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Extraction and Hydrolysis of Naringin from Citrus fruit peels

Akrati Sant¹, Imran Ahmad¹, Sonika Bhatia^{*1}

¹Department of Chemistry, Isabella Thoburn College, Lucknow 226007, UP, India

*Email: sonikaitc2@gmail.com

Abstract. Naringenin, a flavonoid belonging to the flavanones subclass has been reported to have several promising biological activities like antioxidant, antitumor, anticancer, antiviral, anti-inflammatory & estrogenic activities. Naringin is found in the glycosidic form in citrus fruits like grapefruit, orange, and lemons. Naringenin is obtained by the hydrolysis of the glycoside naringin. In the present study, environmentally benign extraction methods have been reported for the extraction of naringin from the peels of grapefruit (*Citrus X paradisi*), an agricultural waste of the fruit juice industry. Successful isolation of naringin from both fresh and dry albedo of grapefruit has been carried out under three different conditions to yield pure white crystalline naringin. Further, simple acidic hydrolysis under three different conditions has been standardized to yield naringenin in a relatively good yield. Characterization of both naringin and naringenin has been done using NMR and thin layer chromatography.

Keywords: naringin, extraction, hydrolysis, naringenin

1. Introduction

In today's growing world, the waste output is increasing in huge volumes on a daily basis, affecting the ecosystem and eventually human health. Citrus fruits are one of the most extensively grown fruits the world over, resulting in an enormous number of byproducts that are rich in flavonoids, cellulose, pectin, soluble sugars, polyphenols, dietary fibers, and essential oils [1,2]. Unfortunately, a substantial number of leftovers from citrus fruits end up as garbage in farm fields and disposal bins. Every year, the citrus processing industry generates massive volumes of trash, with citrus peel waste accounting for over half of the wet fruit mass [2]. The waste citrus fruit peels are of great interest among researchers and scientists as they are a rich source of phytochemicals that are beneficial in treating various long-standing diseases. Flavonoids are potent phytochemicals present in vegetables and fruits with many health benefits. Subclasses of flavonoids are flavanols, flavones, flavanones, flavonols, anthocyanins and isoflavones [3]. One of the promising phytochemicals is naringin and its a-glycolate form naringenin. They are flavanones, a subclass of flavonoids, and possess many biological activities like anticancer, antidiabetic, anti-inflammatory, antimicrobial, antiosteoporosis, anti-estrogenic, anti-oxidant, and have been found to be effective in treating cardio and neurovascular diseases [4]. Applications of flavonoids are also reported in the pharmaceuticals, medicinal, nutraceutical and cosmetic industries [5]. Various flavonoids are found in grapefruit, lemon, oranges, and lime, naringin being one of them [4]. Grapefruit which is the result of the crossing of pomelo and the sweet orange is a rich source of naringin [6]. Naringin has been extracted from waste grapefruit peels by cold extraction and Soxhlet extraction. Extraction of natural products is quite challenging as they contain heat-labile linkages and some essential oils. In literature, enzymatic hydrolysis of naringin has been used to convert it into its a-aglycone form naringenin [7]. Simple and efficient hydrolysis of naringin under different conditions can also be carried out [8,9]. In this work, hydrolysis of naringin has been carried out by 3 methods to yield the a-aglycone naringenin. In this study, we standardized 3 ways of extraction and hydrolysis methods under different conditions.



2. Materials and Method

2.1 Chemicals and instruments

Commercially available ethanol, methanol, chloroform, (all solvents from Rankem, India), and sulfuric acid (Rankem, India) were used in the process. The extraction process and reactions were monitored using TLC plates. ^1H NMR spectra were recorded at Avll HD-300(FT NMR) on Bruker equipment (Avance) using deuterated methanol and acetone as a solvent.

2.2 Grapefruits and Grapefruit peels

Fresh grapefruits (*Citrus x paradisi* L.) were purchased and grapefruit peels were also collected from juice centers of Dehradun, Uttarakhand. The albedo (inner white spongy part) from the grapefruit peels was separated from the rest of the peel which is flavedo (orange exterior). The albedo was then air-dried for 4-5 days. The fresh albedos were also hand-sliced into small pieces and churned in a grinder for the direct extraction process.

2.3 Extraction of Naringin

The extraction of naringin from the albedos of grapefruit (Figure 1) was carried out with some modifications to the methods described in the literature [6]. The extraction process was carried out by three different methods to get a better yield of naringin using both dry and fresh albedo.

