

Evaluation of Total Phenolic and Flavonoid Content of Pomegranate Juice

¹Arnt Win, ²Aye Mon Thida Nyo

¹ Department of Chemistry, Kyaukse University, the Republic of the Union of Myanmar

² Department of Chemistry, University of Mandalay, the Republic of the Union of Myanmar

Abstract : In this research work, the juice of pomegranate, *Punica granatum* L., Myanmar name Tha-le which evokes various therapeutic effects, was selected for qualitative and quantitative determination of total phenolic and flavonoid content. The fruits of pomegranate were collected from Taw-ma Village, Kyaukse Township, Mandalay Region in Myanmar. Firstly, the seeds of fresh pomegranate were crushed to obtain the expressed juice which is the liquid product. This expressed juice was checked for qualitative tests of phenol and flavonoid. In addition, total phenolic content of the juice of pomegranate was evaluated by the Folin-Ciocalteu reagent using UV spectrophotometer (UV-1800, SHIMADZU, UV spectrophotometer) at 765 nm. Moreover, the total flavonoid content of pomegranate was determined by the Aluminium chloride (AlCl₃) method using same spectrophotometer at 415 nm.

Index Terms - Pomegranate, *punica granatum* L., phenol, flavonoid, Folin-Ciocalteu reagent, UV spectrophotometer.

I. INTRODUCTION

The pomegranate (*Punica granatum* L.), which belongs to the Punicaceae family, is a nutrient dense food source rich in phytochemical compounds [1-2]. It is an important fruit of tropical and subtropical regions, which originated in the Middle East and India and has been used for centuries in ancient cultures for its medicinal purposes. It is widely reported that pomegranate exhibits antiviral, antioxidant, anticancer, and antiproliferative activities [3-5].

Pomegranates are popularly consumed as fresh fruit, beverages, food products (jams and jellies), and extracts wherein they are used as botanical ingredients in herbal medicines and dietary supplements. Several studies reported that phytochemicals have been identified from various parts of the pomegranate tree and from pomegranate fruit: peel, juice, and seeds. Pomegranate polyphenols include flavonoids (flavonols, flavanols, anthocyanins.), condensed tannins (proanthocyanidins), and hydrolyzable tannins (ellagitannins and gallotannins). Other phytochemicals identified from the pomegranate are organic and phenolic acids, sterols and triterpenoids, fatty acids, triglycerides, and alkaloids [6].

Other major components of pomegranate juice are ellagic, caffeic, and punicalic acids. These phenolic compounds belong to different representative chemical classes with known bioactivities. Apart from flavonoids and tannins pomegranate also contains anthocyanins. Anthocyanins possess known pharmacological properties and are used by humans for therapeutic purposes [7].

Anthocyanins are the water-soluble pigments responsible for the bright red color of pomegranate. Several anthocyanin compounds identified in pomegranate, include pelargonidin-3-glucoside, cyanidin-3-glucoside, delphinidin-3-glucoside, pelargonidin 3,5- diglucoside, cyanidin 3,5-diglucoside and delphinidin 3,5-diglucoside [8]. Anthocyanins can be insect attractants in flowers but can also be insecticidal and antimicrobial at the same time. Tannins as well as anthocyanins have significant antiproliferative and proapoptotic effects in several different types of cancer cells in vitro, including colon cancer, prostate cancer, and head and neck cancer [9].

Popularity of pomegranate has increased tremendously especially in the last decade because of anti-microbial, anti-viral, anti-cancer, potent anti-oxidant, and anti mutagenic effects of the fruit. The antioxidants contained in fruits and vegetables are including ascorbic acid, carotenoids, flavonoids, and hydrolysable tannins [10]. However, pomegranates also have some anti-nutritional factors such as tannins and other secondary compounds. Tannins are polyphenolic substances with various molecular weights and a variable complexity [11].

Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins. Plant phenolics include phenolics acids, flavonoids, tannins and the less common stilbenes and lignans [12].

Several epidemiological and intervention studies have reported a direct relationship between consumption of fresh fruits and vegetables, and prevention of most degenerative diseases as well as slowing of the ageing process. Fruits and vegetables are rich in polyphenols, which do not only play physiological roles in plants but also act as antioxidants by donating a hydrogen atom or an electron to other compounds, scavenging free radicals, quenching singlet oxygen, and maintaining a balance between oxidants and antioxidants to improve human health [13-15].

