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# Flavonoids content estimation in different parts of grapefruit

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**Abstract**
The present study was carried out to extract and estimate the different flavonoid compounds in different parts of grapefruit. Grapefruit used in this study was procured from Dehradun (UK). Peel of fruit was removed manually followed by collection of pulp and seed. Peel, pulp and seed collected were dried by two methods: Oven drying at 45-50 $^0$C (OD) and freeze drying at -76 $^0$C (FD). Dried samples were grounded to powder prior to extraction of flavonoids by using two methods: Soxhlet assisted extraction (SAE) and Ultrasonic assisted Extraction (UAE) using methanol as a solvent. The estimation of flavonoids was carried out on UPLC. Apigenin (4192.7 µg/g), Naringenin (19656 µg/g), Hesperidin (2999.95 µg/g), Quercetin (1429.19 µg/g), Rutin (994.17 µg/g), Epicatechin (984.33 µg/g), Daidzein (307.21 µg/g) were recorded in oven dried peels extracted via SAE method. Flavonoid content in freeze dried pulp sample extracted via SAE method had shown the maximum content of Naringenin (21109.9 µg/g) followed by Epicatechin (1080.32 µg/g), Apigenin (591.58 µg/g), Daidzein (552.92 µg/g), Hesperidin (391.83 µg/g), Rutin (277.75 µg/g) and Quercetin (252.21 µg/g). Daidzein 14.1 µg/g was least recorded flavonoids in oven dried seed sample processed via SAE method. While the maximum flavonoids reported in oven dried content was also processed via UAE seed sample method that is Naringenin (15800.4 µg/g).

**Keywords:** Grapefruit, ultrasonic assisted extraction, Soxhlet extraction, flavonoids

## 1. Introduction
Grapefruit is a hardy fruit grown in northeastern India, and some southern regions. It lasts for two weeks at room temperature and three to eight weeks when refrigerated. This large size with thick peel is a natural cross between pomelo and orange. Presence of Naringin, Limonoids, and Furanocoumarins contributes to its typical citrus fruit taste with bitter tinges. Carotenoids or anthocyanins content present in fruit are responsible for white, yellow, orange, and red colour. Large size seeds constitute around 4.5% of fruit weight. Vitamin C, provitamin A (carotenoids), carbohydrates, fiber, organic acids, essential amino acids, and fatty acids are the most common nutrients in grapefruit. Limonoids, flavonoids, monoterpenes, phytosterols, and polyphenols are also present in this fruit (Murthy *et al.*, 2020) [7]. Flavonoids and phenols are bioactive compounds found in fruits and vegetables, offering health benefits through radical scavenging and chelating activities (Seal, 2016) [2]. Flavonoids are a subclass of polyphenols with common structural features. They are found in fruits, vegetables, grains, and processed products. Flavonoids are categorized into subclasses based on their molecular structure. Ultrasound is used to remove phenolics from plant materials in both static and dynamic modes (Kivilompolo *et al.*, 2009) [10]. To utilize them in foods, pharmaceuticals, and cosmetic industries, their content must be known. Middledleton *et al.*, (1998) [11] discovered flavonoids have low atomic weight and three-ring structure. Solid-phase extraction is widely used, while high-performance liquid chromatography (HPLC) is a convenient technique for qualitative and quantitative analysis (Abidin *et al.*, 2014) [1]. Ultra-performance liquid chromatography (UPLC) offers high resolution, speed, and sensitivity as compared to HPLC (Chawla and Ranjan, 2016) [4]. Castor *et al.*, (2016) found that albedo has the highest concentration of flavonoids followed by flavedo, pulp, and seeds. Flavonoids are found in both edible and non-eatable plants and are extracted using various solvents. Tapiero *et al.*, (2002) [12] found flavonoids and phenol acids prevent carcinogenesis, inflammation, atherosclerosis, and thrombosis, with high cell reinforcement limit. Flavonoids are pervasive polyphenolic compounds found in nature, classified into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins, and chalcones.

