Determination of Elemental Analysis and Antioxidant Activity of *Cydonia oblonga* Mill. (Quince)

Twal Tar Soe

Abstract

In this research work, quince was selected for determination of ash, moisture, pH, fibre, mineral composition, vitamin C content and antioxidant activity. Ash, moisture, pH and fibre contents in quince were determined by using AOAC (Association of Official Analytical Chemistry) method. Mineral composition of quince was determined by EDXRF (Energy Dispersive X-ray Fluorescence) spectroscopy. Ascorbic acid content in quince was quantitatively determined by spectrophotometric method, using of potassium chromate diphenylcarbazide. In antioxidant activity study, ethanolic extract of quince was used to investigate radical scavenging activity by (1, 1-diphenyl-2-picryl- hydrozyl) DPPH assay.

Keyword: Vitamin C, Antioxidant, EDXRF, AOAC

Introduction

Quinces belong to the group of the oldest cultural plants. They originate from central Asia and were gradually penetrated also to other parts of the world. Quinces are less suitable for the direct consumption and for that reason they are mostly used either cooked or preserved. However, as compared with other species, this fruit specie is less popular throughout the world. Its predisposition to turning brown and decay is a limiting factor in the long-term storage of this fruit. The nutritional value of the fruit is high. It is well known that quince fruit shows antioxidant effects which result from the presence of a number of polyphenolic substances and also from increased levels of vitamin C.

Quinces are important also for the canning industry, especially due to their high contents of pectins. Quince fruit can also be used for the production of an aromatic distillate. At present, the possibilities of a commercial use of quinces are intensively studied. The nutritional values of the individual quince varieties may be really processing (Rop *et al.*, 2011).

Fruits are also recommended as a source of vitamin C and potassium. Traditionally, fruits, as foodstuffs were available for a limited time and, when ripe, were sometimes difficult to collect and transport. When ripe, they have a short period of acceptability before senescence intervenes. Thus, many fruits consumed in today's world are processed, frozen, canned, or dried (Farnsworth and Bunyapraphatsara 1992).

Botanical Description

Figure 1. The fruits of *Cydonia oblonga* Mill. (Chin-saw-ka)

Daw, Demonstrator, Department of Chemistry, Mandalar University

Scientific name : *Cydonia oblonga* Mill
Family name : Rosaceae
English name : Quince
Myanmar name : Chin-saw-ka
Part used : fruit

Juice made from the fruit is said to have antiseptic, analeptic, astringent, diuretic and tonic properties. High concentration of dietary fibre is beneficial in weight loss programs. Consumption of quince fruit on a daily basis shows excellent results in people afflicted with tuberculosis, hepatic insufficiency, diarrhea, dysentery, gastric ulcer, liver and eye diseases. Quince juice is helpful in cases of anemia, cardiovascular, respiratory and gastrointestinal disorders. Its antioxidant properties could prove to be beneficial in reducing risk of cancer appearance.

Materials and Methods

Sample Collection

Quince fruits were collected from Myitkyina, Kachin state. The quince fruits were cut into small pieces and air-dried in the shade for few days. These pieces were used as the sample.

Determination of Ash Content

The ash content of sample was determined by oven drying method. The ash of a sample is the inorganic residue remaining after the organic matter has been burnt away.

Sample (5 g) was introduced into a predried and cooled porcelain crucible in air-tight desiccator and accurately weighed. Then, it was heated gently over a burner until the sample was thoroughly charred. The crucible and sample content were then transferred to the muffle furnace at 600˚C for two hours until the residue was free from carbon. Then the crucible containing residue was cooled in a desiccator and weighed. Heating, cooling and weighing were repeated until constant weight was attained.

The ash content of the sample was calculated using the following equations.

$$\text{Ash (\%)} = \frac{\text{weight of residue} \times 100}{\text{weight of sample}}$$

Determination of Moisture Content

The moisture content of sample was determined by oven drying method. The moisture content of sample is the weight lost due to the evaporation of water at the drying temperature.

