red cabbage, pomegranates and violet flower petals.Within each plant source, these pigment complexes vary in concentration, proportions, and chemical structure, all of which influence use for color in food or beverage.Gladiolus flowers are attractedto their beauty by showing their attractive color. They are rich in polyphenolic compounds that bear cyanic colors including Pink, red, violet, and dark blue of most flowers, fruits, leaves and stems. Polyphenols are organic compounds containing aromatic ring(s) with two or more hydroxyl groups, and their derivatives, which include a broad class of compounds known as flavonoids and tannins.

Anthocyaninsare widely distributed throughout higher plants, ferns, and mosses [2]. They comprise one of the largest groups of the water-soluble pigments in the plant kingdom [3]. In nature, anthocyanins exist in association with or without sugars. Anthocyanidins are those that exist without sugar, also called anthocyan aglycones. Anthocyanins may also have other potential physiologic effects as antineoplastic agents [4], radiation-protective agents [5,6], vasotonic agents [7], vasoprotective and anti-inflammatory agents [8], chemoprotective agents against platinum toxicity in anticancer therapy [9], hepatoprotective agents against carbon tetrachloride damage [10], and possible other beneficial effects due to their interaction with various enzymes and metabolic processes [11,12,13,14]. No adverse effects were observed in animals fed a grape color extract containing principally anthocyanins[15], nor were there any adverse effects on humans consuming a grape peel extract that has been approved by the Food and Drug Administration as a food pigment [16]. There is a growing mandate in the food industry for use of natural fooddyes, and different color flowers are considered an excellent source of stable anthocyanins that can be utilized as a red food colorant [17,18]. As a food additive, anthocyanins are high in this color compared with cochineal pigments [17,18].

The structure is one underlying inherent property of a compound that distinguishes it from others and defines its properties. The structure of anthocyaninsis based on the C_6-C_3-C_6 skeleton. They share the same carbon skeleton configuration, which is composed of two aromatic rings (A and B), joined to a C ring (Figure 1). The positively charged core structures common to all anthocyanins is called the flavyliumcation (2-phenylbenzopyrylium), or interchangeably, 2-phenylchromenylium, which is a type of oxonium ion (Figure 1). Based on the kind of reaction this molecule undergoes with glucose, hydroxyl, or acetyl group, numerous molecules exist as a product that gives rise to the class of anthocyanins. Diversity within the anthocyanins is achieved by substitutions of hydroxyl groups, by methylation, acylation, and/or glycosylation reactions [19]. As a result, six most common compounds of anthocyanins are known; namely, pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin, which only vary by the hydroxylation and methoxylation pattern on their B-rings (Figure 2) [20]. Most anthocyaninsare mono-, di-, or tri-glycosylated at the C-3 hydroxyl group (Figure 2). In plants, anthocyanins occur as solutions in cell vacuoles. Purified solutions of anthocyanins in acidic (pH 4-6) aqueous environment, typical of the plant vacuole, can undergo hydration, acid-base, and ring-chain tautomeric modifications. As a result of the hydration reactions, anthocyanins are thought to exist as a mixture of four main secondary structures in equilibrium (Figure 3): the flavylium cation (AH^+), the quinoida base (A), the carbinol pseudobase (B), and the chalcone pseudobase (**C**) (21). At low pH values (pH 1-3) the red colored flavylium structures are dominant. The plant cell vacuole is maintained at approximately pH 5.5, at which 90 % of the total anthocyanin content would be expected to exist in the colorless carbinolpseudobase form, and a smaller proportion in the quinoidal base form [21,22]. However, anthocyaninsnormally exhibit visible coloration *in situ*, and the occurrence of natural colorless pseudobases is extremely rare [23]. The reason for this has been postulated to be due to various stabilization mechanisms, which allow the formation of the stable, and complex, tertiary structures, especially with sugars. Recently there is an increasing tendency in the literature regarding anthocyanin studies which may be due to reported health benefits or an increased improvement in analytical methods. Documentation of the anthocyanin content of different botanical sources is, therefore, necessary for the determination of structural diversity as well as for food and health perspectives. In this work, the flower part of five gladiolus genotypes have been examined to assess the anthocyanin contents of each type. Thus, the information on anthocyanin compositions will be used for promoting uses as natural food colorants in food and related industries.