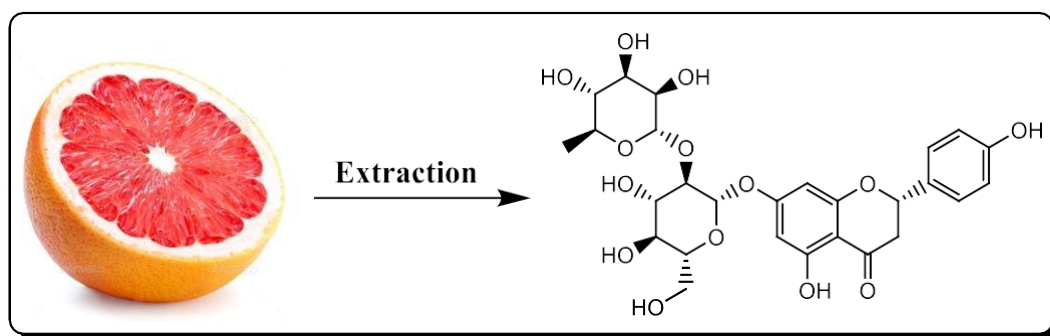


Figure 1: Extraction of Naringin from grapefruit albedo

2.3.1 Method 1: Soxhlet extraction using dry albedo in methanol: The extraction was carried out using the Soxhlet apparatus with 40g of the dried albedo of grapefruit (figure 2). The solvent used for Soxhlet extraction was methanol and temperature was maintained at 55-65 °C for approximately 3 h. After leaving the extract overnight, colourless crystals of pectin (figure 3) were collected from the extract and the remaining total methanolic extract was reduced to 10-12 g under reduced pressure at 45-50 °C and 20-25 cm³ of water added to that extract. After stirring for 30 min at 60-70 °C, this methanolic extract was left for 3 days in a stoppered flask after adding 3 cm³ of chloroform in it. On standing for 3 days, fine naringin crystals were formed and were filtered out using sintered crucible and collected. The progress of extraction was monitored and the purity of the obtained compound was checked using glass TLC plates coated with silica gel-G by qualigens in the solvent system methanol, water, and acetic acid (5:4:1) as shown in figure 4 and validated using NMR spectroscopy (figure 5).

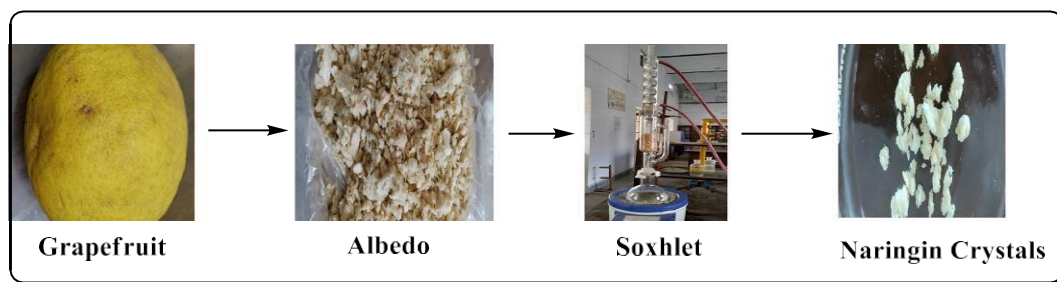


Figure 2: Pictorial representation of the conducted experiment for the extraction