Accordingly, the main objective of this research was to evaluate the total phenols and flavonoids (natural antioxidants) content from pomegranate juice which is phenolic rich fruit by using spectrophotometric method.

II. MATERIALS AND METHODS

2.1 Sample Collection

The fruits of *P. granatum* L. were collected from Taw-ma Village, Kyaukse Township, Mandalay Region in Myanmar.

2.2 Preparation of Fruit Juice of Pomegranate

100 g of seeds of fresh pomegranate fruit were crushed by blender. These juices were squeezed, filtered and then centrifuged with 5000 rpm for 30 minutes. 58 mL of expressed juice which is the liquid product was obtained. Then 1 mL of this expressed juice was diluted with 9 mL of distilled water.

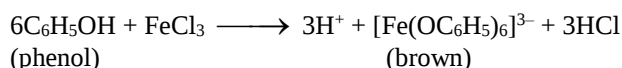
2.3 Qualitative Test for Phenols

Group Test:

The fresh juice of pomegranate was tested by blue litmus paper. This blue litmus paper turns red.

Colour with FeCl_3 :

1mL of fresh juice of pomegranate was taken and a few drops of very dilute solution of ferric chloride were added. The colour changes to brown which indicates the presence of phenol. The reaction takes place as follows [16].



2.4 Quantitative Determination of Total Phenolic Content

2.4.1 Principle

Phenols in alkaline medium react with phosphomolybdic acid of Folin- Ciocalteu reagent producing a blue coloured complex.

2.4.2 Preparation and Determination of Standard Gallic Acid

10 mg of the standard gallic acid was taken in a test tube. 10 mL of distilled water was added to the standard compound. 1 mL of this standard solution was taken in another test tube. The volume of this solution was made up to 10 mL with distilled water. The standard solution was taken by micro-pipette into a series of test tubes 20 μ L, 40 μ L, 60 μ L, 80 μ L and 100 μ L respectively.

The volume was made up to 1.6 mL with distilled water in each test tube. And then, 100 μ L of Folin-Ciocalteu reagent and 300 μ L of saturated Na_2CO_3 (20%) solution were added. After the each standard solution was heated in the water bath at 40°C for 30 minutes, the absorbances of these prepared solutions were measured with a UV/ Visible spectrophotometer at 765 nm with respect to the blank solution [17-18]. The calibration curve of standard gallic acid is shown in Fig 1 [17-18].

2.4.3 Determination of Total Phenolic Content of Pomegranate Juice

The total phenolic content of expressed pomegranate juice was measured with the Folin-Ciocalteu reagent. Firstly, 20 μL of expressed juice was taken in a test tube. It was made up to 1.6 mL with distilled water. 100 μL of Folin-Ciocalteu reagent was mixed, then 300 μL of saturated Na_2CO_3 (20%) was added.

The mixture was heated in a water bath at 40°C for 30 minutes and then cooled in an ice-bath. The absorbance of this prepared sample solution was measured at 765 nm by using UV/ Visible spectrophotometer. The results are shown in Table 1. The assay was carried out in triplicate. The total phenolic content of pomegranate fresh juice was expressed as mg gallic acid equivalent (GAE) /L [17-18].

2.5 Qualitative Test for Flavonoids

Ferric Chloride Test:

A few drops of neutral ferric chloride solution were added to 1 mL of fresh juice of pomegranate. Formation of blackish red color indicates the presence of flavonoids.

Shinoda's Test:

To 1 mL of fresh juice of pomegranate, a small piece of magnesium ribbon or magnesium foil was added and a few drops of concentrated HCl were added. Change in colour (from red to pink) shows the presence of flavonoids.

Lead- acetate Test:

To 1 mL of fresh juice of pomegranate, a few drops of aqueous basic lead acetate solution were added. Reddish brown bulky precipitate indicates the presence of flavonoids.

