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Original article

Phytochemical profiling and HPLC quantification of citrus peel from different varieties

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Summary. Nutraceutics are well-known for the individuals anxious to attenuate the impact of aging and mitigate the adverse effects of unhealthy lifestyle. Plant-based bioactive compounds are known to offer many health benefits. The objective of this study was to characterize selected phytochemicals and assess antioxidant capacity from citrus processing by-product. Processing waste from different varieties of citrus (oranges, grapefruit and musami) was utilized for the antioxidant indices for extraction with ethanol, methanol and water. The highest antioxidant potential in the form of TPC (206.53±6.82 mg GAE/100 g), flavonoids (83.06±2.74 mg/100 g), DPPH (62.80±2.07 µmole TE/g), ABTS (10.35±0.34 µmole TE/g) and FRAP (18.54±0.61 µmole TE/g) were found in grapefruit ethanolic extract, followed by oranges and musami peel extract. The highest ascorbic acid content (51.3±1.6 mg/100g) was observed in orange peel followed by that in grapefruit and musami peel extracts. On the basis of antioxidant profiling the ethanolic extract of each variety was quantified through HPLC that showed the highest concentration of hesperidin and nobiletin in grape fruit extract as 28.51 and 9.92 mg/g, respectively followed by oranges (24.96 and 7.31 mg/g) and musami (21.38 and 6.08 mg/g) extract.

Key words: extraction, ethanolic extract, TPC, flavonoids, DPPH, ABTS, FRAP

Introduction

Contemporary nutritional approaches are becoming a key for the prevention of chronic diseases. In this context, functional foods and nutraceuticals have been claimed to reduce the mounting incidences of various life threatening ailments owing to their bioactive moieties. In everyday life, humans are readily exposed to toxicants derived from various sources such as food, pesticides and intermediate metabolites. These toxic substances trigger oxidative stress in various biological systems, which consequently lead to acute or chronic diseases (1). Plants are shown to have therapeutic potential due to the presence of secondary metabolites that are used in industries for prevention of health related disorders. The inherent chemical erraticism in plants is the main reason of its industrial usage that are produced under stress in the form of secondary metabolites and medically proven as health boosting ingredient (2).

The phytoceutics that consist of phenolics, flavonoids and secondary metabolites have the ability to act as free radical scavengers (3). However, the role of flavonoids in response of oxidative stress is still being investigated. Mostly, these entities enhance the antioxidant activity in response to stress. The mode of action of flavonoids is through chelation process (4). A number of nutritional studies focused on the effect of foods for their health promoting and protective potential. Epidemiological studies depicted that fruits/veg-

etables are rich source of natural antioxidants that can prevent the chances of man metabolic disarrays namely, cardiovascular diseases, diabetes, ulcer and cancer (5). These bioactive entities such as phenolics, flavonoids, alkaloids and many other nitrogenous compounds that are present in fruits and vegetables have been reported to exhibit bioactivities such as antioxidant, antibacterial, antifungal and antiviral activities (6).

Fresh fruits are an excellent source of energy, vitamins, minerals and fiber. The nutritional value of fruits is greatly depended on the quality as well as quantity of these nutrients. Among all fruits, guava, citrus and pineapple are rich source of vitamins and some of minerals (7). At present, there is a significant demand for fresh fruits and their processed products. Due to seasonal nature, fruits have limited shelf life; their processing becomes necessary to keep the quality and to ensure its availability throughout the year (8). Amongst all fruits and vegetables waste, citrus peel and other by-products (bagasse and seeds) have the strongest health boosting potential. Therefore, citrus waste is gaining more attention due to the antioxidant and nutritional value (9).

The total worldwide production of citrus fruit is estimated as 153 million tons per year (10). Amongst this production half quantity is utilized for juice extraction whilst, the remaining production after squeezing of juice include bagasse, peel and other byproducts that is commonly known as citrus waste. A few decades ago, this citrus waste was only used for cattle feeding that did not have any significant importance to cover production and transportation expenditures. Thus, a huge amount of citrus waste was deposited that lead health problems for humans due to the environmental pollution and many economic disadvantage (11).

