

Isolation and Functional Groups Determination of Pectin from the Peels of *Citrus Maxima*

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Abstract

In this research work, the peels of *Citrus maxima* were selected for chemical analysis. Firstly, the sample was tested by phytochemical screening which gave rise to positive results for glycoside, phenolic compound, polyphenol, lipophenol, flavonoid, alkaloid and carbohydrate. After that, Pectin was isolated from peels of citrus maxima by using different concentrations of hydrochloric acid and different refluxing times. Finally, the isolated compound was characterized by FTIR (Fourier Transform Infrared) spectroscopic method.

Key word: *Citrus maxima*, concentrations, refluxing, pectin, FTIR

1. Introduction

Pectin is one of the most versatile stabilizers available. It's gelling, thickening and stabilizing is an essential additive in the production of many food products. Traditionally, pectin was primary used in the production of jams and fruit jellies industrially as well as domestically. Pectin is a key stabilizer in many food and organic food products as well as fruit applications, bakery fillings and toppings, dairy applications, confectionery, beverages, nutritional and health products and pharmaceutical and medical applications. Furthermore, Pectin can be applied in pharmaceutical industry as carrier for drug delivery to the gastrointestinal tract, such as matrix tablet, gel beads, film-coated dosage form.[3]. Pectin favorably influences cholesterol levels in blood. It has been reported to help reduce blood cholesterol in a wide variety of subject and experimental conditions as comprehensively reviewed. For consumption of fat, at least 6g/day of pectin is necessary to have a significant effect in cholesterol reduction. Many techniques have been used to manufacture the pectin- based delivery systems, especially gel coating. There are several ways to extract pectin from different materials and solvents. The extraction of pectin usually uses two kinds of solvents: inorganic acid solvents such as hydrochloric, sulfuric or nitric acid and organic acid solvent such as oxalic, tartaric or acetic acid. Pectin, naturally polysaccharides is 20-40% of the dry matter content of citrus peel (eg. pomelo, lemon, lime and orange peels etc.). Commercial pectin is extracted at high temperature by hydrolyzing protopectin using acid.[4]. In this research, pectin was extracted from *Citrus maxima* peel by using different concentrations of

extraction solvent and extraction time. In addition the chemical constituents of *Citrus maxima* peel were detected by phytochemical test. The extraction of pectin was characterized by FT-IR spectroscopic method.

Botanical Description[1]

| | |
|----------------|------------------------|
| Botanical Name | : <i>Citrus maxima</i> |
| Family Name | : Rutaceae |
| English Name | : Pomelo |
| Myanmar Name | : Kywe-gaw |
| Part Used | : Peels |



Figure 1. The Fruits of *Citrus maxima*

2. Experimental

2.1 Sample Collection

The *Citrus maxima* (pomelo) were collected from local market, Mandalay, Mandalay Region. The *Citrus maxima* (Pomelo) was cut to get the peels. The peels of pomelo were dried in the air and ground into finely powder by using a blender. The powder was sieved by using 100 mesh sieves. Then the sample was packed in a stoppered bottle and it was used throughout the experiment.



Figure 2. Dried Peels and Powder of *Citrus maxima*

2.2 Preliminary Phytochemical Test [2]

Preliminary phytochemical tests were carried out to detect the presence or absence of organic constituents in the *Citrus maxima* (pomelo) peels.

2.3 Extraction of Pectin from *Citrus maxima* Peel Powder by Using Different Concentrations of HCl

50 milliliter (mL) of 0.1 Molarity (M) hydrochloric acid was added to 1 gram (g) sample powder and stirred for 1 hour and reflux for about 30 minutes. Then, the mixture was stand for 24 hours at room temperature. The mixture was filtered. 30 mL of 95 % ethanol was added to the filtrate and this mixture was placed in the dark room for 24 hours. After 24 hours, the mixture was filtered and the precipitate was washed with 20 mL of acetone and dried. The yield percent was calculated.

$$\text{Percent yield} = \frac{\text{weight of pectin}}{\text{weight of sample}} \times 100$$

Similarly, the same procedure was carried out by using 0.2 M HCl, 0.3 M HCl, 0.4 M HCl, 0.5 M HCl, 1.0 M HCl, 2.0 M HCl, 3.0 M HCl, 4.0 M HCl and 5.0 M HCl respectively. The yield percent was calculated and the data was shown in Table 1.

2.4 Extraction of Pectin from *Citrus maxima* Peel Powder by Different Refluxing Times

The sample powder 1 g was mixed with 50 ml of 2.0 M hydrochloric acid in a flask. Then the mixture was refluxed for 1 hour. After refluxing period, the mixture was stand for 24 hours and then filtered. 30 mL of 95 % ethanol was added to the filtrate and the mixture was placed in a dark condition for 24 hours. After 24 hours the mixture was filtered and the precipitate was washed with 20 mL of acetone and dried. The yield percent was calculated.

$$\text{Percent yield} = \frac{\text{weight of pectin}}{\text{weight of sample}} \times 100$$

In order to know the effect of time taken, refluxing period was changed 60 minutes, 90 minutes and 120 minutes. The yield percent were calculated.