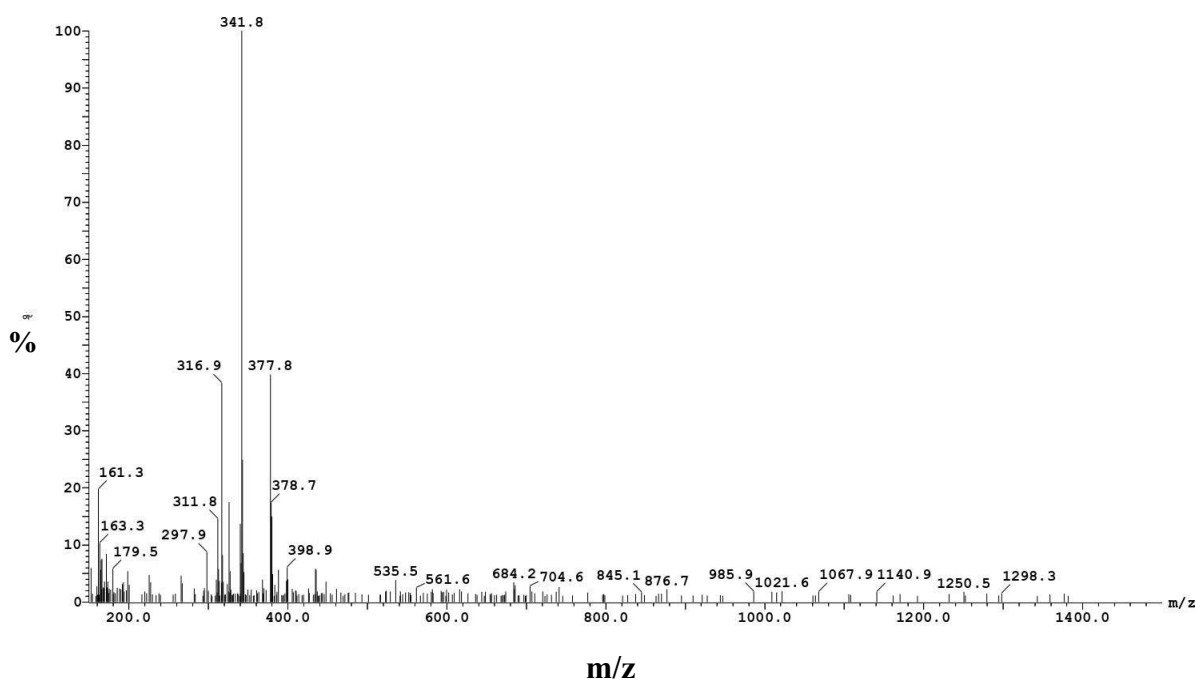


Figure 3: Mass Spectra of Pectin

2.3.2 Method 2: Soxhlet extraction using fresh albedo in methanol: The extraction was carried out using the Soxhlet apparatus with 40 g of the fresh albedo of grapefruit. The solvent used for Soxhlet extraction was methanol and the temperature was maintained at 55-65 °C for approximately 3 h. After that methanolic extract was reduced to 10-12 g under reduced pressure at 45-50 °C, and 20-25 cm³ of water was added to the extract. After stirring for 30 min at 60-70 °C this methanolic extract was left for 3 days in a stoppered flask after adding 3 cm³ of chloroform in it. After 3 days, fine naringin crystals were filtered using sintered and collected. Characterization was done as described in method 1.

2.3.3 Method 3: Cold extraction of dry albedo: Cold extraction using 30g of the dried albedo of grapefruit has been carried out in methanol. The albedo was immersed in methanol and left for 4 days in a stoppered flask. After four days, the methanolic extract was decanted and reduced to 10-12 cm³ under reduced pressure of 45-50 °C. After the addition of 20-25 cm³ water to the methanolic extract, it

was stirred for 30 min at 60-70 °C temperature. The methanolic extract was left undisturbed for 3 days in a stoppered flask after adding 3 cm³ of chloroform. After 3 days, naringin crystals were filtered using sintered and collected. The characterization was done as described in method 1.