Sample (5 g) was placed in the crucible. The crucible with the sample placed in an oven and dried for 30 minutes at 100°C. Then, they were removed from the oven and cooled in the air-tight desiccator at room temperature and weighed. The procedure was repeated until the loss in weight had not been changed.

The moisture content can be calculated by the following formula.

$$\text{Moisture (\%)} = \frac{\text{loss in weight (g)} \times 100}{\text{weight of sample (g)}}$$

Determination of pH Value

The pH value of the sample was determined by pH meter.

Determination of Fibre Content

The fiber content of sample was determined by AOAC method.

About 1g of defatted sample was weighed and added to 200ml of 1.25 % sulphuric acid held in a 500 ml beaker and bolied for 30 min on a hot plate. Any loss

in volume during boiling was made up with distilled water. To the residue, 200 ml of 1.25 % sodium hydroxide was added and boiled for 30 min. The liquor was filtered through a cotton cloth and the residue washed with distilled water until the washing was no alkaline. The residue was dried at 105˚C for 3h and weighed again.

$$\text{Crude fibre (\%)} = \left(\frac{B - A}{S}\right) \times 100$$

B =weight of fibre + dish
A =weight of empty dish
S =sample of weight

Determination of Ascorbic Acid Content

Ascorbic acid content in quince was determined by using UV-visible spectrophotometer.

Five milliliters of standard ascorbic acid and sample solution were pipette into 25 ml volumetric flask and 5 ml of 5.2 µg/ml standard chromate ion in 0.8 M HNO_3 solution was added. After mixing, 4 ml of diphenylcarbazide solution was added and solution was diluted to the mark with distilled water. Absorbance of violet color of Cr-diphenylcarbazide complex was measured at 548 nm using UV-Vis spectrophotometer.

Determination of Antioxidant Activity Content

DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of plant materials.

Preparation of Crude Extracts

Ethanol extract of quince was prepared by the following procedure.

The dried powdered sample (100 g) was extracted with 95 % ethanol for one month and filtered. Evaporation of the solvent was provided ethanol crude extract of quince.

Preparation of Test Sample Solution

Quince crude extract (5 mg) was dissolved in 95% ethanol (10 ml) under vigorous shaking. After filtration, the filtrate was used as a stock solution (500µg/ml). Desired concentrations (400, 200, 100, 50, 25 µg/ml) of sample solutions were prepared from this stock solution by dilution with appropriate amount of 95 % ethanol.

Preparation of 60 µM DPPH Solution

DPPH (2.364 mg) was thoroughly dissolved in 95 % ethanol (100 ml). This solution was freshly prepared in the brown colored flask and kept in refrigeration for no longer than 24 hours.

Preparation of Standard Solution

Ascorbic acid (5 mg) was dissolved in 10 ml of 95 % EtOH and it was used as a standard solution.

Measurement of Absorbance

i. Sample solution was prepared by thoroughly mixing 1.5 ml of 60 µM DPPH solution and 1.5 ml of test sample solution. The solution was then allowed to stand at room temperature for 30 minutes.
ii. Control solution was prepared by mixing 1.5 ml of 60 µM DPPH solution and 1.5 ml of 95 % ethanol. The solution was then allowed to stand at room temperature for 30 minutes.
iii. Blank solution was prepared by mixing 1.5 ml of test sample solution and 1.5 ml of 95 % ethanol. The solution was then allowed to stand at room temperature for 30 minutes.

Absorbance of these solutions was measured at 517 nm by using UV-7504 sepectrophotometer. Experiment was done in triplicate for quince sample solution and % inhibition was calculated by using the following equation.

$$\% \text{ Inhibition of Oxidation} = \frac{A - B}{A} \times 100\%$$

A = Absorbance of DPPH solution

B = Absorbance of sample + DPPH solution

Finally, IC_{50} (50 % inhibition concentration) was determined by using linear regressive excel program.

Ascorbic acid was used as a standard antioxidant for comparison purpose.