Citrus being an important crop consists of oranges, grapefruits, lemons and mandarins consists of 98% of citrus used in industries for juice extracts and other processed products (12). Among all the citrus family oranges are the more abundant with an amount of 82% however, the most common use of citrus fruit waste is in the processing of other citrus based products e.g., marmalade. Citrus peel as well as bagasse or pomace are also rich sources of bioactive compounds that have antioxidant potential such as phenolic compounds and flavonoids that are important for human nutrition (13). Moreover, juice only constitutes half of citrus total weight and it has a larger amount of by-products and wastes that is used as molasses for animal feeding, fuel production as well as source of fiber due to the presence of pectin (14).

The bioactive moieties present in citrus wastes are flavonoids such as hesperidin, nobiletin, alkaloids, naringin and synephrine that have medical impact on human health stratum (15). Furthermore, citrus waste has abundant quantity of essential oils that are used in food flavors and perfumes. It is also used in traditional medicines to prevent indigestion, cancer, constipation, nausea, sedative and cardiovascular disease. Although, the citrus waste especially peel is well known and popular for bitter essential oil that has the ability to replace ephedra stimulant that is banned around the globe. Owing to this property the citrus waste is also considered as a power source of antioxidants (16). The objective of present study was to quantify the content of selected bioactive compounds in citrus waste using different extraction regime and assess their antioxidant potential.

Materials and Methods

In the present research, waste material like peel of three varieties of citrus (oranges, grapefruit and musami) was used for the extraction and characterization of bioactive compounds. Moreover, the antioxidant potential of citrus waste extracts was evaluated. Citrus waste (Peel and Bagasse) was procured from local fruit market of Faisalabad. Reagents (analytical and HPLC grade) and standards were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma Aldrich (Sigma-Aldrich, Tokyo, Japan). All the reagents used were of analytical grade.

Sample preparation

Citrus waste was shredded into small pieces and dried in air cabinet dryer at 60±5 °C for 6-8 hours. Then dried citrus waste was ground into powder and stored in polyethylene bags to avoid rehydration. Resultant citrus waste powder was analyzed for their chemical characteristics.

Preparation of citrus waste extracts

Citrus extracts were prepared using water, aqueous methanol (50% v/v) and aqueous ethanol (50% v/v) as solvents at 60°C following method of Park *et al.* (17). Afterwards the resultant extracts were subjected to rotary evaporator (Eyela, Japan) to remove water and solvent then stored for further analysis.

Total phenolic contents (TPC)

Total phenolic contents (TPC) in citrus waste extracts were measured using Folin-Ciocalteu method as described by Ghafoor and Choi, (18). Briefly, 50 µL of citrus waste extract was separately added to test tube containing 250 µL of Folin-Ciocalteu's reagent, 750 µL of 20% sodium carbonate solution and volume was made up to 5 mL with distilled water. After two hours, absorbance was measured at 765 nm using UV/visible light Spectrophotometer (CECIL CE7200, Waltham, MA, USA) against control that has all reaction reagents except sample extract. Total polyphenols was estimated and values were verbalized as gallic acid equivalent (mg gallic acid/100g).

Total phenolic compounds of each extract as gallic acid equivalents (GAE) was calculated by following formula:

C= c×V/ m

C = Total phenolic contents (mg/g plant extract, in GAE)

c = Concentration of gallic acid (mg/mL)

V = Volume of extract (mL)

M = Weight of citrus waste extract (g)

Flavonoids

Total flavonoids content of citrus waste extract was determined spectrophotometrically using method that based on the development of a flavonoid-aluminum complex (Bushra *et al*. (19). Quercetin was used as a standard to measure total flavonoids in citrus waste extracts. 1 mL extract was added to a 10 mL volumetric flask and volume was made up to 5 mL with distilled water followed by addition of 0.3 mL of 5% (w/v) sodium nitrite. After 5 min, 0.6 mL of 10% (w/v) $AlCl_3$ was added and then at 6 min 2 mL of 1M NaOH was mixed in, followed by the addition of 2.1 mL distilled water. Absorbance was measured immediately at 510 nm on UV/visible light spectrophotometer. Data was expressed as quercetin equivalents in mg per 100 gram of extract.