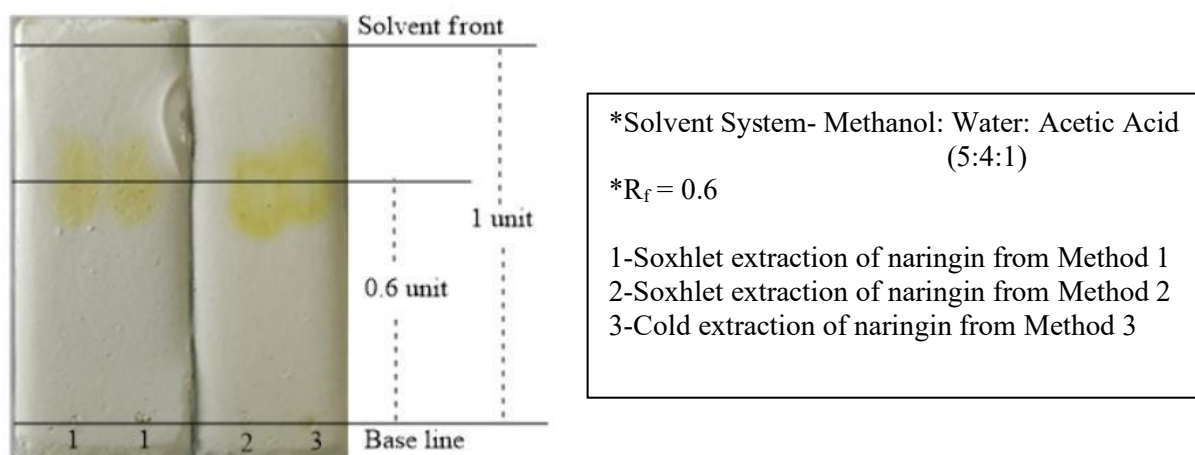


Figure 4. Comparative TLC of naringin extracted by methods 1, 2 & 3

2.3.4 Analytical data of Naringin: White solid, M.P- 169.1°C- 170°C. ¹H NMR (MeOD, 300 MHz): δ 1.3 (dd, J= 6.3 Hz, 3H), 2.8-2.7 (dd, J = 17.1 Hz and 3 Hz, 1H), 3.9-3.1 (m, 10H), 3.89(dd, 1H), 5.25 (s, 1H), 5.1 (dd, 1H), 5.35 (dd, J = 13.5 Hz and 11.7 Hz, 1H), 6.18 (dd, J = 2.1 Hz, 1H), 6.15 (dd, J = 4.8 Hz, 1H), 6.85 (d, J = 7.2 Hz, 2H), 7.35 (d, J = 7.2 Hz, 2H).

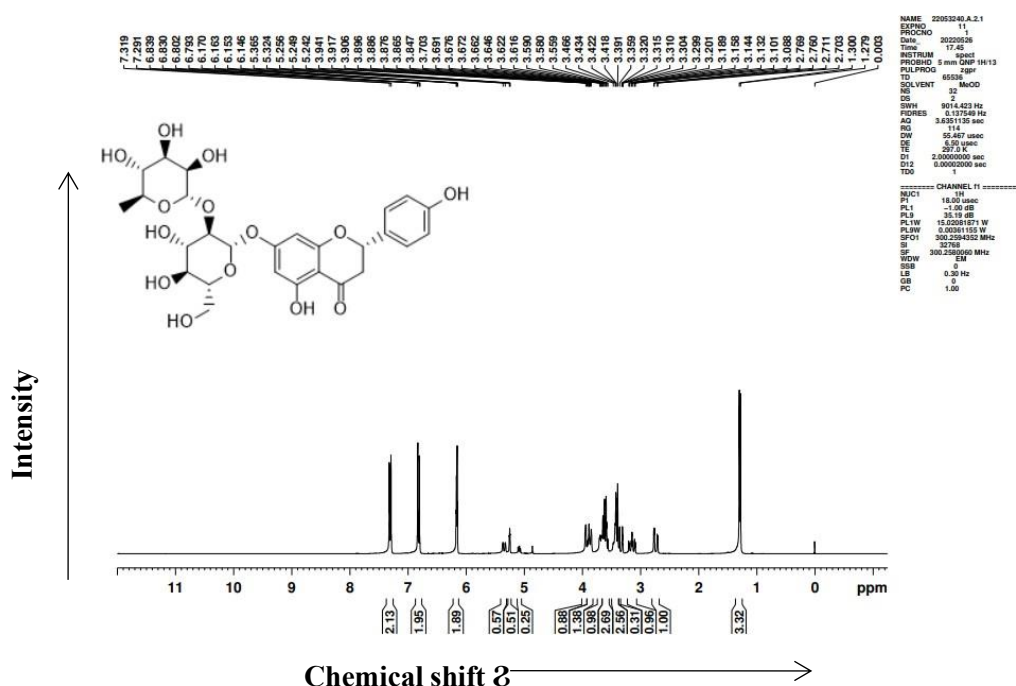


Figure 5: ¹H NMR of Naringin obtained from grapefruit albedo

2.4 Hydrolysis of Naringin

Naringenin is derived from the hydrolysis of naringin (figure 6). Literature shows enzymatic hydrolysis of naringin to yield naringenin. Simple and efficient hydrolysis of naringin under different conditions can also be carried out to get a better yield without further purification.