Flavonoids are known for their therapeutic properties, particularly in preventing diseases and cardiovascular diseases. They can improve the body's response to allergens, infections, and cancer-causing agents, indicating resistance to harmful microbes and harmful pathogens. Flavonoids, like epicatechin, quercetin, and luteolin, can also help prevent intestinal cystic fibrosis by reducing the production of harmful substances. This makes flavonoids a valuable addition to various food sources. Consuming grapefruit three times. daily can lead to weight loss over a 12-week period. A detailed study regarding extraction and quantification of flavonoids from underutilized grapefruit would be of great interest. The investigation compares individual compounds' responses to different techniques and quantifies flavonoids and phenols using soxhlet-assisted extraction, and ultrasound-assisted extraction.

## Materials and Methods

Grapefruits were procured from local market of Dehradun (Uttrakhand), India in the month of December (2017) and January (2018). Fruits were thoroughly washed with running tap water and sorted for uniform size, colour and free from any physical damage or visible signs of disease occurrence. Selected grapefruits were kept in open air for removal of water droplets from the outer surface followed by blotting with tissue paper. Peel was removed manually with knife, after juice extraction pulp and seed of grapefruit were separated and stored separately for further work. Drying of these parts was carried out by two methods viz; Hot air oven and Freeze drying. Grapefruits (seed, pulp and peel) were dried in tray (Oven 300, NSW 354) at 45 °C for 3 days. Dried samples were grounded using a commercial grinder and were stored in air-tight glass containers separately at 4 °C for further analysis. For freeze drying grapefruits (seed, pulp and peel) were placed in ultra-low temperature freezer at – 76 °C (U410) for 24 hours in Petri plates separately. A vertical freezer, (CHRIST- Alpha 2/4 LD plus Germany) was used to freeze-dry the samples. Samples were subjected to freeze-drying for 24 hours at -75 °C. Then each sample was ground using a commercial grinder and stored in air-tight glass containers at 4 °C individually, until further analysis.

For determination of flavonoids most important step was extraction. For extraction, Ultrasonicator and Soxhlet assisted extractor techniques were used. For each sample extraction was repeated three times under same conditions. After extraction filtration was done for ultrasonic assisted extraction. One gram powder was accurately weighed and placed in the sealed vessel by adding 70 ml of 90% methanol solvent, and then the vessel was placed in an ultrasonic cleaning bath (Power-Sonic 410) for extraction for 60 min at (40° C). After extraction, mixture was filtered through Whatman filter paper no. 1 for the removal of peel, pulp and seed particles. The residue was re-extracted twice to ensure complete extraction. The extracts were filtered and evaporated to dryness by the oven at 40 $^0$C. The extract was stored in amber coloured glass bottles at refrigerated temperature for further analysis. For soxhlet assisted extraction powder of the sample (1 gm) was accurately weighed and placed in a thimble. The 90% methanol solvent was used, followed by extraction for 2 hrs by Solvent Extractor (VELP Scientifica SER148 Solvent Extractor). The mixture was filtered through Whatman filter paper no. 1 for the removal of peel, pulp and seed particles. The extracts were filtered and evaporated to dryness by oven at 40 $^0$C.the extract was stored in amber colored glass bottles at refrigerated temperature for further analysis.

### Quantification of Flavonoids from purified extracts

UPLC modified version of HPLC was used for quantification of Flavonoids from grapefruit. Quantitative determination of individual Flavonoids in foodstuff is not possible as these compounds are not available commercially. All the flavonoids were identified by comparing the retention time and quantified by comparing the peak area with standard Catechin, Epicatechin, Rutin, Hesperidin, Quercetin, Apigenin, Kaempferol, Daidzein of known concentrations, as shown in Fig 1. For the estimation of different Flavonoids from solvent i.e, methanol UPLC model (Acquity- H) equipped with acuity photodiode array (PDA, 3 CH 1 275 nm @4.8 nm) detector. Peak was integrated using Empower $^{TM}$ 3 software. a phase ethylene bridged hybrid (BEH C- 18 RP (50 mm x2.1 MM I'd. and 1.7 µ columns were used in the experiments). The solvent mobile phase consisted of 1% methanol solution (A) and acetonitrile (B) was used at a flow rate of 0.5ml/min. the flow rate maintained in gradient mode as per the details are given below: 95% A, 5% B in 0-6 min. 70% A, 30% B in 6-6.30 min. 10% A, 90% B IN 7-8 min and 95% A, 5% B in 8-10 min. injection volume for sample analysis was 1 µl with the run time of 10 min and wavelength was 275 nm. Flavonoids acids are generally detected between at wavelength between 200-320 nm (Haminiuk *et al.*, 2014) [5]. The following standards were used Catechin, Epicatechin, Rutin, Hesperidin, Quercetin, Apigenin, Kaempferol, Daidzein, stock solution (200 ppm) of all standards were prepared in methanol (100%) and injected into the UPLC individually as well as in mixture (equal ratio of each standard). Dried extract, after weighing was dissolved in methanol (100%) and filtered through 0.22 µm Nylon syringe filters (Axiva). total volume 5 ml was prepared for each sample separately in vials and stored under refrigerated conditions for further UPLC analysis. Amount of each compound in the sample was calculated by using the following formula.