Free radical scavenging activity (DPPH assay)

Protocol of Ghafoor, (20) was followed to determine DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity of citrus waste extract. Sample solution was prepared by dissolving 0.025 mL of sample extract in 10 mL of respective solvent. 3 mL of freshly prepared DPPH solution in respective solvent (6×10-5M) was mixed with 77 µL sample extract. Each sample was kept in dark place for about 15 minutes at room temperature and decrease in absorbance was measured at 517 nm on UV/visible light spectrophotometer. Similarly, blank sample absorbance having the same amount of solvent and DPPH solution except extract was prepared and absorbance was read at the same wavelength on UV/visible light spectrophotometer. The free radical-scavenging activity of each citrus waste extract can be presented as percentage reduction in DPPH due to given amount of each extract.

Reduction of absorbance (%) = [(AB - AA)/AB] × 100

AB = Absorbance of blank sample at t = 0 minute

AA = Absorbance of tested extract solution at t = 15 minutes

ABTS (2, 2-azino-bis, 3-ethylbenzothiazoline-6-sulphonic acid) assay

ABTS assay is a decolorizing method, according to Vaio *et al.* (21) the ABTS radical was freshly prepared by adding 5 mL of a 4.9 mM potassium persulfate solution to 5 mL of a 14 mM ABTS solution and keeping the mixture in the dark for 16 hr. This solution was diluted further with respective solvent to yield an absorbance of 0.7±0.02 at 734 nm and was used for antioxidant assay. The final reaction mixture (1 mL) comprised of 950 µL of ABTS solution and 50 µL of the extract or water was mixed for 30 sec and allowed to stay for 5 min at ambient temperature. After the absorbance was recorded at 734 nm using a UV-visible spectrophotometer (Shimadzu UV-160A, Kyoto, Japan) and compared with the control ABTS solution. A calibration curve was made by making various concentration of Trolox. ABTS radical scavenging activity was expressed as µmol trolox

equivalent antioxidant capacity (TEAC) per gram of sample.

FRAP

Ferrous ions chelating activity of extracts was estimated following the guidelines of Ramful *et al.* (22). Extracts (0.1 mL) were added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.1 mL) and 2.75 mL of distilled water. The mixture was shaken vigorously and let to stand at room temperature for 10 min. The absorbance of the solution was then measured at 562 nm. The scavenging activity was calculated as follows;

Where,

A blank = absorbance of the control reaction

A sample = absorbance in the presence of plant extract

Samples were analyzed in triplicate.

Determination of ascorbic acid content

Ascorbic acid content of resultant extracts was analyzed by using method of Ghafoor *et al.* (23). Took 0.1g of ascorbic acid in volumetric flask and make the volume 100 mL. After that, took 0.04g 2, 6-chlorophenol indophenol dye and dissolved in 100 mL of distilled water. Dissolve 4 g oxalic acid in distilled water and made the volume up to 1L. Ascorbic acid was determined by oxidizing it in acidic medium with dye 2, 6-chloro-phenol indophenol that changed L-ascorbic acid to D-ascorbic acid. First we determined the value for standard ascorbic acid. Took 1 mL of the standard ascorbic acid solution and add 1.5 mL of oxalic acid. Shake well and titrated it against dye which was taken in burette. Titrated it until light pink color appeared and noted the value R1 as standard value. For determination of ascorbic acid, took blended sample and made volume 100 mL by adding 0.4% oxalic acid solution. It was filtered due to presence of fibrous material. Took 10 mL of filtrate and added 15 mL of oxalic acid in sample. Then titrated it against dye and note the volume of dye used R.