Determination of Mineral Content

The mineral contents of quince were determined by Energy Dispersive X-rays Fluorescence Spectroscopy measured at Department of Physics, University of Mandalay.

Results and Discussion

The main aim of this research work, quince was selected for determination of ash, moisture, pH, fiber, mineral composition, vitamin C content and antioxidant activity.

Quince also provides small amounts of vitamin C. Quince contains vitamin C (0.101) and antioxidant (271.81) that prevents aging and beautifies for skin. The amount of fiber (0.88٪) found in quince can help feed the healthy bacteria that live in the digestive tract, which in turn helps reduce inflammation and improve resistance to infection. The quince is a goof source of minerals. This mineral is necessary for proper functioning of the nervous system and muscles.

Determination of Elemental Compositions in Quince by EDXRF

The sample was found to be highest value of potassium (1.580 %), the second highest was calcium (0.4353 %) and the third was the silicon (0.2750 %). The toxic metals were observed in this sample but the amounts of these metals were very trace. So, they were not toxic for human, the results are shown in Table 1.

Table 1. Results of Elemental Compositions of Quince

No.	Element	Symbols	Concentration (%)
1	Potassium	K	1.580
2	Calcium	Ca	0.435
3	Silicon	Si	0.275
4	Iron	Fe	0.128
5	Aluminum	Al	0.114
6	Phosphorus	P	0.090
7	Chlorine	Cl	0.067
8	Sulfur	S	0.012
9	Titanium	Ti	0.012
10	Manganese	Mn	0.003

Determination of Vitamin C Content in Quince

Vitamin C content in quince was determined by spectrophotometry using potassium chromate-diphenylcarbazide. In this case, the determinations were repeated three times and the mean value was taken. The result is shown in Table 2.

Table 2. Vitamin C Content in Quince

Sample	Vitamin C Content
Quince	0.101%

Determination of Ash, Moisture, Fibre and pH Contents in Quince

Ash, moisture, fibre and pH contents in quince were determined. The results are shown in Table 3.

Table 3. Result of Ash, Moisture, Fibre and pH Contents in Quince

No.	Parameters	Content
1	Ash	6.5%
2	Moisture	9.02%
3	Fibre	0.88%
4	pH	4.7

Standard Curve for Cr-diphenylcarbazide Complex with Respect to Ascorbic Acid Concentration

The standard curve was constructed using concentrations of standard ascorbic acid solution. Absorbance of Cr-dipenylcarbazide complex was related to ascorbic acid concentration. Ascorbic acid concentration was inversely proportional to absorbance of the complex at 548 nm.

Table 4. Concentration of Ascorbic Acid and Absorbance of Cr-diphenylcarbazide Complex

No.	Concentration of Ascorbic Acid	Absorbance at 548 nm
1	2	1.87
2	3	1.62
3	4	1.33
4	5	0.99
5	6	0.76
6	7	0.48

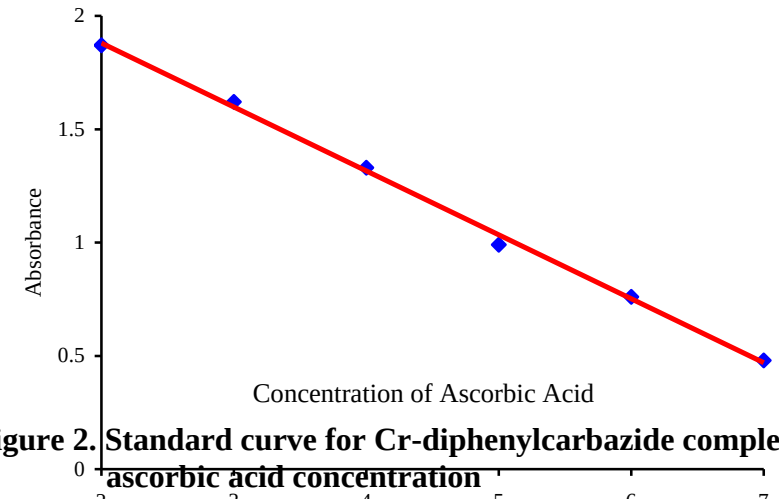

Figure 2. Standard curve for Cr-diphenylcarbazide complex with respect to ascorbic acid concentration