$$\text{Concentration } (\mu/g) = \frac{\text{Conc. of stand. Sol. x Area of the sample x Final volume of sample}}{\text{Area of standard x Weight of sample}}$$

## Results and Discussion

**Table 1** had indicated the presence and quantity of various flavonoids (apigenin, catechin, daidzein, epicatechin, hesperidin, kaempferol, naringin, Rutin and quercetin) present in different parts of grape fruit, processed via OD and FD followed by different method of extraction.

**Flavonoid content in peel:** Freeze dried peel had shown the maximum content of flavonoid was Naringenin (18106.5 µg/g) extracted by the UAE method. While the Hesperidin content was reported in the least amount in the peel extracted via UAE. It was observed that in the freeze-dried peel the different flavonoid content was comparatively higher in UAE method than SEA except Hesperidin (SAE for 477.56 µg/g and UAE 49.62 µg/g) and Apigenin (SAE 520.79 µg/g and UAE 414.56 µg/g), as shown in Fig 2. The Epicatechin (120.9 µg/g), Rutin (26593 µg/g), Quercetin (368.48 µg/g) and Daidzein (580.77 µg/g) were traced in freeze dried peel. Zainol *et al*. (2009) [6] reported least loss

of flavonoids in freeze drying.

In oven dried peel, SAE method had resulted in better extraction of different flavonoids then UAE in contrary to freeze dried peel where UAE had been found as a better method of extraction. Apigenin (4192.7 µg/g), Naringenin (19656 µg/g), Hesperidin (2999.95 µg/g), Quercetin (1429.19 µg/g), Rutin (994.17 µg/g), Epicatechin (984.33 µg/g), Daidzein (307.21 µg/g) were recorded in oven dried peels extracted via SAE method.

**Flavonoid content in pulp:** Flavonoid content in freeze dried pulp sample extracted via SAE method had shown the maximum content of Naringenin (21109.9 µg/g) followed by Epicatechin (1080.32 µg/g), Apigenin (591.58 µg/g), Daidzein (552.92 µg/g), Hesperidin (391.83 µg/g), Rutin (277.75 µg/g) and Quercetin (252.21 µg/g). SAE method had shown better retention of flavonoids in freeze dried pulp sample then UAE method except epicatechin which is higher in UAE processed sample that is (1429.05 µg/g) in comparison to (11080.32 µg/g) in SAE processed sample. The oven dried sample had shown the maximum quantity of Naringenin (13448.8 µg/g) and minimum quantity of hesperidin (74.5 µg/g) among the all-recorded flavonoids. Kaempepherol were not traced in grapefruit in any extraction methods like UAE and SAE. For Epicatechin water was more viable though for daidzein and Rutin methanol had great productivity (Bimakr *et al*., 2011; Haminiuk *et al*., 2014) [5].