Calculation:

Ascorbic acid (mg/100 mL) = 1 × R × V / R1 × W × V1 × 100

Where

R = Volume of dye used against sample titration

V = Volume of sample by adding 0.4% oxalic acid

R1= Volume of dye used against standard titration

W = weight of sample

V1= volume of filtrate used

Selection of best treatment for HPLC analysis

Out of nine treatments three best treatments from each variety were selected on the basis of phytochemical screening test and in vitro studies for HPLC analysis. Best selected citrus waste extracts were analyzed for active ingredients (hesperidin and nobiletin), following the protocol of Tumbas *et al.* (24) by HPLC (PerkinElmer, Series 200, USA) containing shim-pack CLC-ODS C18 column (15 cm x 4.6 mm, 5.0 µm particle size) and an auto sampler. The mobile phase was comprised of isocratic HPLC grade water (H_2O) at a flow rate of 1.0 mL/min. Sample amount of 10 µL and column temperature at 40 °C were kept during the whole analysis. The eluent was analyzed at 345 nm for hesperidin and 330 nm for nobiletin using UV detector (PerkinElmer, Series 200, USA). Quantification of both active ingredients was achieved by comparing the retention time of peaks of sample extracts to those of hesperidin and nobiletin standards.

Results and Discussion

Total Phenolic Content (TPC)

The values for citrus peel varieties in Table 1 showed that highest TPC 206.53±6.82 mg GAE/100 g was observed in grapefruit peel in methanol followed by 181.11±5.80 and 154.94±5.27 mg GAE/100 g in oranges and musami in methanol extract, respectively. The same trend was observed for ethanolic extract in which the maximum phenolic contents were observed as 172.44±5.69, 159.93±5.12 and 119.02±4.05 mg GAE/100 g in grapefruit, oranges and musami extracts, respectively. Similarly, for water extract the TPC were 100.76±3.33 mg GAE/100 g of grapefruit extract, 92.86±2.97 mg GAE/100 g in oranges and 83.42±2.84 mg GAE/100 g of musami extract.

The current findings were similar to those reported by Safdar *et al.* (25) who evaluated the extraction and quantification of citrus peel waste. They concluded that the TPC of citrus peel were 28.04±0.33 mg GAE/g in

Table 1. Antioxidant profiling of citrus peel

Bioactive compounds	Extraction medium		
	Ethanol	Methanol	Water
Total Phenolic (mg GAE/100g)			
Oranges	159.93±5.12Ba	154.94±5.27Ba	92.86±2.97Bb
Grapefruit	172.44±5.69Ab	206.53±6.82Aa	100.76±3.33Ac
Musami	119.02±4.05Cb	181.11±5.80Ca	83.42±2.84Cc
Flavonoids (mg/100 g)			
Oranges	60.23±1.93Bb	77.41±2.48Ba	46.97±1.50Bc
Grapefruit	69.52±2.29Ab	83.06±2.74Aa	53.89±1.78Ac
Musami	54.90±1.87Cb	68.36±2.32Ca	38.10±1.30Cc
Ascorbic acid (mg/100 g)			
Oranges	49.8±1.6Ab	51.3±1.6Aa	47.6±1.5Ac
Grapefruit	41.4±1.4Bb	43.2±1.4Ba	40.1±1.3Bc
Musami	34.2±1.2Cb	36.2±1.2Ca	32.9±1.1Cc

Different upper cap letters in each column for the respective compound show significant difference ($p<0.05$)
Different lower cap letters in each row for the respective extraction medium show significant difference ($p<0.05$)

methanolic extract. Nonetheless, Esparza-Martinez *et al.* (26) conducted research work on orange peel and suggested that the TPC of orange peel was 74.56±0.73 mg/g. Likewise, Kim and Kim, (27) reported that the total phenolic contents of citrus peel extracted with water was 41.6 g/100 g. Moreover, Garcia-Castello *et al.* (28) suggested that by using different concentration of ethanol and water, the total phenolic assay of grapefruit peel varied from 25.3 to 55.8 mg/g of peel. Similarly, the total phenolic content of ethanolic fresh orange peel extract was 5255.02±24.04 mg GAE/100 g that reduced to 3026±6.26 and 2453.75±9.72 mg GAE/100 g in microwave dried and air oven dried orange peel. Previously, Lagha-Benamrouche and Madani, (29) suggested that TPC of musami peel were 31.62±0.88 mg GAE/g of musami peel.

