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Extraction and estimation of anthocyanin content in pomegranate flower (*Punica granatum*)

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Abstract
The present study was designed with the aim of extraction and estimation of anthocyanin content in Pomegranate flower (*Punica granatum*). Pomegranate flowers were obtained from a farm located in Bangalore, Karnataka. 100 g of pomegranate flowers was dried in oven at 100 °C for 20 min. It was then powdered using a blender and stored in an airtight container under refrigerated conditions (4 °C) for further analysis. 0.05g of pomegranate flower extract was diluted with 20.50 ml of deionized water and the absorbance of the diluted extract using pH 1.0 and pH 4.5 buffer was read at 520 nm using a spectrophotometer. The results revealed that, the anthocyanin content of pomegranate flower *(Punica granatum*) was estimated as 72.49 mg CGE/g of dry weight. In conclusion, our findings delineated the pomegranate flower samples collected from a farm are rich sources of anthocyanin and hence pomegranate flower extract having potential health benefits could be used as natural dyes.

Keywords: *Punica granatum*, anthocyanin, extraction, natural dye, health

Introduction
It is widely accepted that fruit and vegetable consumption have beneficial health effects associated with disease prevention due to their high content of bioactive components. Red fruits, such as strawberries, cherries, grapes and pomegranates, have been characterized as rich sources of phenolic compounds such as phenolic acids, flavonoids and tannins [1]. In particular, pomegranate has gained widespread popularity as a functional food due to the high content of bioactive components of the whole fruit, as well as its juice and extracts. Anthocyanins (ACNs) are the largest and most important group of flavonoids present in pomegranate juice (PJ) and together with hydrolysable tannins (HTs) they constitute the most valuable bioactive compounds. Anthocyanins are defined as floral secondary metabolite pigment ranging from blue to red intensity depending on pH and exists as glycosides in combination with glucose or cellulose molecules [2]. Anthocyanins are stored in vacuoles which are slightly acidic in nature [3]. They counter check the imbalance of oxidative and antioxidative factors, thus protecting health against higher risk of several cancer forms. They help in lowering blood glucose by improving insulin resistance, protecting beta cells and also reduces obesity [4]. Anthocyanins are found to have 150 types of flavonoids having antioxidant property, hormone supplementation property (reduces menopausal symptoms and osteoporosis) stimulating property for few enzymes and interference with DNA replications [5].
Acylated and co pigmentated anthocyanidins have higher heat stability, thus they help in maintaining the structure even in different pH conditions. Anthocyanins are the value-added colorants that can be used for preventing several diseases, including cardiovascular diseases, cancers, diabetes, some metabolic diseases and microbial infection. These compounds also improve visual ability and have neuro protective effect. In a nutshell, free-radical scavenging, changes in blood biomarkers, cyclooxygenase (COX) and Mitogen-activated protein kinase (MAPKs) pathways, as well as inflammatory cytokines signaling are the typical mechanisms of action of these coloured pigments in the prevention of diseases [6]. With this background, the present study was undertaken with the main purpose of extraction and estimation of anthocyanin content in Pomegranate flowers (*Punica granatum*).

Materials and Methods
Collection of Pomegranate flowers and preparation
Pomegranate flowers (*Punica granatum*) were used as a source of anthocyanins, a natural pigment in the present study. Petals of pomegranate flowers were collected from a farm located in Bangalore, Karnataka. 100 g of pomegranate petals was dried in oven at

100 °C for 20 min. It was then powdered using a blender and stored in an airtight container under refrigerated conditions (4 °C) for further analysis.

Extraction of anthocyanins

40g of dried pomegranate petal powder was mixed with 500 ml of distilled water and was centrifuged at 4200 rpm for 15 min. It was then subjected to vacuum evaporation for 4 hrs at 70 °C. The powder was kept in airtight container under refrigeration at 4 °C for further analysis as described by Metivier RP *et al*. [7].

Estimation of anthocyanin content

Preparation of buffers

The pH 1.0 buffer (0.025 M potassium chloride) was prepared by using an analytical balance to weigh 1.86 g of KCl in a 1000 mL beaker. After that, 980 mL of distilled water was added and mixed. The pH was measured using the pH meter and adjusted to pH 1.0 using HCl. The solution was transferred to a 1000 mL volumetric flask and the volume was made up to 1000 mL using distilled water.

The pH 4.5 buffer (0.4 M sodium acetate) was prepared by using an analytical balance to weigh 54.43 g of $CH_3CO_2Na{\cdot}3H_2O$ in a 1000 mL beaker. After that, 980 mL of distilled water was added and mixed. The pH was measured using the pH meter and adjusted to pH 4.5 using HCl. The solution was transferred to a 1000 mL volumetric flask and the volume was made up to 1000 mL using distilled water [8].

Preparation of test solutions

0.05g of anthocyanin extract was diluted with 20.50 ml of deionized water. The dilutions were done by using the volumetric pipette to measure and add the test portion in a 50 mL volumetric flask. The dilution factor was measured by diluting the test portion by using pH 1.0 buffer until the absorbance of the spectrophotometer (Model: UV-Spectrophotometer, Spectronic® Genesys™ 2 Instruments, USA) measured at 520 nm was within the linear range (0.2-1.4 AU). The test portion added was not more than 10 mL which means that 1 test portion was added to 4 parts of the buffer to ensure that the buffer capacity of the reagents was not exceeded. Using the calculated dilution factor two test solutions were prepared, the first one with a pH 1.0 buffer and the other test solution with pH 4.5 buffer.