**Flavonoid content in seed:** Flavonoid content of seed was found comparatively lower than peel and pulp in almost all studied sample. Among the studied sample freeze dried sample had shown comparatively lesser extractions then oven dried samples. Apegenin was not recorded in seed sample. Daidzein 14.1 µg/g was least recorded flavonoids in oven dried seed sample processed via SAE method (Fig 3). While the maximum flavonoids reported in oven dried content was also processed via UAE seed sample method that is Naringenin (15800.4 µg/g). In most of the cases UAE method of extraction was found better then SAE method in case of oven dried seeds and pulp for better retention of flavonoids. While as for freeze dried seed sample SAE method had shown better result than UAE, Example quercetin (SAE 145.39 µg/g, UAE 133.16 µg/g), epicatechin (SAE165.85 µg/g, UAE 116.11 µg/g). Quercetin showed degradation in grapefruit was also shown by (Zhang *et al*., 2007) [9].

**Principal component analysis**

Principal component analysis is a useful tool for diagnosing correlations within a dataset. Biplots utilize the principal component analysis to illustrate the relationship between the independent variables (correlation), similarities of individual data points (clustering) and relative importance of the observations to each independent variable. The data obtained from the various treatments was evaluated using the principal components analysis using PAST software. The data was grouped according to the extraction methods, whereas various parts freeze dried/oven dried are considered as components to evaluate the variance-covariance at disregard grouping. Several important trends can be inferred from the scatter biplot (Fig. 4). The scatter plots clearly show the treatment in 3 groups, except N-UAE and N-SAE are grouped. The closely placed vectors are more positively correlated, whereas far placed vectors are negatively correlated. The length of the respective vector approximates the variance. The angle between the vectors approximates the correlation between different variables. The closer the angle is to 90º, or 270º, the smaller the correlation. An angle of 0º or 180º reflects a correlation of +1 (positive) or -1 (negative), respectively. The scree plot showing 76.87% of the variance was explained by PC-I and inclusion of the PC-II (22.19%) explained 99.06% of the variance.

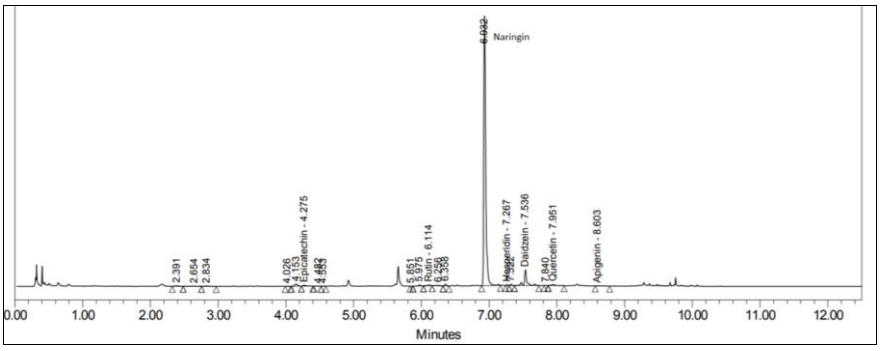
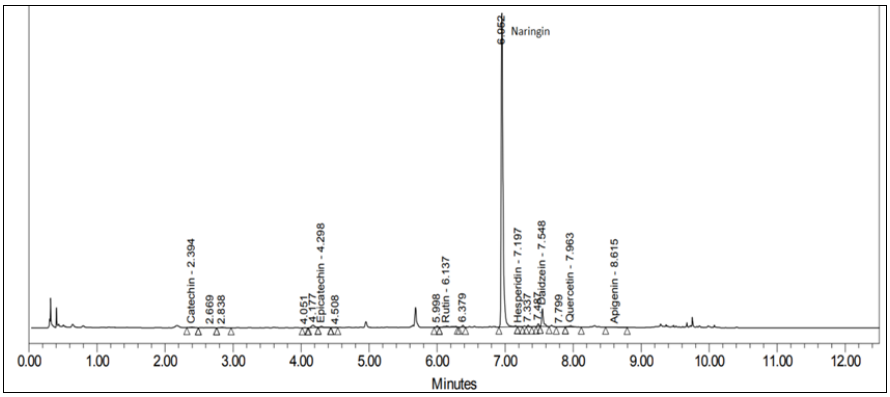