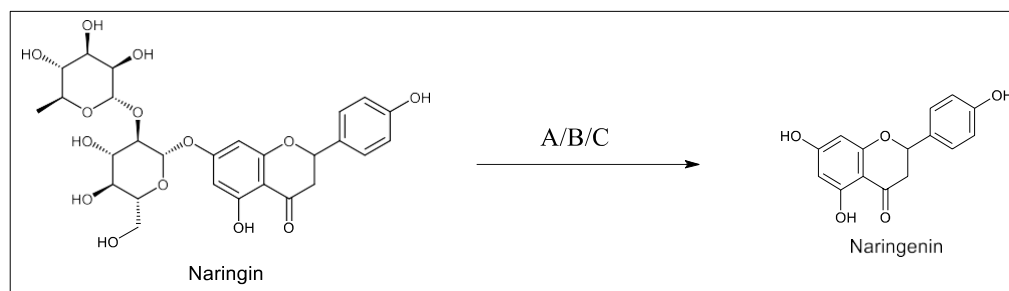


Figure 6: Hydrolysis of Naringin into Naringenin

2.4.1 : Method A: Acidic Hydrolysis in Ethanol. 2% 15 cm³ H₂SO₄ ethanolic solution was added to 0.1 g of naringin in the round bottom flask. The mixture was then stirred and refluxed at 80 °C to 85 °C for 13h. The progress of the reaction was monitored via TLC. The reaction was monitored on glass TLC plates coated with silica gel-G using chloroform and methanol (9:1) as a solvent. The reaction was run until a single spot-on TLC was obtained, as shown in figure 7. The overall yield of 65 % was obtained and purification was done by column chromatography using ethyl acetate and hexane (50:50) as solvent. The purity of the product was checked using thin layer chromatography (solvent system) with an authentic sample of naringenin (Tokyo Chemical Company/Japan). Further validation was done by NMR spectroscopy (figure 8).

2.4.2 : Method B: Acidic Hydrolysis in Water. 2% 15 cm³ H₂SO₄ water solution was added to 0.1 g of naringin in a round bottom flask. The mixture was then stirred and refluxed at 81°C to 85 °C for 14 h. Progress of the reaction was monitored via TLC. Characterization of the compound was done as described in method A. Purification of the product was done by column chromatography. The overall yield obtained was 45 %.

2.4.3 : Method C: Acidic Hydrolysis using the biphasic system. 2% 50 cm³ H₂SO₄ water solution was added to 0.1 g of naringin in a round bottom flask with 100 cm³ chloroform in it. The mixture was stirred and refluxed at 81 °C to 85 °C for 5h. The progress of the reaction was monitored via TLC until a single spot came. Characterization of the compound was done as described in method A. White crystalline product was obtained requiring no further purification. The overall yield from this method was 67 %. Comparative TLC of hydrolysis of naringin into naringenin by methods A, B & C is shown in figure 7.

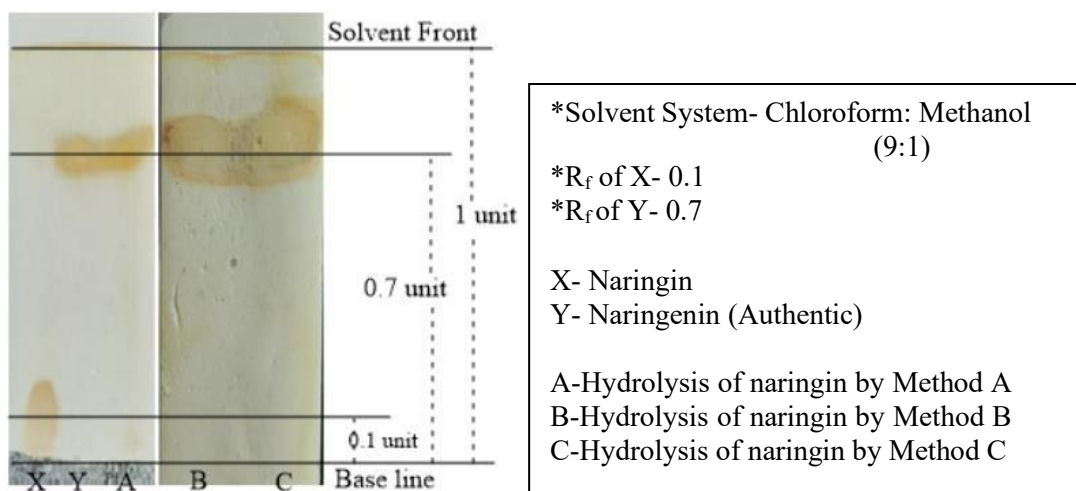


Figure 7: Comparative TLC of hydrolysis of Naringin into naringenin by methods A, B & C

2.4.4 : *Analytical data of Naringenin*: White solid, M.P- 247°C- 248°C. ¹H NMR (Acetone, 300 MHz) δ 2.7 (dd, J = 17.1 Hz, 1H), 3.2 (m, J = 12 Hz, 1H), 5.5 (dd, J = 12.9 Hz, 1H), 5.93 (s, 2H), 6.9 (dd, J = 2.7 Hz and 1.8 Hz, 2H), 7.4 (dd, J = 8.7 Hz, 2H).