Determination

The absorbance was measured within 20-50 min of preparation for the 2 test solutions pH 1.0 and 4.5 using a spectrophotometer at 520 nm. Absorbance is also measured at 700 nm for the correction of haze. The diluted test solution that was found to be excessively turbid was filtered before measuring the absorbance using filter papers with $\leq$1.2 mm pore size to ensure that anthocyanins do not get absorbed. The blank used was distilled water and the test solutions were read versus it. The anthocyanin pigment concentration (as cyanidin-3-glucoside equivalents) was calculated using the following equation [9]:

Anthocyanin pigment [cyanidin-3-glucoside equivalents

$$(\text{CGE, mg/L}] = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times L}$$

Where:

A = $(A_{520\ nm}-A_{700\ nm})\ pH_{1.0}-(A_{520\ nm}-A_{700\ nm})\ pH_{4.5}$

MW = 449.2 g moL^{-1} for cyanidin-3-glucoside

DF = Dilution factor

l = Path-length (cm)

ε = 26,900 molar extinction coefficient, in L/mol cm for cyanidin-3-glucoside

10^3 = Factor for conversion from g to mg

Results and Discussion

The spectrophotometric analysis of anthocyanin pigment in pomegranate flower extract with pH 1.0 and pH 4.5 is depicted in Fig 1 and 2.

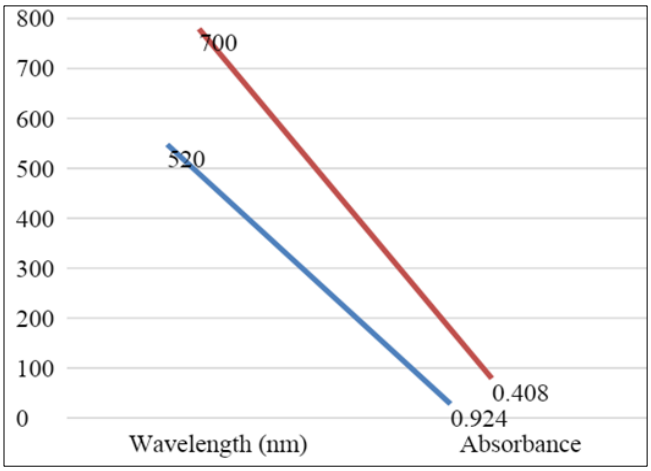

Fig 1: Spectrophotometric analysis of anthocyanin pigment in pomegranate flower extract (pH 1.0)

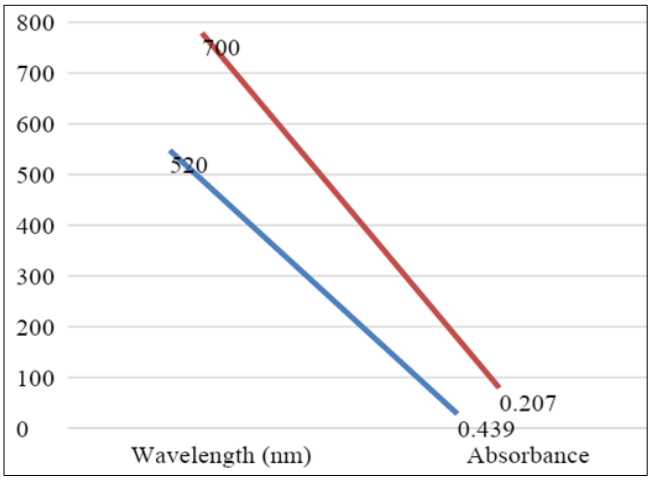

Fig 2: Spectrophotometric analysis of anthocyanin pigment in pomegranate flower extract (pH 4.5)

The results revealed that, the anthocyanins content of pomegranate flower (*Punica granatum*) was estimated as 72.49 mg CGE/g of dry weight. These findings are in accordance with the results obtained by Elfalleh W *et al*., who found that, the total anthocyanin content of pomegranate flower was 80.20 ± 7.02mg CGE/g of dry weight [10]. The total anthocyanins content present in pomegranate peel in the study of Sharifiyan F *et al*. was found to be 69 mg CGE/g dry weight which is similar to our study results [11]. Anthocyanins are phenolic compounds which are water-soluble pigments. The main anthocyanins in pomegranates are cyanidin-3, 5-diglucoside and cyanidin-3-glucoside. They are responsible for imparting a variety of colors to the plants like orange, red, pink, blue and purple. Anthocyanins are known to possess antioxidant activity and for the quantification, separation, identification and purification of anthocyanins, many methods

are used. The UV-V spectrophotometry and High-Performance Liquid Chromatography (HPLC) are mainly used for the quantification [9].

Anthocyanins can be used industrially as natural colors and can be used for a wide variety of foods, cosmetics and drugs. As a natural product, anthocyanins are good for health due to their antioxidant properties and may have a role in immunity by boosting our immune system. In addition, it has a role in disease prevention and maintaining health. The use of anthocyanin-based colorants in yogurt drink and some mixed fruit juice is becoming more popular. Some companies did use synthetic dyes in their products. However, these synthetic dyes may be toxic if over consumed. Recently, acylated anthocyanins are food colorants used in the food industry due to their high stability over non acylated anthocyanins [12]. These commodities have potential as colorants for use in the food industry. Besides the use of anthocyanins as natural dyes, these coloured pigments are potential pharmaceutical ingredients that give various beneficial health effects.

Conclusions

In summary, our results suggest, the pomegranate flowers collected from a farm are a rich source of anthocyanins and therefore, pomegranate flower extract could be used as an ingredient in functional foods and nutraceuticals.

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