**Fig 1:** UPLC chromatogram for standards (Flavonoids)



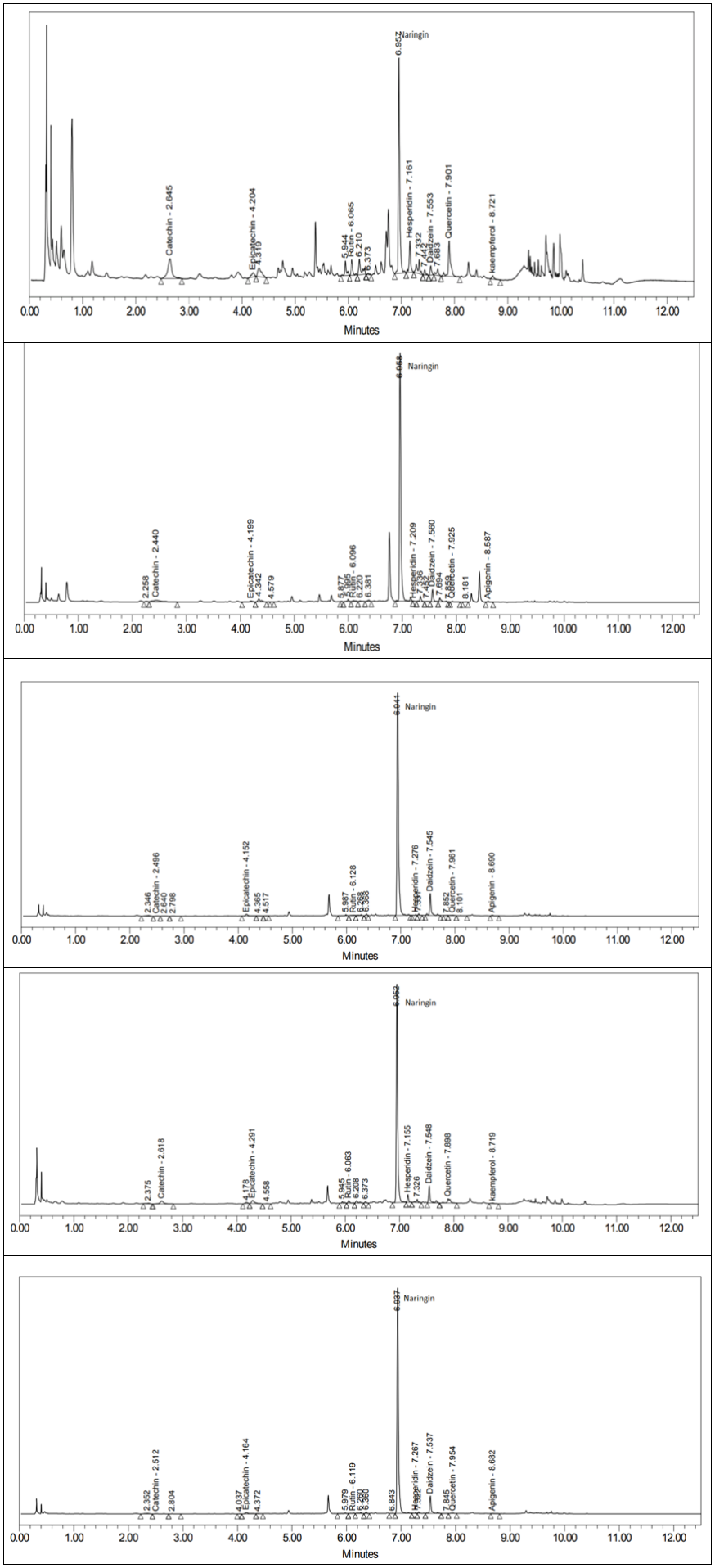
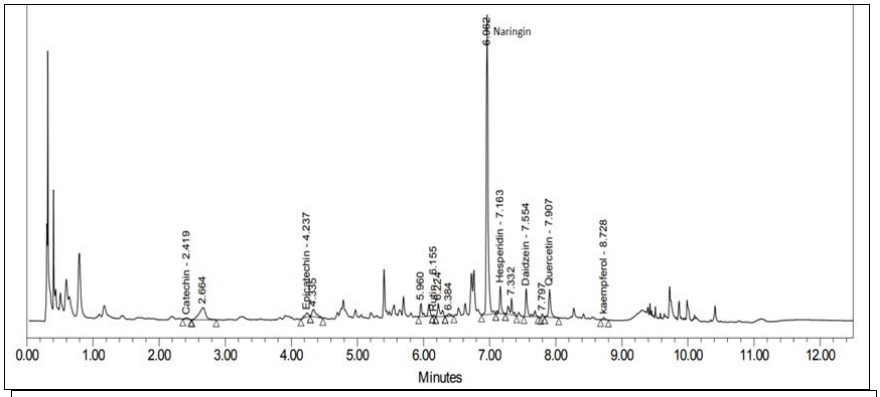
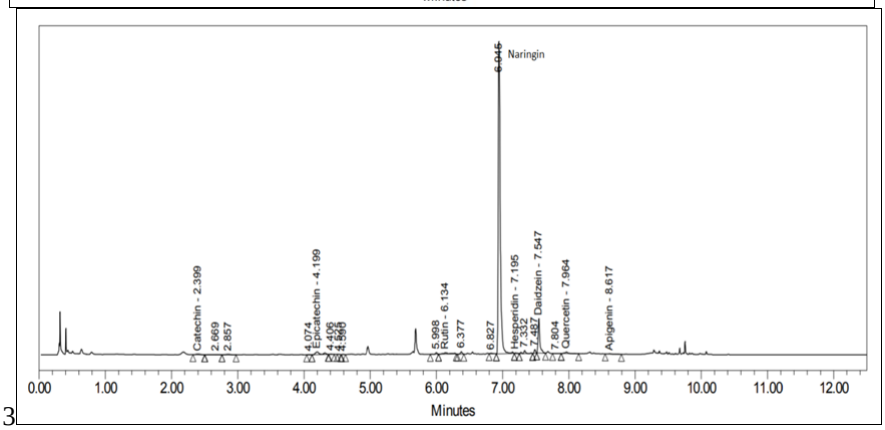
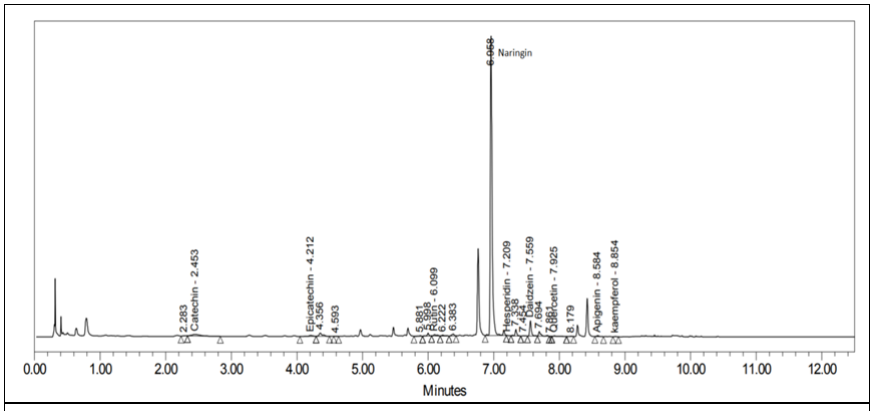