Determination of Antioxidant Activity of Crude Extract from Quince

According to pre-test, it was found that ethanol; ethyl acetate and n-Hexane extracts could change the color of DPPH from purple into yellow. Therefore, it can be inferred that, ethanol extracts may possess the DPPH free radical

scavenging activity. Consequently, the antioxidant activity was studied on the ethanol extracted by DPPH assay.

The results obtained are tabulated in Table 5 and 6. The inhibition of ethanol extract of quince was also compared with that of ascorbic acid.

In addition, from these results, IC_{50} values (50% inhibition concentration) for ethanol extract of quince and ascorbic acid were determined by linear regressive excel program and the results are described in Table 5. The IC_{50} value was found to be 271.81µg/mL for ethanol extract of quince. Figure 3. shows the bar graph of IC_{50} values or ethanol crude extract and standard ascorbic acid.

According to Figure 4. IC_{50} value of quince is lower than that of standard ascorbic acid (IC_{50} = 2.23).

Table 5. % Inhibition of Various Concentration and IC_{50}Values for Standard Ascorbic Acid

Extracts	Concentration (µg/mL)	Mean Absorbance	Mean % inhibition	IC_{50} (µg/mL)
Ascorbic acid	1.25	0.221	28.71	2.23
	2.5	0.152	50.97	
	5.0	0.091	70.65	
	10.0	0.026	91.61	

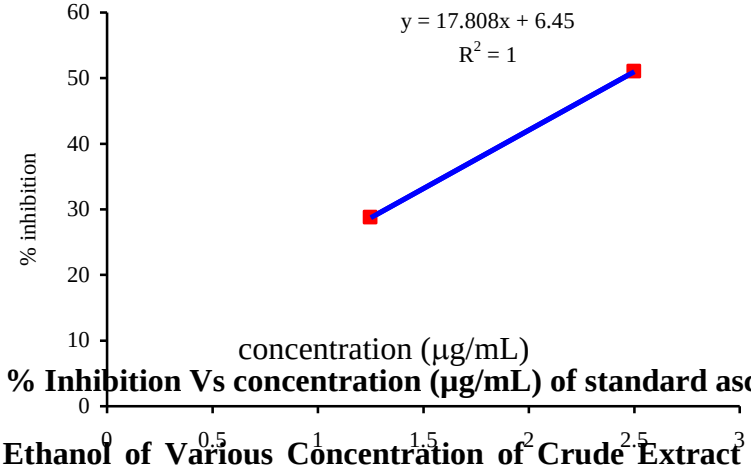

Figure 3. % Inhibition Vs concentration (µg/mL) of standard ascorbic acid

Table 6. Ethanol of Various Concentration of Crude Extract and IC_{50} Values (Quince Fruits)

Extracts	Concentration (µg/mL)	Mean Absorbance	Mean % inhibition	IC_{50} (µg/mL)
Ethanol extract (sample)	25	0.4915	8.98	271.81
	50	0.4655	13.80	
	100	0.4125	23.61	
	200	0.327	39.44	
	400	0.1665	69.165	