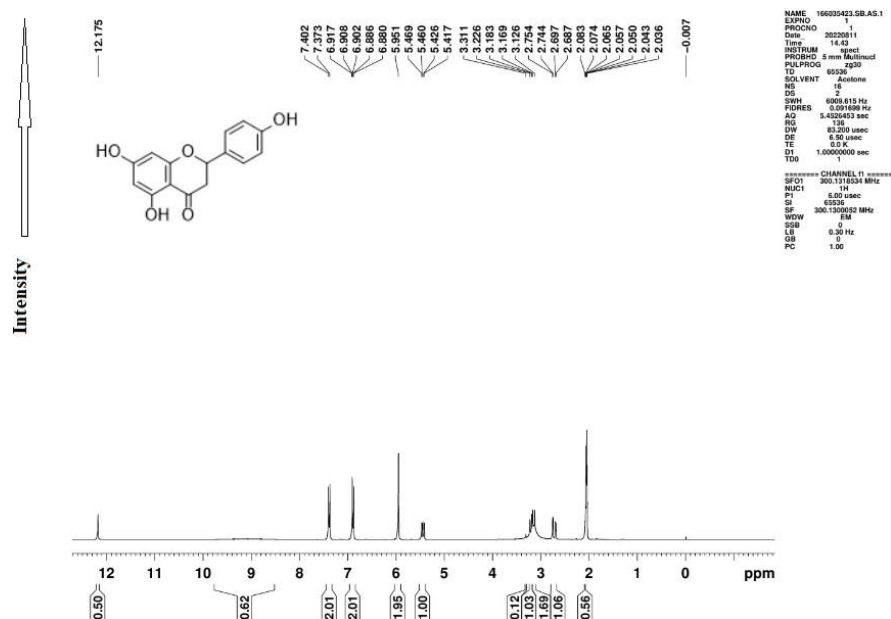


Figure 8: ¹H NMR (Acetone, 300 MHz) of Naringenin

3. Results & Discussion

Naringenin, the aglycon form of naringin has shown many biological activities like anti-cancer, anti-diabetic, anti-inflammatory, anti-microbial, anti-osteoporosis, anti-estrogenic, anti-oxidant, and in treating cardio and neurovascular diseases. It is thus a promising phytochemical. The derivatives of naringenin are easy to synthesize as compared to the glycolate naringin because naringin contains glucose units that are heat-labile, so the reactions which require high temperatures are less favourable.

Thus, naringenin is a better starting material for the derivatization and further development of pharmaceuticals.

In this study, firstly we validated the different extraction and hydrolysis methods of naringin from grapefruit peels and then made minor changes in conditions to improve the yield. In extraction *Method A*, along with naringin, we also got pectin thus it is a one-pot extraction of two very useful phytochemicals naringin and pectin. The extraction from the fresh albedo gave marginally more yield than the dry albedo which is 19.75 mg/gm. In the hydrolysis process, Biphasic-acidic hydrolysis yielded a pure compound in comparison to single-phase acid hydrolysis thus eliminating the need for further purification.

Table 1: Standardization of different extraction methods of Naringin from grapefruit albedo

S.No	Conditions	Solvent	Yield (Naringin/Albedo) (mg/g)	Yield reported in [6] (Naringin/Albedo) (mg/g)	Yield Pectin/Albedo (mg/g)
1.	Soxhlet extraction from Dry Albedo	Methanol	18	26	164
2.	Soxhlet extraction from Fresh Albedo	Methanol	19.75	10	negligible
3.	Cold extraction of Dry albedo	Methanol	11	22	negligible

Table 2: Standardisation of different methods of hydrolysis of naringenin

S. No.	Conditions	Yield (%)
A	Acidic Hydrolysis (Water)	45
B	Acidic Hydrolysis (ethanolic)	64
C	Bi-phasic Acidic Hydrolysis	67

4. Conclusion

From this study, it can be concluded that although extraction of naringin from wet albedo gave a better yield, though extraction from the dry albedo is more favorable as the content of

essential oils is negligible in dry albedo so the naringin obtained requires less purification. Handling and storage of dry albedo are much easier as compared to wet albedo which can develop fungal growth on keeping. Biphasic acid hydrolysis is the preferred technique in comparison to single-phase as it yields a pure white crystalline product. So, from the waste, the best can be extracted. Naringin & its aglycone naringenin can be used in treating various chronic illnesses and pectin can be used in the food industry.

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