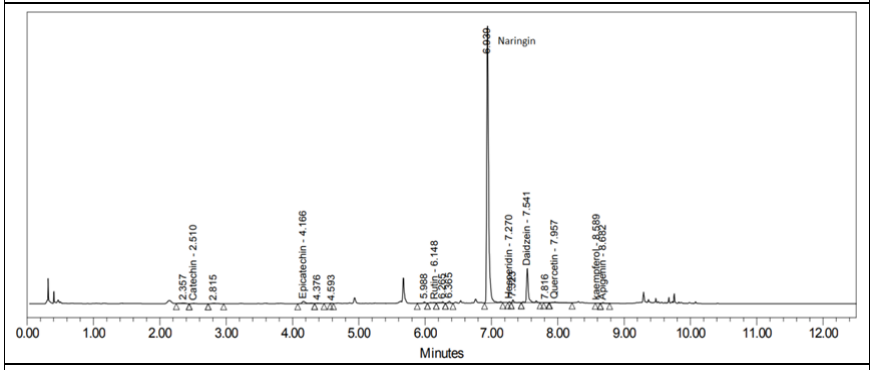

**Fig 2:** UPLC chromatograms of Soxhlet assisted extraction A-OD peel, B-OD seed, C-OD pulp, D-FD peel, E-FD seed, F-Fd pulp