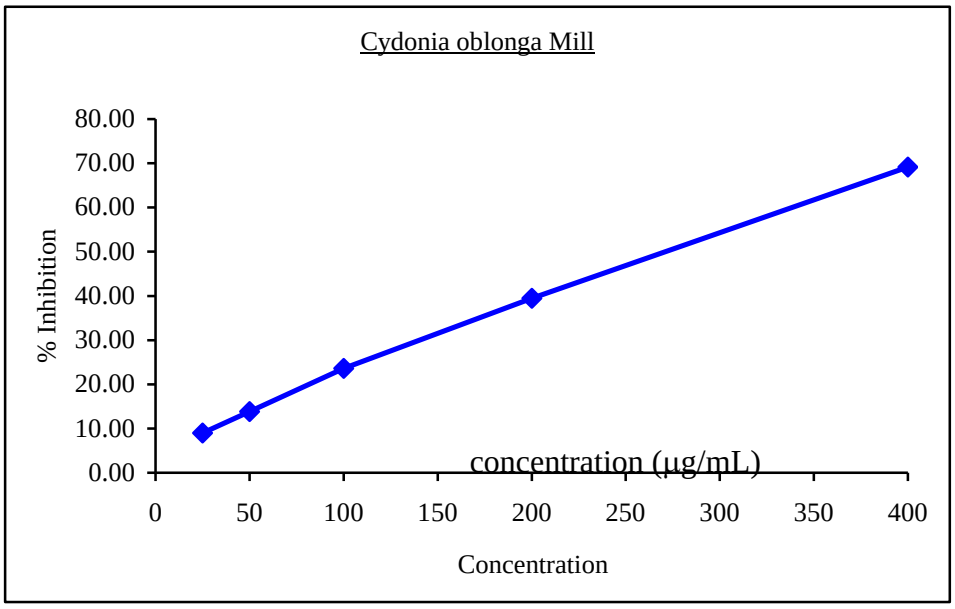

Figure 4. % inhibition Vs concentration (µg/mL) of crude ethanol extract of quince

Conclusion

In this research work, quince was selected for chemical analysis. Ash, moisture, pH and fibre contents of quince were determined and it was found that ash content (6.5%), moisture content (9.02%), pH v alue (4.7) and fibre content (0.88%). Ascorbic acid content of quince was quantitatively determined by spectrophotometry method using potassium chromate diphenylcarbazide reagents. Vitamin C content of quince was 0.101 %. Vitamin C is an essential vitamin for improving skin health.

In addition, the determination of antioxidant activity of ethanol crude extract was performed by DPPH assay. Ascorbic acid was used as standard antioxidant. IC_{50} values were also calculated and the comparison of antioxidant activity of ethanol crude extract with ascorbic acid was described. It is observed that antioxidant activity of ethanol extract of quince is lower than that of ascorbic acid. From EDXRF study, it can be found that quince contains ten principal elements K, Ca, Si, Fe, Al, P, Cl, S, Ti, Mn. It can be concluded that quince should be taken daily to get minerals which are beneficial for human health.

Acknowledgements

I would like to express my sincere gratitude to the Rector Dr Thar Tun Maung, Dagon University, for his kind permission to the submission of this paper. I would like to express my sincere thanks to Dr Thidar Kyaw, Professor and Head, Department of Chemistry, Mandalar University and Dr Hnin Hnin Than, Professor and Head, Department of Chemistry, Dagon University, for their encouragement and assistance.

References

Farnsworth, NR. and N Bunyapraphatsara., (1992) “Thai Medicinal Plants Recommended for Primary Health Care System”. First Edition, Mehidol University, Thailand, Medicinal Plants Information Centers, Page 276-304.

Kaur, C. and HC, Kapoor., “Antioxidants in fruits and vegetables the millennium’s health”, Intl J Food Sci Tech, Volume 36, Issue 7, Page 703-25

Khin Thandar Htun. (2014), "Determination of Vitamin C Content and Antioxidant Activity of *Spinaceae oleracea* L. (Spinach)," MSc Thesis, Yadanabon University, Page 18-29

Rop O., Balik J., Reznicek V., Jurikova T., Skardova P., Salas P., Sochor J., Mlcek J., Kramarova D. (2011): Chemical characteristics of fruits of some selected quince (*Cydonia oblonga* Mill.) cultivars. Czech J. Food Sci., Volume 29, Issue 1, Page 65-73

Yin Mar Htay. (2014), "Investigation on the Vitamin C Content and Antioxidant Activity of *Hibiscus sabdariffa* L. (Roselle)", MSc Thesis, Yadanabon University, Page 13-28.