Flavonoids

The values for flavonoids are given in Table 1, which shows flavonoid contents in grapefruit extract as 83.06±2.74 mg QE/100 g in methanol, 69.52±2029 mg QE/100 g in ethanol and 53.89±1.78 mg/100 g in water extract. Likewise, in oranges extracts of methanol, ethanol and water extracts the flavonoid contents were 77.41±2.48, 60.23±1.93 and 46.97±1.50mg QE/100 g, correspondingly. However, the lowest flavonoids were observed in musami as 68.36±2.32, 54.90±1.87 and 38.10±1.30 mg QE/100 g in ethanol, methanol and water extracts, accordingly.

The results of present study work were in line with the findings of Esparza-Martinez *et al.* (26), who performed their research work on orange peel and suggested that the flavonoid contents were 21.56±0.24 mg/g. One of their peers, Abou-Arab *et al.* (30) concluded that the flavonoids varied from variety to variety. They reported that the flavonoid contents of *C. valencia* were 455.83±3.82 mg QE/100 g whilst, the flavonoid contents for methanolic extract of C. balady were 486.67±12.83 mg QE/100 g. Moreover, Lagha-Benamrouche and Madani, (29) reported that total

flavonoid contents were 1.29±0.02 mg QE/g in musami peel.

Ascorbic acid

The content of ascorbic acid was determined in orange peel followed by grapefruit and musami (Table 1). The maximum ascorbic acid contents were 51.3±1.6 mg/100 g in methanol extract of orange peel followed by 49.8±1.6 mg/100 g in ethanol and 47.6±1.5 mg/100 g in water extract. After oranges, the ascorbic acid contents in grapefruit peel were 43.2±1.4, 41.4±1.4 and 40.1±1.3 mg/100 g in methanolic, ethanolic and water extract, respectively. The lowest content of ascorbic acid was estimated in musami that was 36.2±1.2 mg/100 g in methanol, 34.2±1.2 mg/100 g in ethanol and 32.9±1.1 mg/100 g in water extract, respectively.
The findings of current work are in agreement to the outcomes of Abou-Arab *et al.* (30) who performed their research work to evaluate the effect of different drying processes on the ascorbic acid content of citrus peel extracts. They reported that the ascorbic acid of methanolic extract of different varieties varies significantly. According to this, the ascorbic acid content varies from 89.79±2.13 to 139.81±10.35 mg/100 g in different varieties. Similarly, El-Ghfar *et al.* (31) reported that the ascorbic acid content of fresh orange peel extract was 127.70±0.04 mg/100 g that reduced to 66.55±0.006 mg/100 g when the peel was dried under microwave although the ascorbic acid content decreased to 66.50±0.05 mg/100 g in air dried oven.

DPPH

The DPPH assay of citrus waste is depicted in Figure 1. It was observed that the highest DPPH assay was in methanolic extract as 62.80±2.07 µmole TE/g for grapefruit, 57.48±1.84 µmole TE/g for oranges, 38.36±1.30 µmole TE/g for musami followed by ethanolic extract as 50.69±1.67, 47.18±1.51 and 34.98±1.19 µmole TE/g in grapefruit, oranges and musami extracts, respectively. The lowest DPPH assay was observed in water extract of all varieties, as 41.13±1.36 µmole TE/g (grapefruit), 35.85±1.15 µmole TE/g (oranges) and 27.61±0.94 µmole TE/g (musami).

Our results were in close agreement to those reported by Safdar *et al.* (25) who worked on extracted and quantification of citrus peel and concluded that the DPPH assay was 60.67±1.24 µmole TE/g. Furthermore, Kim and Kim, (27) reported that the DPPH assay of citrus waste was 1.1±0.08 mg/mL. Likewise, Castro-Vazquez *et al.* (32) depicted that the DPPH assay of fresh grapefruit was 25.18±8.52 mg TE/g however, in freeze dried grapefruit peel based methanolic extract, DPPH assay was 110.98±13.76 mg TE/g. Likewise, El-Ghfar *et al.* (31) worked on the methanolic and ethanolic extract of orange peel and reported that the DPPH assay of fresh, microwave oven dried and air oven dried extract in methanolic extract were 99.79±0.95, 69.83±0.04 and 56.29±0.30% however, the DPPH assay of ethanolic extract was 98.6±0.36 (fresh orange peel), 68.85±0.25 (microwave oven dried) and 53.83±0.04 (air oven dried) orange peel.