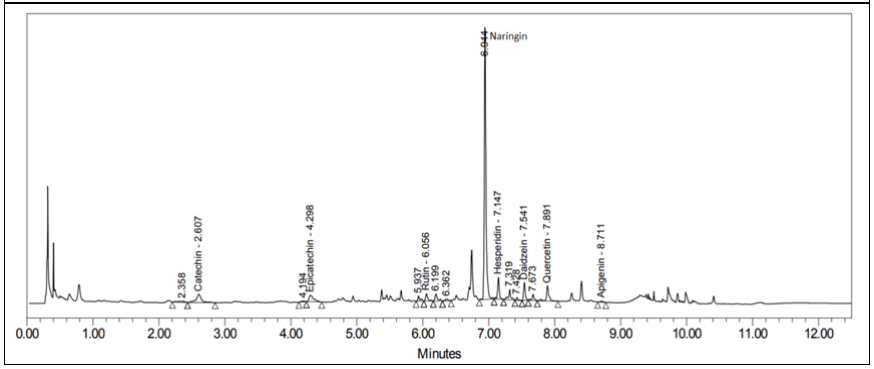

Naringin
Catechin - 2.419
2.664
Epicatechin - 4.237
4.355
5.960
6.155
6.384
Hesperidin - 7.163
7.332
Daidzein - 7.554
7.790
Quercetin - 7.907
kaempferol - 8.728
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00 11.00 12.00
Minutes


Naringin
Catechin - 2.399
2.669
2.857
Epicatechin - 4.199
4.406
Rutin - 6.134
6.377
6.827
Hesperidin - 7.195
7.332
Daidzein - 7.547
7.804
Quercetin - 7.964
Apigenin - 8.617
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00 11.00 12.00
Minutes

3


Naringin
2.283
Catechin - 2.453
Epicatechin - 4.212
4.356
4.593
Rutin - 6.099
6.383
Hesperidin - 7.209
7.338
Daidzein - 7.559
7.864
Quercetin - 7.925
8.179
Apigenin - 8.584
kaempferol - 8.854
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00 11.00 12.00
Minutes


Naringin
2.357
Catechin - 2.510
2.815
Epicatechin - 4.166
4.376
4.593
5.988
Rutin - 6.148
6.395
Hesperidin - 7.270
Daidzein - 7.541
7.816
Quercetin - 7.957
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00 11.00 12.00
Minutes


Naringin
2.358
Catechin - 2.607
4.194
Epicatechin - 4.298
5.937
Rutin - 6.056
6.199
6.362
Hesperidin - 7.147
7.319
Daidzein - 7.541
7.673
Quercetin - 7.891
Apigenin - 8.711
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00 11.00 12.00
Minutes

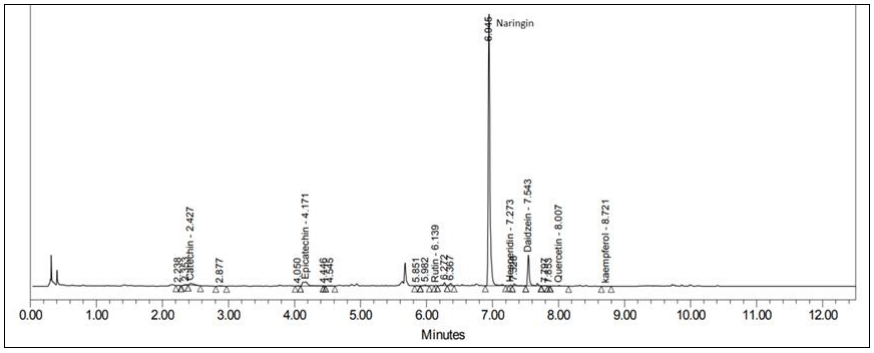



**Fig 3:** UPLC chromatograms of ultrasound-assisted extraction G-OD peel, H-OD seed, I-OD pulp, J-FD peel, K-FD seed, L-FD pulp