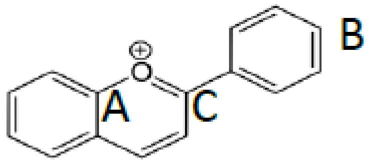
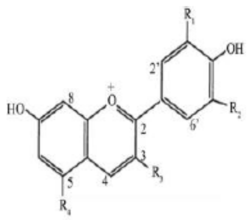


Figure 1. Flavylium Ion



	R1	R2	R3	R4
Delphinidin	OH	OH	OH	OH
Pelargonidin	-	OH	-	OH
Malvidin	-OCH3	OH	-OCH3	OH
Cyanidin	OH	OH	-	OH
Petunidin	OH	OH	-OCH3	OH
Peonidin	-OCH3	OH	-	OH

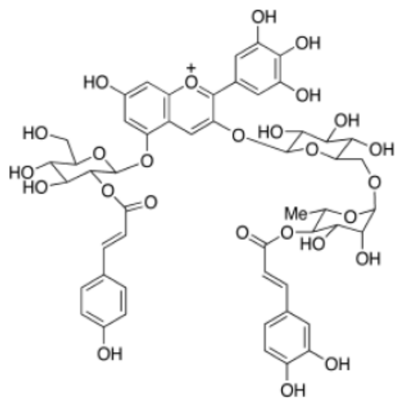


Figure 2. Structure of most common anthocyanins

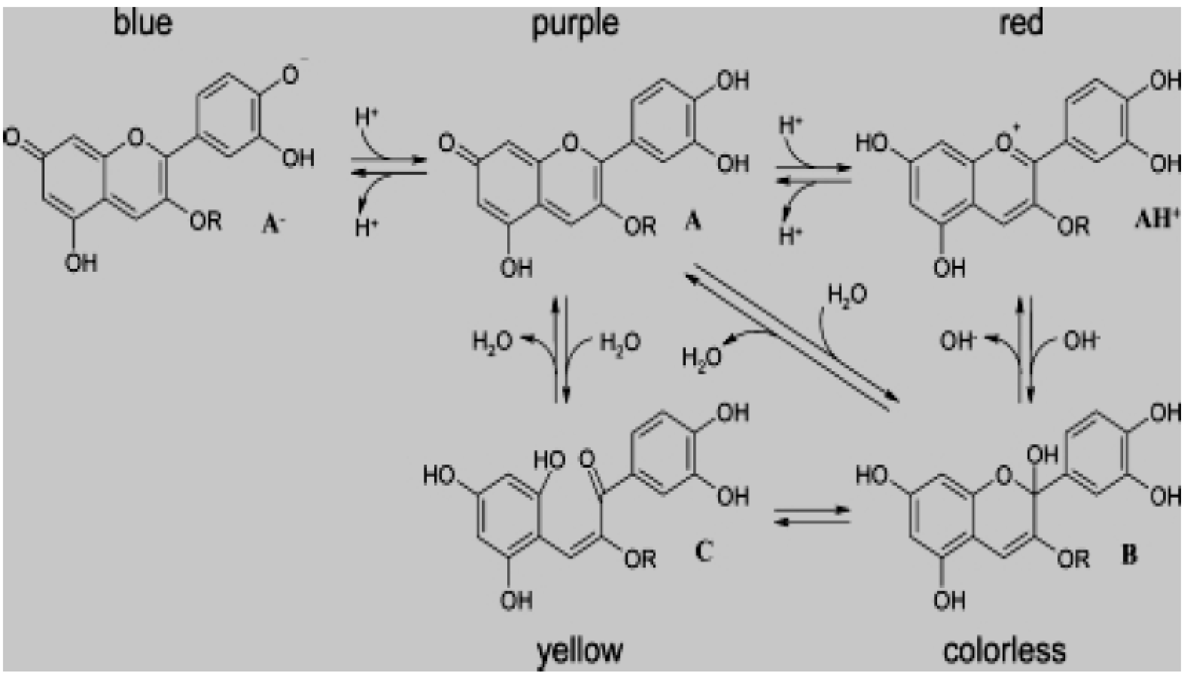


Figure 3. Anthocyanin Equilibrium Structures

2. Materials and Methods

2.1.Materials

The resources used to conduct the experiment consisted of 5 kinds of *Gladiolus* corms, each of them representing a variety. The genotypes used were *Gladiolus Green Star, Gladiolus Red Flair, Gladiolus Pink Event, Gladiolus Violetta, and Gladiolus Ice Cap*. The corms for each variety were obtained from Harris Seed Company, New York City, were received late January. The cormswere kept at a temperature of 40°C till planting. The fertilizer type used was liquid fertilizer of 20-10-20 PLS. It was obtained from Sierra Chemical Company, Milpitas, California. Potting soil and bulb pots have been achieved from Hummert International, Earth City, Missouri. The growing media (Scotts Metro Mix 366) was obtained from Scotts-Serra Horticultural Products Company, Marysville, Ohio. The greenhouse was monitored by Growmaster Green House System. The system was a product of Micro-Grow, Green House Inc., Temecula, California. Planting, field preparation, and transplanting of the corms were done as per our previous paper (24). At full blossom, the petals of each variety were collected and freeze dried. The dried samples were ground and extracted by sonicating with 3% formic acid in methanol. The extract was centrifuged, and the supernatant was filtered using 0.45µm syringe filter and the filtrate was used for analysis. The chemicals and the filter syringes were obtained from Fisher Scientific, USA.

2.2. Measurement of Total Anthocyanin

