Qualitative and Quantitative Determination of Total Flavonoid Contents from the Peels of *Psidium guajava* L. Fruits (Guava)

Wah Wah Moe[1], Mon Mon Thaw[2], Naing Thaw Zin Thu[3],Thandar Htun[3]

Abstract

In this research work, one of Myanmar well known fruits, *Psidium guajava* L., English name (Guava), Myanmar name Malaka was selected for chemical analysis. The fruits sample were collected from near the Chamery, Shwebo University Campus, Shwebo Township, Sagaing Region. The aim of this research is to observe phytochemical constituents and total flavonoid contents in the peels of *Psidium guajava* L. fruits (Guava). Firstly, the preliminary phytochemical screening of selected sample was performed. According to the phytochemical test, the peels of *Psidium guajava* L. fruits (Guava) consists of alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugar, saponins, steroids, tannins and terpenoids respectively. Moreover, the elemental compositions in the peels of *Psidium guajava* L. fruits (Guava) were measured by Energy Dispersive X - ray Fluorescence method. In addition, qualitative determination of flavonoids content in the peels of *Psidium guajava* L. fruits (Guava) were carried out by test tube method. Finally, the content of total flavonoid compound in the sample was measured by using UV-vis spectrophotometer at 415 nm. The total flavonoid content from the peels of *Psidium guajava* L. fruits (Guava) was found to be 190.20 ± 0.41 mg/100 g fresh weight.

***Keywords*:** *Psidium guajava* L., phytochemical screening, flavonoid, UV-vis spectrophotometric method

Introduction

Psidium guajava L. known as guava is a medicinal plant belonging to the family Myrtaceae. *Psidium guajava* is a well-known traditional medicinal plant used in various indigenous systems of medicine. It is widely distributed throughout the world. It has about 133 genera and more than 3,800 species (Nwinyi *et al*., 2008). Guava is considered a commercial and nutritional values. Guava is considered a common man's fruits and is called Malaka. The chemical composition of the fruits depends on factors such as variety, maturity and the environmental conditions within which they are grown. Nutritional value of guavas is often included among super fruits, being rich in dietary fiber, vitamins A and C, folic acid and the dietary minerals, potassium, copper and manganese. Having a generally broad, low calories profile of essential nutrients, a single common guava fruit contains about four times the amount of vitamin C as an orange (Razzaque *et al*., 2000).

Guava contains a large number of antioxidants and phytochemicals including essential oils, polysaccharides, minerals, vitamins, enzymes, and triterpenoid acid alkaloids, steroids, glycosides, tannins, flavonoids and saponins. Guava contains a higher content of vitamin C and vitamin A. Guava is also a very good source of the pectin which is an important dietary fiber (Bag and Devi, 2015). It is a native of central America but is now widely cultivated, distributed and the fruits enrich the diets of millions of people in the tropics of the world (Vernin *et al*., 1991).

The main constituents of guava are vitamin, tannins, phenolic compounds, flavonoids, essential oils and triterpenoid acids. Phenolic and flavonoid compounds are example of herbal compounds with widely reported antioxidant and anti-

[1] Associate Professor, Dr, Department of Chemistry, Shwebo University
[2] Demonstrator, Department of Chemistry, Shwebo University
[3] Demonstrator, Department of Chemistry, Shwebo University

inflammatory properties. Flavonoids exhibit pharmaceutical activities like anti-allergic, anti-microbial and anti-cancer activity (Krishnaiah *et al*., 2007). The aim of the present study was to evaluate the total flavonoid content in the peels of *Psidium guajava* L. fruits qualitatively and quantitatively.

Botantical Description

Scientific Name : *Psidium guajava* L.
Kingdom : Plantae
Family : Myrtaceae
Myanmar Name : Malaka
English Name : Guava
Part of Used : Peels of Fruits

Figure 1. Peels and plant of *Psidium guajava* L. (Guava)

Materials and Methods

Sample Collection

The fruits of *Psidium guajava* L. (Guava) were collected from near the Chamery Shwebo University Campus, Shwebo Township, Sagaing Region. These samples were thoroughly washed with fresh water and it was peeled. The peeled samples 50 g was obtained. These samples were crushed with 50 mL of distilled water by blender. These juices were used throughout the experiment. Figure 1 represents the peels and fruits of *Psidium guajava* L. (Guava).

Phytochemical Test of Samples

Phytochemical tests were carried out on the various extracts of fresh juice solutions according to the reported procedures (Harbone,1976).