2.5 FT-IR Spectrophotometric Analysis

The FT-IR Spectra of isolated pectin was recorded by using Perkin Elmer GX system FT-IR spectrophotometer at the Department of Chemistry, University of Mandalay.

3. Results and Discussion

3.1 Preliminary Phytochemical Test for the Peel

Powder of *Citrus maxima* (Pomelo)

The peels of *Citrus maxima* (pomelo) were tested by phytochemical screening and these results are shown in Table 1.

Table 1. The Results of Phytochemical Tests for *Citrus maxima*

| Tests | Reagent used | Observation | Result |
|-------------------|--|------------------------------|--------|
| Tannin | 10% FeCl ₃ , dil (H ₂ SO ₄) | no ppt | - |
| Saponin | Distilled water | no froth | - |
| Glycoside | 10% lead acetate | White ppt | + |
| Phenolic compound | 10% FeCl ₃ | Brown color solution | + |
| Polyphenol | 1% FeCl ₃ + 1% K ₃ [Fe(CN) ₆] | Greenish blue color solution | + |
| Lipophenol | 0.5 M KOH, NaOH | Pale brown color solution | + |
| Steroid | Acetic anhydride, Conc: H ₂ SO ₄ , CHCl ₃ | no green color solution | - |
| Flavonoid | Conc: HCl, Mg | Pink color solution | + |
| Terpene | Conc: H ₂ SO ₄ , CHCl ₃ | no pink color solution | - |
| Alkaloids | (i) Dragendorff's reagent (ii) Wagner's reagent | Orange ppt Brown ppt | + |
| Reducing Sugar | Benedict's solution | no ppt | - |
| Carbohydrate | Fehling's (A)+(B) | Yellow ppt | + |

(+) = presence, (-) = absence

According to this table, peels of *Citrus maxima* extract consist of glycoside, phenolic compound, polyphenol, lipophenol, flavonoid, alkaloids and carbohydrate respectively.

3.2 Extraction of Pectin from *Citrus maxima* Peel Powder by using various Concentrations of HCl

Pectin extraction was performed using various concentrations of HCl such as 0.1 M HCl, 0.2 M HCl, 0.3 M HCl, 0.4 M HCl, 0.5 M HCl, 1.0 M HCl, 2.0 M

HCl, 3.0 M HCl, 4.0 M HCl and 5.0 M HCl respectively. The yield percent of pectin was shown in Table 2.

Table 2. Yield Percent of Pectin from *Citrus maxima* Peel Powder by using Various Concentrations of HCl

| Weight of sample (g) | Concentration of HCl (M) | Yield percent of pectin (%) |
|----------------------|--------------------------|-----------------------------|
| 1 | 0.1 | 27.27 |
| 1 | 0.2 | 32.14 |
| 1 | 0.3 | 34.81 |
| 1 | 0.4 | 35.70 |
| 1 | 0.5 | 39.83 |
| 1 | 1.0 | 41.05 |
| 1 | 2.0 | 42.20 |
| 1 | 3.0 | 38.17 |
| 1 | 4.0 | 33.14 |
| 1 | 5.0 | 29.82 |

According to these results the maximum yield from *Citrus maxima* was found to be 42.20 % at 2.0 M HCl.

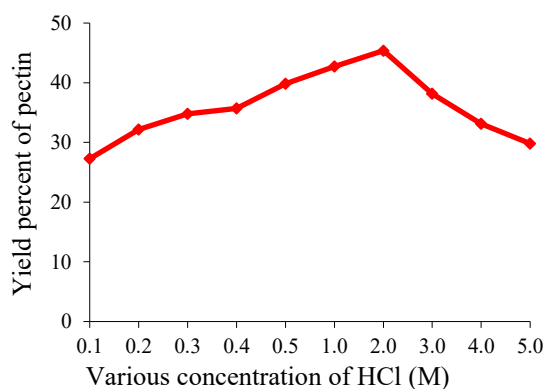


Figure 3. Yield Percent of Pectin from *Citrus maxima* Peel Powder by Using Various Concentrations of HCl

According to these results, the maximum yield percent was obtained by using 2.0 M HCl. Therefore the optimum condition was chosen that 2.0 M HCl.

3.3 Extraction of Pectin from *Citrus maxima* Peel Powder by Different Extraction Times

Pectin extraction was carried out by using 2.0 M HCl at different refluxing period such as 60 minutes, 90 minutes and 120 minutes respectively. The results were shown in Table 3.