A 500 mg leaf powder was taken and add 10 ml of 0.5% H_2SO_4 solution and steeped overnight at room temperature. Centrifuge for 15 min and filtrated through filter paper and collect the supernatant as samples. The supernatant diluted fourfold with a Mcllvaine's buffer solution [25] and pH adjusted to 3 .0, was used for the measurement of the optical densities at 530 nm with a spectrophotometer. A color value (CV) for the pigment extract, which is a commercial pointer of total anthocyanin, was intended using the following formula: CV= 0.1 x OD530 x 4 x 20g^{-1}DW, where OD530 is a spectrophotometric interpretation at 530 nm, 4 and 20 are the levels of dilution, and DW is leaf dry weight [26].

2.3. Assessment of Anthocyanin Compositions by Reversed-phaseHigh-pressure Liquid Chromatography (RP-HPLC)

For the assessment of anthocyanins in the five Gladiolus varieties, the High-Performance Liquid Chromatographic method was adopted. The crude extract was vacuum dried. The remainder was redissolved in 15% acetic acid and filtered through a 0.2 µm filter membrane and used for anthocyanin identification and quality analysis. The HPLC analysis was performed according to the method described in a previous paper [27,28] with slight modifications. Analytical HPLC was run on an Inertsil ODS-2 column (250 X 4.6 mm, GL Sciences Inc.) at 35°C and monitored at 530 nm. The following solvents in water with a flow rate of 1 ml min^{-1}were used: (A) 1.5% phosphoric acid, and (B) 1.5% phosphoric acid, 20% acetic acid, 25% acetonitrile. The elution profile was a linear gradient elution for B of 25% to 85% during 40 min solvent A. The chromatograms were recorded, and the comparative concentration of pigments was calculated from the peak areas. Identification of anthocyaninswas carried out comparing the peaks with standard peaks of the anthocyanin compounds used for calibration of HPLC include malvidin, cyanin, delphinidin, and pelargonidin. All of the compounds were supplied by Indofine Chemical Company, Inc. Hillsborough, New Jersey, USA. The chromatograms for the standards and the samples were recorded and were given in Figure 5.