Elemental Analysis of the Peels of *Psidium guajava* L. Fruits (Guava)

Elemental compositions in the peels of fruits samples, *Psidium guajava* L. were measured at Department of Chemistry, Monywa University by applying EDXRF (Energy Dispersive X-ray Fluorescence) Spectroscopic method.

Special Qualitative Test of Flavonoids

(i) Alkaline reagent test

A few drops of dilute sodium hydroxide were added to one milliliter of fresh juice. An intense white precipitate was appeared, which became colorless on the addition of a few drops of dilute hydrochloric acid. It indicates the presence of flavonoids.

(ii) Shinoda's test

To one milliliter of fresh juice, a small piece of magnesium ribbon and a few drops of concentrated hydrochloric acid were added. Formation of pale yellow color indicates the presence of flavonoids.

(iii) Ferric chloride test

A few drops of ferric chloride solution were added to one milliliter of fresh juice. Development of intense pale-green color indicates the presence of flavonoids.

(iv) Lead acetate test

A few drops of 10 % lead acetate solution were added to one milliliter of fresh juice. An intense white bulky precipitate was produced. It indicates the presence of flavonoids.

Quantitative Determination of Total Flavonoid Content

Principle

The basic principle of aluminium chloride chlorimetric method that aluminium chloride forms acid stable complexes with the C-4 keto group an either the C-3 or C-5 hydroxyl group of flavones and flavanols. In addition, it also forms acid labile complexes with the ortho-dihydroxyl group in the A-or B-ring of flavones and flavanols. 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.

Preparation and determination of standard quercetin

The standard quercetin, 10 mg was taken in a test tube. 100 mL of methanol was added to the standard compound. The stock solution was obtained. It was diluted with methanol in various ratios to be 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 tube.

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-vis 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 was shown in Figure 2.

Determination of Total Flavonoid Content in the Peels *Psidium guajava* L. Fruits

The total flavonoid content in fresh juice of *Psidium guajava* L. 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 *Psidium guajava* L. was taken in test tube and 1.5 mL distilled water, 0.1 mL of 10 % aluminium chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water were added into this test 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-vis spectrophotometer. The assay was performed in triplicate. The total flavonoid content of fresh juice sample was expressed as mg quercetin equivalent (QE)/100 g fresh weight.

Results and Discussion

Preliminary phytochemical screening of the peels of *Psidium guajava* L**.** fruits (Guava) was carried out to determine the presence or absence of organic constituents in it. The results are shown in Table 1.

Table 1. The Results of Phytochemical Test from the Peels of *Psidium guajava* L. Fruits (Guava)

No.	Constituents	Reagents	Observation	Remark
1	Alkaloids	Dragendroff's solution	yellow ppt	+
2	Carbohydrates	10 % α -naphthol	red ring	+
3	Flavonoids	EtOH, Mg turning	Pale yellow color	+
4	Glycosides	10 % lead acetate	brown color	+
5	Phenolics	10 % $FeCl_3$	green color	+
6	Reducing Sugars	Benedict's solution	yellow ppt	+
7	Saponins	Distilled Water	frothy	+
8	Steroids	$(CH_3CO)_2O$, Conc: H_2SO_4	yellow color	+
9	Tannins	2 % gelatin solution	green color	+
10	Terpenoids	$CHCl_3$, $(CH_3CO)_2O$, Conc: H_2SO_4	red color	+

+ = presence ppt = precipitate

According to phytochemical tests, the peels of *Psidium guajava* L. fruits (Guava) consists of alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids respectively.

Determination of Elemental Compositions in the Peels of *Psidium guajava* L. Fruits (Guava)

Elemental compositions of peels from *Psidium guajava* L. fruits were measured by energy dispersive X-ray fluorescence (EDXRF) method. The EDXRF spectra of fruits samples are represented in Table 2 and Figure 2.

Table 2. The Results of Some Elements in the Peels of *Psidium guajava* L. Fruits (Guava)

No.	Element	Relative Abundance (%)
1	K	0.559
2	Ca	0.053
3	S	0.053
4	Fe	0.010
5	Ba	0.003
6	Cu	0.002
7	Rb	0.001
8	Zn	0.001
9	Mn	0.001

