

Study on Total Flavonoid Content of Fruit of *Fragaria × Ananassa Duch*

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ABSTRACT

In this research work, one of Myanmar well known fruits, *Fragaria × ananassa Duch.*, Myanmar name Strawberry was selected to qualify and quantify the flavonoids present in it. The fresh Strawberries were collected from Pyin Oo Lwin Township, Mandalay Region, Myanmar. Firstly, the fresh Strawberries were crushed to obtain the expressed juice with distilled water which is the liquid product. This juice was checked for qualitative test of flavonoids. It responds positive for Ferric Chloride test, Shinoda's test and Lead Acetate test respectively. In addition, total flavonoid content of *Fragaria × ananassa Duch.* was evaluated by the Aluminum chloride (AlCl₃) method using UV spectrophotometer (UV- 1800, SHIMADZU, UV spectrophotometer) at 415 nm. The total flavonoid content of this selected sample was determined as 45.37± 0.25 mg quercetin equivalent (QE) per 100 g fresh weight.

Keywords: *Fragaria × ananassa Duch.*, Strawberry, flavonoids Aluminum chloride (AlCl₃) method, quercetin, UV spectrophotometer.

INTRODUCTION

Several epidemiological studies suggest that diets rich in phytochemicals and antioxidants perform a protective role on health. Phenolics are important secondary plant metabolites and are widely distributed in many fruits and vegetables. [1] Among fruits, citrus fruits are rich sources of bioactive compounds such as flavonoids, phenolic acids and vitamin C, which display potential health-promoting effects. Dietary intake of phenolics has been associated with reduced risk of chronic diseases, such as heart disease and cancer, probably due to their antioxidant properties. [2,3] Many studies have suggested that the major antioxidant activity in fruits is due to the presence of phenolic compounds.

The garden strawberry (*Fragaria × ananassa Duch.*) is a cultivated hybrid

species of the genus *Fragaria* (collectively known as the strawberries) and it is appealing to the human senses of sight and taste due to its bright red color, juicy texture, sweetness and distinct flavor. [4] More recently, there is an increasing interest in colorful berry fruits including strawberry, raspberry, blackberry, mulberry, blueberry, elderberry etc. These fruits are popularly consumed not only in fresh and frozen forms but also as processed and derived products including dried and canned fruits, yogurts, beverages, jams, and jellies. [5]

Phenolic compounds are secondary plant metabolites that are widespread in the vegetable kingdom. In strawberries the phenolic compounds are present as ellagic and *p*-coumaric acid; and the flavonoids as quercetin, kaempferol and myricetin. Anthocyanins consist of a group of phenolic

compounds responsible for the red-blue color of many fruits and vegetables. They are glycosylated polyhydroxy or polymethoxy derivatives of 2-phenylbenzopyrylium or flavilium salts. [6]

Berries provide significant health benefits because of their high levels of polyphenols, antioxidants, vitamins, minerals, and fibers. [7,8] It has been demonstrated that a wide diversity of phytochemical levels and antioxidant capacities exist within and across berries. [9,10] Furthermore, accumulating evidence suggests that genotype has a profound influence on concentrations of bioactive compounds in berries. [7,8]

Some berries, such as strawberries, have been identified as sources of phenolic compounds like gallic and ellagic acids, which have potential cancer chemo preventive activity. [11] These different bioactive phenolic compounds, including flavonoids, tannins, and phenolic acids, have received considerable interest in bearing possible relations to human health. The aim of this work was to evaluate the total flavonoid content in the fruit of *Fragaria* × *ananassa* Duch. qualitatively and quantitatively.

Botanical Description

Family : Rosaceae
Genus : *Fragaria*
Species : *F. × ananassa*
Binomial name: *Fragaria* × *ananassa* Duch.



Figure 1. Flower and fruit of *Fragaria* × *ananassa* Duch.

MATERIALS & METHODS

Sample Collection

The fruits of *F. × ananassa* Duch. were collected from Pyin Oo Lwin Township, Mandalay Region in Myanmar.