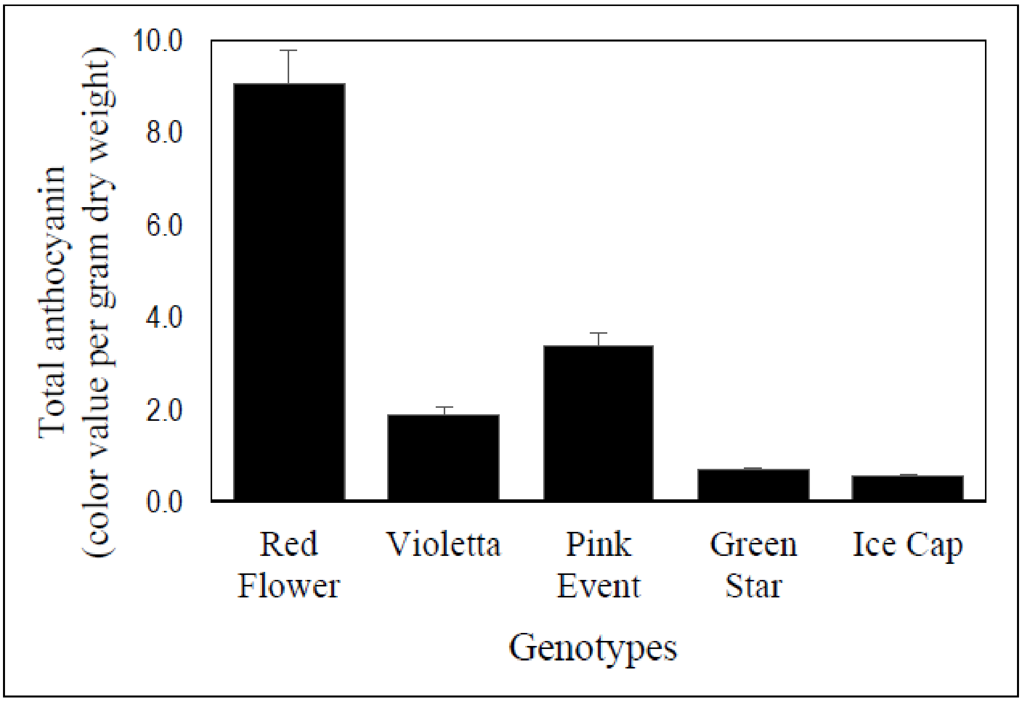


Figure 4. Total anthocyanin pigments (color value per gram dry weight) in different gladiolus flowers studied