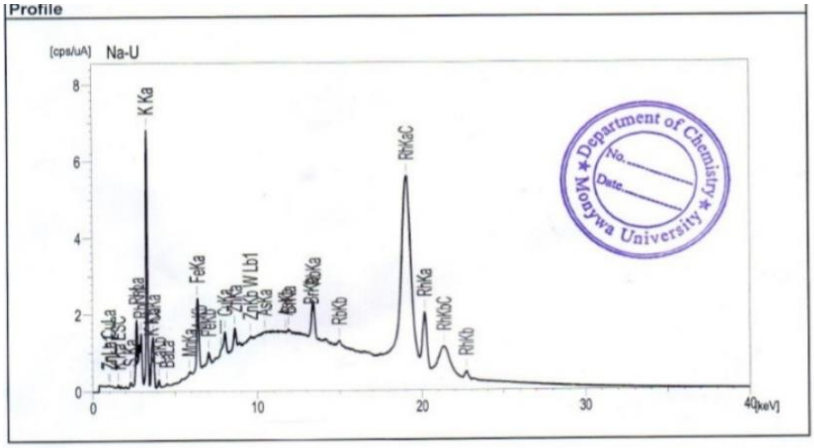

Figure 2. EDXRF spectrum of peels of *Psidium guajava* L. fruits (Guava)

From these results, it can be seen that potassium, calcium, sulphur, iron, barium, copper, rubidium, zinc and manganese were present in the samples. It was found that the peel samples contain highest content of potassium. It observes that the peels of *Psidium guajava* L. fruits (Guava) is a rich source of minerals for health benefit.

Special Test for Flavonoids

The fresh juice obtained by crushing the peels of *Psidium guajava* L. fruits was examined by using the special qualitative tests of flavonoids. The resulted data are tabulated in Table 3.

Table 3. The Results of Qualitative Test for Flavonoids

No.	Experiment	Observation	Inference
1	Alkaline Reagent Test	White ppt was appeared	Flavonoid may be present
2	Ferric Chloride Test	Intense pale-green color was developed	Flavonoid may be present
3	Lead Acetate Test	White bulky precipitate was developed	Flavonoid may be present
4	Shinoda's Test	Pale-Yellow was appeared	Flavonoid may be present

According to these results, the fresh juice of *Psidium guajava* L. consists of flavonoid compounds.

Quantitative Determination of Total Flavonoids Content in the Peels of *Psidium guajava* L. Fruits (Guava)

Determination of total flavonoid content in fresh juice sample, *Psidium guajava* L. was carried out by using spectrophotometer. The calibration curve was plotted against by using the resulting data of standard quercetin solution as shown in Table 4 and Figure 3.

Table 4. The Results of Concentration and Absorbances of Standard Quercetin Solutions

No.	Test sample	Concentration (µg/mL)	Absorbance
1	Std Q1	25	0.165
2	Std Q2	50	0.308
3	Std Q3	75	0.495
4	Std Q4	100	0.636

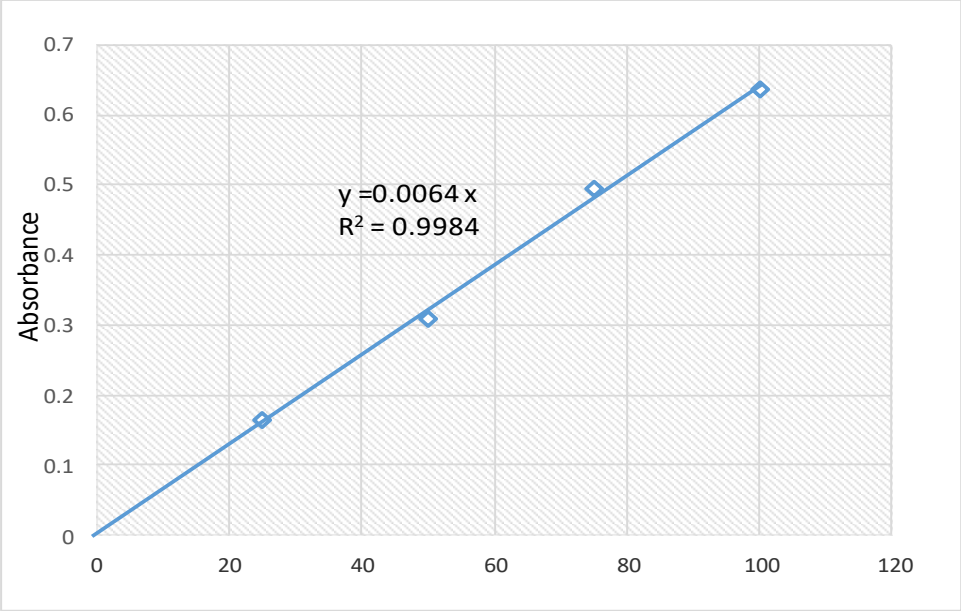

Figure 3. Concentration and absorbance calibration curve for standard quercetin

The total flavonoids content of *Psidium guajava* L. was carried out by aluminium chloride spectrophotometric method using the quercetin as a standard. The absorbances of prepared sample solution were measured with UV-vis spectrophotometer at 415 nm with respect to the blank solution.