2.6 Quantitative Determination of Total Flavonoid Content

2.6.1 Principle

The basic principle of Aluminium chloride colorimetric method is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition it also forms acid labile complexes with the ortho-dihydroxyl groups in the A- or B- ring of flavonoids. Quercetin is reported to be suitable for building the calibration curve. Therefore standard Quercetin solutions of various concentrations were used to build up the calibration curve [19-21].

2.6.2 Preparation and Determination of Standard Quercetin

10 mg of the standard quercetin was taken in a test tube. 100 mL of MeOH was added to the standard compound. The stock solution was obtained. It was diluted with MeOH in various ratios to obtained four ranges of concentration, such as 25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL respectively. Then, 4.0 mL of solution was prepared for each concentration. 0.5 mL of each standard quercetin solution was taken in test tube and 1.5 mL methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL distilled water were added separately to each tubes.

These tubes were left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm with UV/ Visible spectrophotometer. The calibration curve was plotted by using the resulted absorbance data of standard quercetin solutions at concentrations 25 µg/ mL to 100 µg/ mL in methanol. The calibration curve of standard quercetin is shown in Fig. 2 [19-21].

2.6.3 Determination of Total Flavonoid Content of Pomegranate Juice

The total flavonoid content of fresh juice of pomegranate was measured by aluminium chloride (AlCl_3) according to the spectrophotometric method using quercetin as a standard. Firstly, 0.5 mL of fresh juice of seed of pomegranate was taken in test tube and 1.5 mL methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1M potassium acetate and 2.8 mL distilled water were added into tube.

This tube was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415 nm with UV/ Visible spectrophotometer. The assay was performed in triplicate. The total flavonoid content of fresh juice of seed of pomegranate was expressed as mg quercetin equivalent (QE) /L [19-21].

III. RESULTS AND DISCUSSION

Evaluation of Total Phenolic Content in Pomegranate Juice

Special Test for Phenol

The pomegranate juice obtained by crushing was determined by using the special qualitative tests of phenol. The results of these tests are tabulated in table 1.

Table 1 Special Qualitative Test for Phenol

No	Experiment	Observation	Inference
1.	Group Test	Blue litmus paper turns red	Phenol may be present.
2.	Color with FeCl_3	Brown color was observed	Phenol is present.

In accordance with these results, it was found that the pomegranate juice consists of phenolic compounds.

Total Phenolic Content in Pomegranate Juice

The calibration curve was plotted against by using the resulting data of standard gallic acid solution as shown in table 2 and Fig 1.

Table 2 The Results of Absorbances of Standard Gallic Acid Solutions

No.	Test Sample	Concentration (µg/mL)	Absorbance
1.	Std GA 1	2	0.14
2.	Std GA 2	4	0.233
3.	Std GA 3	6	0.348
4.	Std GA 4	8	0.43
5.	Std GA 5	10	0.508

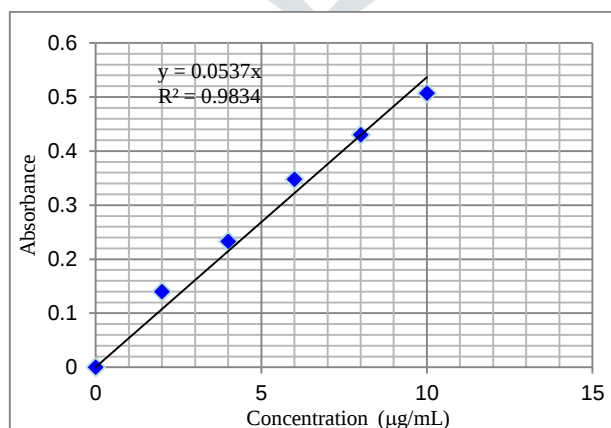


Figure 1 Concentration Absorbance Calibration Curve for Standard Gallic Acid

The total phenolic content of the fresh juice was carried out by spectrophotometric method using the Folin-Ciocalteu reagent. The absorbances of prepared sample solutions were measured with UV/ Visible spectrophotometer at 765 nm with respect to the blank solution. The results are described in table 3.