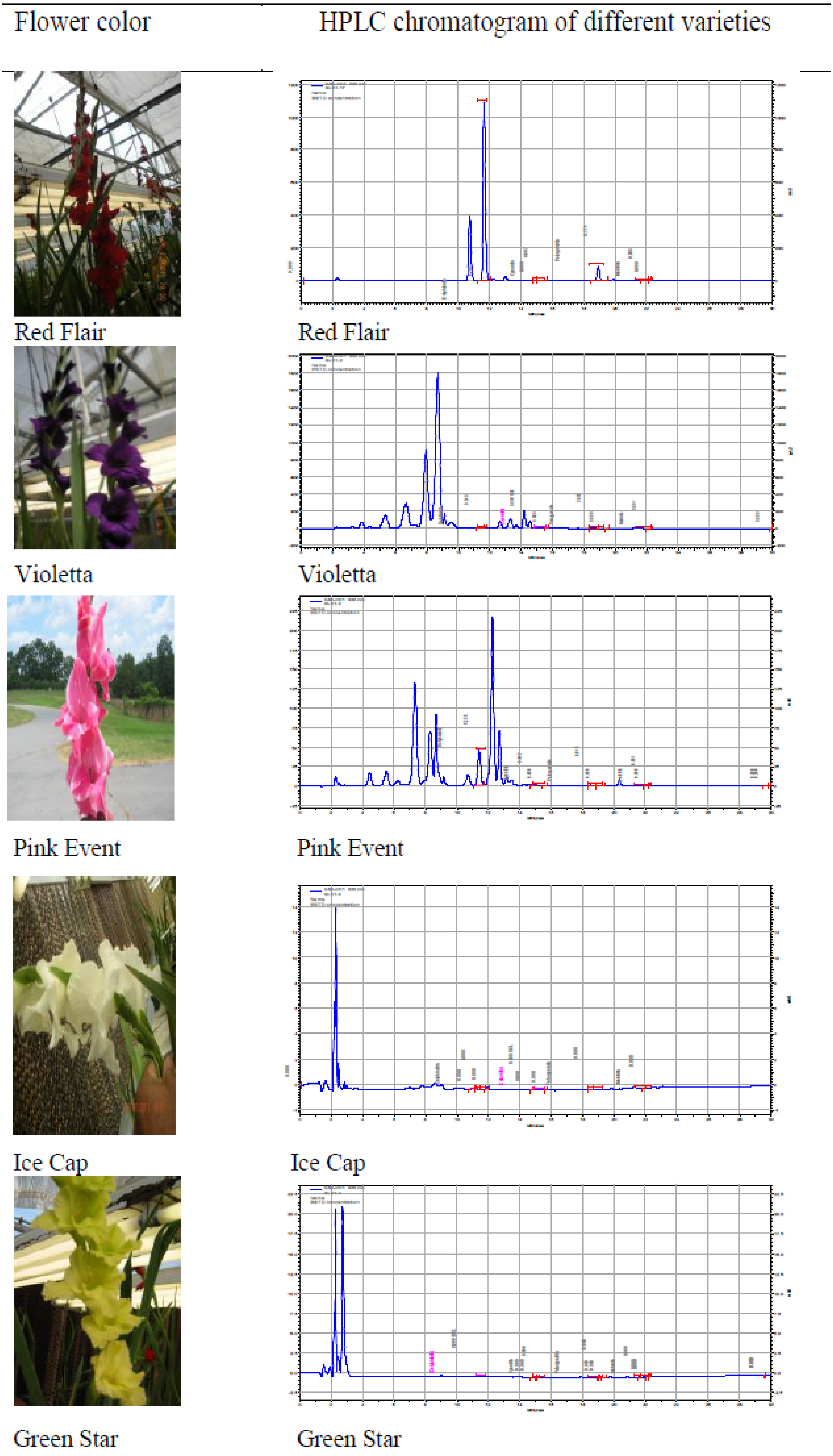


Figure 5. High-performance liquid chromatography chromatograms for anthocyanins of the flowers of gladiolus genotypes studied

3. Results and Discussion

3.1. Total Anthocyanin Pigments and Anthocyanin Compositions

The effect of genotypes on the total anthocyanin content in the gladiolus flowers is shown in Figure 4. The anthocyanin content differed significantly ($p<0.01$) among the genotypes studied. The flower from Red Flower had the highest (9.05 color value per gram dry weight) total anthocyanin content followed by Pink Event (3.37 color value per gram dry weight) and Violetta (1.88 color value per gram dry weight). The anthocyanins are ubiquitous bioactive compounds found in plant food and beverages and have received increased attention because of their potential medicinal importance [29,30,31]. For five gladiolus varieties are grown in Agricultural Experiment Station of the University of Arkansas at Pine Bluff, chromatographic assessment of four common anthocyanidins, namely, pelargonidin, delphinidin, malvidin, and cyanidinwas conducted. The varieties Red Flair, Violetta and Pink Event, contain one or more of the anthocyanins, while in Ice Cap and Green Star none of the anthocyaninswere detected. The different anthocyanin compounds were identified and quantified by one run HPLC analysis (Figure 5). Most of the pigments from the flower extract were hydrophilic and appeared on the ODS-column HPLC with a lower retention time than the anthocyanins of some other plant parts [29,30,31,32]. All the HPLC profiles of the varieties tested showed the peaks at the same retention time, but the peak areas differed with the varieties and individual anthocyanin. As per the quantity in the sample, the major anthocyanin in gladiolus flowers studied are respectively in the order ofDelphinidin>Pelargonidin>Cyanidin>Malvidin. The majority of the pigments were identical to some other plant parts reported by several authors [29,30,31]. Many ornamental flowers are natural sources of imperativebioactive compounds of advantage to the human health and their possible role as dietary components have been reported[33,34,35,36]. The most of theflower pigments are anthocyanin, often present in higher concentration compared with the most common fruits and vegetables [33].