Preparation of Fruit Juice of *F. × ananassa* Duch.

50 g of fruits of *F. × ananassa* Duch. were crushed with 100 mL of distilled water by blender. These juices were squeezed, filtered and then centrifuged with 5000 rpm for 30 minutes. 57 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.

Quantitative Determination of Total Flavonoid Content

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. [12-15]

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 methanol 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 figure 2. [12-15]

Determination of Total Flavonoid Content of *F. × ananassa Duch*.

The total flavonoid content of fresh juice of *F. × ananassa Duch*. was measured by aluminium chloride (AlCl₃) according to the spectrophotometric method using quercetin as a standard. Firstly, 0.5 mL of fresh juice of *F. × ananassa Duch*. was taken in test tube and 1.5 mL diatilled water, 0.1 mL of 10 % aluminium chloride, 0.1 mL of 1 M 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 *F. × ananassa Duch*. was expressed as mg quercetin equivalent (QE) /100 g fresh weight. [12-15]

RESULTS AND DISCUSSION

Evaluation of Total Flavonoid Content in *F. x ananassa Duch*.

Special Test for Flavonoid

The fresh juice of *F. × ananassa Duch*. was examined by using the special qualitative tests of flavonoid. The resulted data are tabulated in table 1.

Table 1 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 *F. × ananassa Duch*.

Table 2 The Results of Absorbances of Standard Quercetin Solutions

No	Test Sample	Concentration (µg / 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

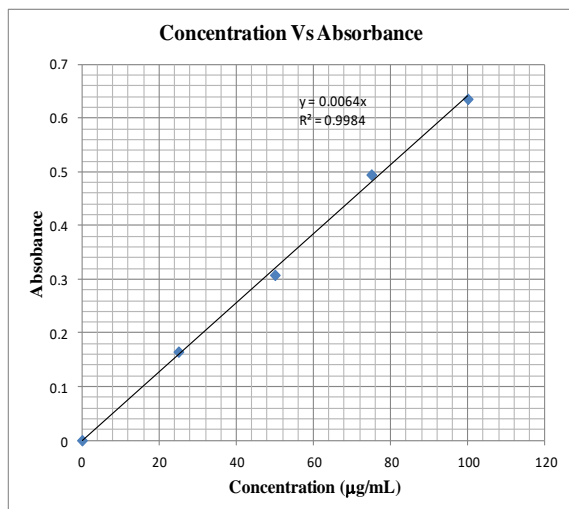


Figure 2 Concentration Absorbance Calibration Curve for Standard Quercetin

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

In addition, the total flavonoid content of *F. × ananassa Duch*. was carried out by aluminium chloride spectrophotometric method using the quercetin as a standard. The absorbance of prepared sample solution (500 µL) was measured by UV- 1800, SHIMADZU, UV spectrophotometer at 415 nm with respect to the blank solution. The results are described in table 3.

Table 3 The Results of Concentrations of Extract solutions of *F. × ananassa Duch*.

Name of Sample	Flavonoid (mg/100 g)	Flavonoid (mg/100 g) Mean ± Standard Deviation
<i>F. × ananassa Duch</i> .	45.4	45.37± 0.25
	45.6	
	45.1	

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 45.37± 0.25 mg quercetin equivalent (QE) per 100 g fresh weight.

CONCLUSION

In this research work, one of the most commonly consumed fruits by the Myanmar population, *F. × ananassa Duch*. (Strawberry) which is flavonoid rich fruit, was selected to qualify and quantify the flavonoid present in it. The total flavonoid content of the fresh juice obtained from the selected sample could be evaluated by UV spectrophotometer using the aluminium chloride method at 415 nm. It was found that the total flavonoid content of *F. × ananassa Duch*. is 45.37 ± 0.25 mg quercetin equivalent (QE) per 100 g fresh weight. The resulted data of the current study showed that the selected sample, the Strawberry, had the considerable amount of total flavonoid compounds. Flavonoid compounds that are secondary metabolites are antioxidants. Antioxidant compounds scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases.

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