Table 3 The Results of Absorbances and Concentrations of Fresh Juice Solution

No	Name of Sample	Phenol (mg/L)	Phenol (mg/L) Mean $\pm$ Standard Deviation
1.	Pomegranate juice	1255	1256.67 $\pm$ 7.64
		1265	
		1250	

From this result, the amount of total phenolic content of analyzed sample was obtained by using the standard graph. The total phenolic content presented in the pomegranate juice was found to be 1256.67 $\pm$ 7.64 mg gallic acid equivalent (GAE) per L.

Evaluation of Total Flavonoid Content in Pomegranate Juice

Special Test for Flavonoid

The fresh pomegranate juice was examined by using the special qualitative tests of flavonoid. The resulted data are tabulated in table 4.

Table 4 The Results of Qualitative Test for Flavonoid

No	Experiment	Observation	Inference
1.	Ferric Chloride Test:	Blackish red colour was appeared	Flavonoid may be present
2.	Shinoda's Test:	Red colour turns to pink	Flavonoid is present
3.	Lead Acetate Test:	Reddish brown bulky ppt was produced	Flavonoid is present

From these results, it was observed that the fresh juice of the selected sample consists of flavonoid compounds.

Total Flavonoid Content in Pomegranate Juice

The calibration curve was plotted against by using the resulting data of standard quercetin solution as shown in table 5 and Fig 2.

Table 5 The Results of Absorbances of Standard Quercetin Solutions

No	Test Sample	Concentration ($\mu\text{g} / \text{mL}$)	Absorbance
1	Std 1	25	0.165
2	Std 2	50	0.308
3	Std 3	75	0.495
4	Std 4	100	0.636

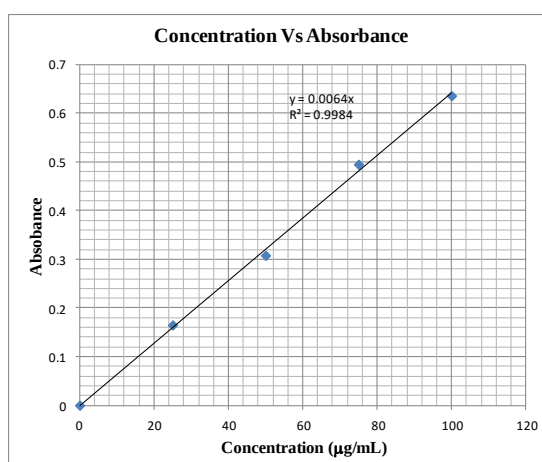


Figure 2 Concentration Absorbance Calibration Curve for Standard Quercetin

In addition, the total flavonoid content of the pomegranate was carried out by aluminium chloride spectrophotometric method using the quercetin as a standard. The absorbance of prepared sample solution (500 μ L) was measured by UV- 1800, SHIMADZU, UV spectrophotometer at 415 nm with respect to the blank solution. The results are described in table 6.

Table 6 The Results of Absorbances and Concentrations of Extract solutions of Pomegranate

No	Name of Sample	Flavonoid (mg/L)	Flavonoid (mg/L) Mean $\pm$ Standard Deviation
1.	Pomegranate juice	510.9	506.27 $\pm$ 4.65
		501.6	
		506.3	

From this result, the amount of total flavonoid content of analyzed sample was obtained by using the standard graph. The total flavonoid content present in the selected fruit juice was found as 506.27 $\pm$ 4.65 mg quercetin equivalent (QE) per L.

IV. CONCLUSION

In this research work, one of the most commonly consumed fruits by the Myanmar people, pomegranate, which is phenolic rich fruit was selected for qualitative and quantitative determination of phenolic and flavonoid content. In accordance with the qualitative tests, it was confirmed that this fresh juice contains the phenolic and flavonoid compounds. It was found that the total phenolic and flavonoid content of *P. granatum* L. is 1256.67 $\pm$ 7.64 mg gallic acid equivalent (GAE) per L and 506.27 $\pm$ 4.65 mg quercetin equivalent (QE) per L respectively. The results of the current investigation showed that the analyzed sample, the pomegranate juice had the significant amount of total phenolic and flavonoid compounds. Flavonoids are phenolic compounds which are the secondary metabolites and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these natural antioxidant compounds (phenolic and flavonoid compounds) were found to be present in the pomegranate juice, it might be responsible for the potent antioxidant capacity of pomegranate.

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