Table 5. Amount of Total Flavonoids Content in Extract Solutions of *Psidium guajava* L.

No.	Name of sample	Flavonoids (mg/100 g)	Flavonoids (mg/100 g) Mean ± Standard Deviation
1	*Psidium guajava* L.	190.60 190.00 190.60	190.20 ± 0.41

The amount of total flavonoids content in analyzed sample were obtained by using the standard graph and the resulted data was listed in Table 5. The total

flavonoid content present in the selected fruit juice was found as 190.20 ± 0.41 mg quercetin equivalent (QE) per 100 g fresh weight. The 8.26 ± 0.20 mg quercetin equivalent (QE) per g dry weight of flavonoid content in the peels of *Psidium guajava* L. fruits (Guava) was found in India Research Journal in 2023 (Dewage *et al*., 2023). Because of different locality and maturity of the fruits (Guava) the flavonoid content in the fruits were differ. From these results, the peels of *Psidium guajava* L. fruits (Guava) must be eaten with peels.

Conclusion

In this research work, the peels of *Psidium guajava* L. fruits (Guava) were selected for chemical screening due to its interesting medicinal uses and presence of many bioactive compounds. The objective of this study was to provide the qualitative and quantitative analysis about phytochemical constituents, elemental compositions and total flavonoid content in the peels of *Psidium guajava* L. fruits (Guava). In addition, the total flavonoid content of extract obtained from the selected sample could be evaluated by UV-vis spectrophotometer using the aluminium chloride method at 415 nm. It was found that the total flavonoid content in the peels of *Psidium guajava* L. fruits (Guava) is 190.20 mg quercetin equivalent (QE) per 100 g fresh weight.

The resulted data of the current study showed that analyzed sample, the peels of *Psidium guajava* L. fruits (Guava) had considerable amount of total flavonoid compounds. Flavonoid compounds are secondary metabolites and antioxidant. Antioxidants are helpful in reducing the occurrence of degenerative diseases. Based on the above findings, it can be suggested that this selected fruits sample which is flavonoid rich fruit could be used as vitamin C and antioxidant activity. Owing to the presence of flavonoid in the peel, the guava fruits should be eaten with peels.

Acknowledgements

The authors are extremely grateful to Dr Myo Myo, Rector and Dr Tin Tin Yi, Pro-rector, Shwebo University for their permitting and good guidance on our study. We would like to express our thanks Dr Thi Thi Aung, Professor and Head, Dr Mya Theingi Professor, Department of Chemistry, Shwebo University for their encouragement and understanding throughout this research work.

References

Bag, G.C. and Devi, P.G. (2015).‘‘Assessment of Total Flavonoid Content and Antioxidant Activity of Methanolic Rhizome Extract of Three Hedychium Species of Manipur Valley”. *Int.J.Pharm. Sci. Rev. Res*., vol.30 (1), pp.154-159.

Dewage, D.D.H, Samarakoon, M.G.K., Karunaratne, S.H.S and Rajapakse, C.S.K. (2023). “Psidium guajava L. (Common Guava) Peel, Pulp and Leaves as Natural Sources of Antioxidants, Antimicrobials and Photoprotective Agents for Development of Sun Protection Cosmeceuticals”. *Asian Journal of Chemistry*; vol.35 (1), pp.89-94.

Harbone, J.B. (1976). *Phytochemicals Methods a Guide to Modern Technique of plant Analysis*. 1st edition, London: Cthapman and Hall Ltd, pp. 1-150.

Krishnaiah, D., Sarbatly, R. and Nithyanandam, R. (2007). "Phytochemical Constitutents and Activities of *Morinda citrifolia* L.." Malaysia: *Univesiti Malaysia Sabah*, vol.3 (2), pp.127-150.

Nwinyi, O., Chinedu, S.N. and Ajani, O.O. (2008). “Evaluation of antibacterial activity of *Psidium guajava and Gongroneme latifolium*”. *J Med plants Res*, vol.2 (8), pp. 189-192.

Razzaque, M., Saud, Z., Absar, N., Karin, M. and Hashinaga, F. (2000). “Purification and Characterization of Polyphenoloxidase from Guava Infected with Fruit- rot Disease”, Pakistan: *J. Biol. Sci.*, vol.3, pp. 407-510.

Vernin, G., Vernin, E., Vernin, C. and Metzger, J. (1991). “Extraction and GC-MS-SPECMA Data Bank Analysis of the Aroma of *Psidium guajava L*. Fruit from Egypt”. *Flavour Fragr. J.*, vol.6 (1), pp.143-148.