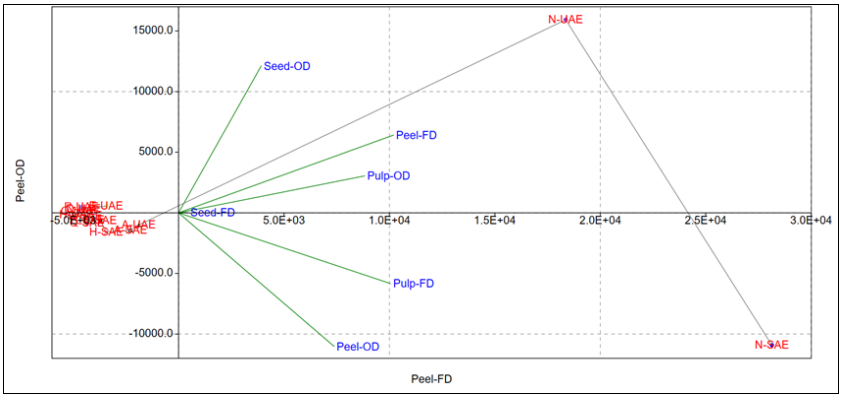



**Fig 4:** Scatter plot

**Table 1:** Flavonoids content in different parts of grapefruits extracted by SAE and UAE methods

| Sr. No | Flavonoid | Method of Extraction | Flavonoid content (µg/g) in different parts of Grapefruit | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Peel | | Pomace | | Seed | |
| | | | Freeze Dried | Oven Dried | Freeze Dried | Oven Dried | Freeze Dried | Oven Dried |
| 1. | Epicatechin | SAE | 662.6 | 894.33 | 1080.32 | 355 | 165.85 | 96.87 |
| 2. | | UAE | 1020.9 | 44.08 | 1429.05 | 399.29 | 116.11 | 1120.64 |
| 3. | Rutin | SAE | 194.23 | 994.17 | 277.75 | 465 | 61.27 | 84.89 |
| 4. | | UAE | 265.93 | 72.9 | 37.67 | 531.03 | 70.94 | 706.73 |
| 5. | Hesperidin | SAE | 477.56 | 2999.95 | 391.83 | 98.65 | 300.46 | 336.24 |
| 6. | | UAE | 149.62 | 351.88 | 113.16 | 74.5 | 296.88 | 191.93 |
| 7. | Quercitin | SAE | 172.87 | 1429.19 | 252.21 | 56.94 | 145.39 | 160.73 |
| 8. | | UAE | 368.48 | 152.77 | 63.42 | 176.38 | 133.16 | 316.48 |
| 9. | Apigenin | SAE | 520.79 | 4192.7 | 591.58 | 695.06 | Nd | Nd |
| 10. | | UAE | 414.56 | ND | Nd | 856.36 | Nd | Nd |
| 11. | Daidzein | SAE | 493.58 | 307.21 | 552.92 | 185.41 | 23.08 | 14.19 |
| 12. | | UAE | 580.77 | 14.68 | 294.1 | 233.43 | 25.99 | 449.53 |
| 13. | Naringin | SAE | 14337.7 | 19656 | 21109.9 | 13769 | 891.32 | 219.03 |
| 14. | | UAE | 18106.5 | 423.77 | 7327.07 | 13448.8 | 715.88 | 15800.4 |

## Conclusion

It was concluded that peel, pulp and seed of grapefruit were rich source flavonoid. All the extraction strategies including SAE and UAE were found appropriate for evaluation of different Flavonoids and Phenols. The yield was more in freeze drying as the loss of phytochemicals were less, though SAE were found most suitable for maximum extraction of individual flavonoids. Extraction method and drying methods do impact the measure of different flavonoids in grapefruit seed, pulp and peel. The result of this study will be exceptionally helpful for the specialists and ventures for acquiring specific compound of intrigue. UPLC showed a simple, quick and delicate method for evaluation of flavonoids from grapefruit. It was finally concluded that grapefruit could be utilized in food industries as a potential source of flavonoids.

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