The chromatogram of each gladiolus variety depicts the presence of chromophores in the samples that absorb radiation in the noticeable range of the electromagnetic spectrum. As could be seen from the figures (Figure 5), the prevalence of the peaks in each sample is somewhat associated with the color of the flowers. The higher the number of peaks in a chromatogram, the sample, contains a greater number of chromophores. In addition to anthocyanins, these chromophores could be chlorophylls or carotenoids. The presence of the sought-for components is evident in three varieties. As shown in Table 1, Red Flair, Violetta, and Pink Event happen to contain delphinidin and pelargonidin. Red Flair appears to contain all the four anthocyanidins. The chromatograms of Ice Cap and Green Star varieties show an insignificant number of peaks of intensity, which is distinct from the color they display. Under the present experimental conditions, the presence of the four anthocyanins is not detected in Green Star and Ice Cap varieties. This complies with the accepted assertion that emphasizes colored plant tissues containing anthocyanins. Compositionally, all anthocyanins have a single aromatic structure, that of cyanidin, and all are derived from this pigment by addition or subtraction of the hydroxyl groups followed by methylation or glycosylation [27,37]. When anthocyaninsare hydrolyzed with an acid, the sugar moieties are removed, and the remaining species are the anthocyanidins, also known as anthocyanin aglycones of the compounds. There are six standard such compounds known to impart characteristic color to the plants. The magenta colored pigment is attributed due to the presence of cyanidin; orange-red due to pelargonidin while mauve, purple and blue colors are caused by the presence of delphinidin. The methylated versions of the cyanides are malvidin, petunidin, and peonidin. Anthocyanins are manufactured within the plants from flavonol resulting structures called anthocyanidins. Examples of anthocyanidins include delphinidin (blue-red), pelargonidin (orange) and cyanidin (orange-red). These anthocyanidins are the structure blocks which are extra reduced, dehydroxylated and glycosylated inside the plant to produce anthocyanins. Anthocyanins further vary in substitution patterns and glycosyl group(s), both of which affect their color and stability. Pigments from vegetable bases characteristically contain more acylated anthocyanins than those from fruit sources, making them more stable. The structure potentials and the variety of anthocyanins present form the characteristics of each anthocyanin color [29,30,31]. Anthocyanins also contain natural antioxidants and other physiological functions [29,30,31,32,38,39].

Table 1. Different anthocyanin content of gladiolus varieties

Gladiolus Variety	Anthocyanidin	μg/100mg dry sample	Gladiolus Variety	Anthocyanidin	μg/100mg dry sample
Red Flair	Delphinidin (11.7)*	4.28	Green Star	Delphinidin	ND
	Cyanidin (15.0)	0.01		Cyanidin	ND
	Pelargonidin (19.0)	0.77		Pelargonidin	ND
	Malvidin (21.8)	0.01		Malvidin	ND
Violetta	Delphinidin (11.7)	0.01	Ice Cap	Delphinidin	ND
	Cyanidin (15.0)	ND*		Cyanidin	ND
	Pelargonidin (19.0)	0.01		Pelargonidin	ND
	Malvidin (21.8)	Trace		Malvidin	ND
Pink Event	Delphinidin (11.7)	0.24			
	Cyanidin (15.0)	0.02			
	Pelargonidin (19.0)	0.01			
	Malvidin (21.8)	ND			

ND = Not detected; * Values in the parentheses are the retention time (min) of the chromatograph.

4. Conclusion

Recently in the food industry, there is a growing demand for use of natural food colorants, and colored flowers have been regarded as an excellent source of stable anthocyanins. The chromatograms of the varieties show that each peak signals the presence of a compound. In the present study, identification of all the peaks was not conducted. It is therefore recommended that comprehensive assessment of the peaks needs to be done to document the compositional content and structural diversity of the compounds present in gladiolus varieties in a future study. Thus, the gladiolus flower contains biologically active anthocyanin compositions, which may have significant medicinal values for certain human diseases and also as stable natural food colorants. The results may be useful for various chemical breeding program improving desiredorganoleptic and nutritional quality characteristics of crop plants and helpful for colorant industries.

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