Table 3. Yield Percent of Pectin from *Citrus maxima* Peel Powder by using Various Extraction Times

| Weight of Sample(g) | Concentration of HCl (M) | Extraction time (minutes) | Yield percent of pectin |
|---------------------|--------------------------|---------------------------|-------------------------|
| 1 | 2.0 | 60 | 42.20 |
| 1 | 2.0 | 90 | 40.29 |
| 1 | 2.0 | 120 | 37.77 |

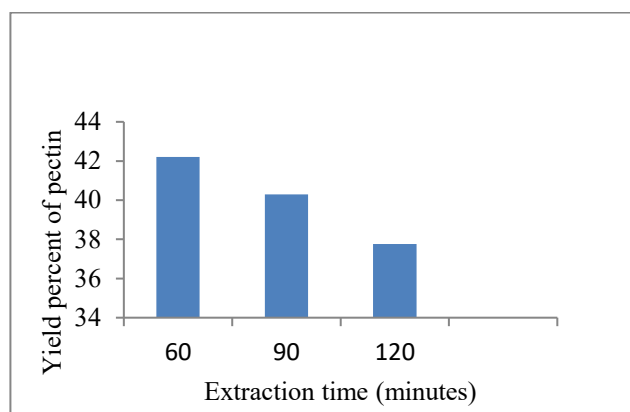


Figure 4. Yield percent of Pectin from *Citrus maxima* peel powder by using different refluxing times

From the results, the maximum value of pectin from *Citrus maxima* was found to be 42.20 % during 60 minutes refluxing time.

3.4 FTIR Assignment for Isolated Compound

Table 4. Characteristics Absorption Peaks of IR and their Assignment

| Bands (cm^{-1}) | Assignments |
|----------------------------|---|
| 3435 | O-H stretching vibration of alcohol and acid group |
| 2935 | C-H stretching vibration of sp^3 hydrocarbon |
| 1741 | C=O stretching vibration of carbonyl group |
| 1334, 1228 | C-C-O stretching vibration of alcohol group |
| 1147, 1099 | C-O-C stretching vibration of ether group |

From the FTIR Spectral data, the strong broad band which appears at 3435 cm^{-1} should be O-H stretching vibration of alcohol and acid groups. The band at 2935 cm^{-1} implies the C-H stretching vibration of sp^3 hydrocarbon. The intense peak at 1741 cm^{-1} indicates C=O stretching vibration of carbonyl group. In addition, the two bands at 1334 cm^{-1} and 1228 cm^{-1} should be C-C-O stretching vibration of alcohol groups. The two

bands which occur at 1147 cm^{-1} and 1099 cm^{-1} should be C – O – C stretching vibration of ether group.

3.5 Comparison of FTIR Spectra of Isolated Compound and Reference Compound

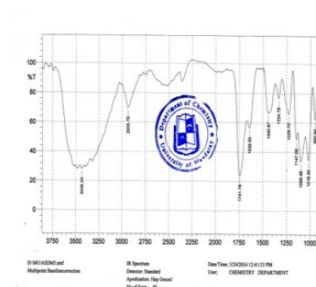


Figure 5. FTIR Spectrum of Isolated Pectin

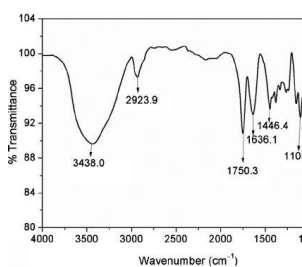


Figure 6. FTIR spectrum of Reference Pectin (Adopted from Savior et.al., 2015)

Moreover the FTIR spectra of isolated compound and reference pectin compound were compared. From the reference spectral data, alcohol group observed at 3438 cm^{-1} . C-H stretching vibration of sp^3 hydrocarbon appeared at 2923.9 cm^{-1} . C=O stretching vibration of carbonyl group found at 1750.3 cm^{-1} and C-C-O stretching vibration of alcohol group indicated at 1446.4 cm^{-1} . Furthermore, C-O-C stretching vibration of ether group implied at 1105.6 cm^{-1} and 1016.2 cm^{-1} . In addition, the isolated compound also contained these functional groups. According to comparison of these spectra, the isolated compound may be Pectin.

4. Conclusion

In this research, the sample was tested by phytochemical screening which gave rise to positive results of glycoside, phenolic compound, polyphenol, lipophenol, alkaloid, flavonoid and carbohydrate.

Pectin was prepared from *Citrus maxima* peel powder by using different concentration of HCl such as 0.1 M HCl, 0.2 M HCl, 0.3 M HCl, 0.4 M HCl, 0.5 M HCl, 1.0 M HCl, 2.0 M HCl, 3.0 M HCl, 4.0 M HCl and 5.0 M HCl respectively. The condition that used 2.0 M HCl was chosen as optimum condition because the maximum amount of yield was obtained at that condition.

In addition the pectin was isolated from *Citrus maxima* peel powder by using 2.0 M HCl with different refluxing time such as 60 minutes, 90 minutes, 120 minutes respectively. It was found that the maximum yield percent (42.20 %) was obtained for 60 min refluxing period.

Finally, the isolated compound was characterized by FTIR (Fourier Transform Infrared) spectroscopic method. According to FT-IR spectral data, the sample consists of alcohol and acid group, sp^3 hydrocarbon, carbonyl group and ether group respectively. FTIR spectrum of isolated compound was compared with reference spectrum. Therefore it may be Pectin. In

addition, pectin can be applied in pharmaceutical industry as carrier for drug delivery to the gastrointestinal tract, such as matrix tablets, gel beads, film-coated dosage form.

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