ABTS

The ABTS assay of citrus peel is shown in Figure 1 in which the maximum ABTS assay was observed in grapefruit as 10.35±0.34, 10.07±0.33 and 9.75±0.32 µmole TE/g in methanol, ethanol and water, accordingly followed by oranges in which the ABTS assay was observed 9.16±0.29 µmole TE/g (methanol), 8.94±0.29 µmole TE/g (ethanol) and 8.60±0.28 µmole TE/g (water). The lowest ABTS activity was observed in musami extract regardless of the solvent used, the value of ABTS in methanolic musami extract was 8.42±0.30 µmole TE/g followed by ethanol 8.11±0.28 µmole TE/g and water 7.97±0.27 µmole TE/g.
The results of present study were in accordance with the findings of El-Ghfar *et al.* (31) who performed research work on citrus peel extract to perform antioxidant assay and reported that the ABTS assay was 1.09±0.05 TE/g in methanolic extract of fresh citrus peel that reduced to 0.68±0.01 and 0.66±0.01 TE/g in microwave and air oven dried citrus peel. Furthermore, in ethanolic extract the ABTS assay was 1.14±0.04 TE/g in fresh citrus peel extract that lowered to 0.63±0.01 and 0.54±0.01 TE/g in microwave dried and air oven dried peel. Nevertheless, Castro-Vaquez *et al.* (32) reported that the ABTS assay of fresh grapefruit peel extract was 99.46±12.09 mg TE/g that increased to 455.38±1.95 mg TE/g in freeze dried extract due to the concentrated use.

FRAP

The FRAP assay of citrus peel was shown in Figure 1 which proved that lowest FRAP essay was observed in musami extracts in which the values were 16.27±0.55 µmole TE/g (methanol), 15.65±0.53 µmole TE/g (ethanol) and 14.90±0.51 µmole TE/g (water). Although, the highest FRAP potential was observed in grapefruit extract as 18.54±0.61, 17.90±0.57 and 16.27±0.55 µmole TE/g in methanol, ethanol and water based extracts followed by orange extracts.

The findings of present work were in line with the findings of Castro-Vazquez *et al.* (32) who worked on the FRAP assay of grapefruit extract of fresh and freeze dried extract and concluded that the FRAP assay of fresh grapefruit extract was 44.82±5.35 mg TE/g that increased to 207.74±14.65 mg TE/g due to higher concentration. Furthermore, Kim and Kim, (27) reported that the FRAP assay of citrus peel extract was 1.0±0.02 mg/mL. Lately, Lagha-Benamrouche and Madani, (29) reported that FRAP assay of musami peel was 25.0±0.75 mg/mL of extract.

HPLC quantification

HPLC (High Performance Liquid Chromatography) evaluation of consequential extract was an obligatory instrumental step for further cataloging and quantification of bioactive moieties. Contingent to the phytochemical profiling and *in vitro* perspectives three best extracts were selected from each category (ethanol, methanol an water) for qualitative and quantitative analyses of bioactive moiety *i.e.* hesperidin and nobiletin from each variety. The quantification of citrus peel extracts through HPLC has revealed that hesperidin was three time more intense as compared to nobiletin and bioactive moieties. Hence, the resultant peaks obtained from HPLC were compared with the standard peak area, retention time and spectral exploration. HPLC assessment for citrus peel (Figure 2) proved that the highest hesperidin value concentration in ethanolic extract of grapefruit 28.51 mg/g followed by orange 24.96 mg/g and least in musami 21.38 mg/g whilst, for nobiletin the maximum concentration was quantified in ethanolic extract as 9.92 mg/g in grapefruit, 7.31 mg/g in methanol and 6.08 mg/g in musami extract (Figure 3). It has been discovered that ethanol was the most effective solvent to solubilize maximum

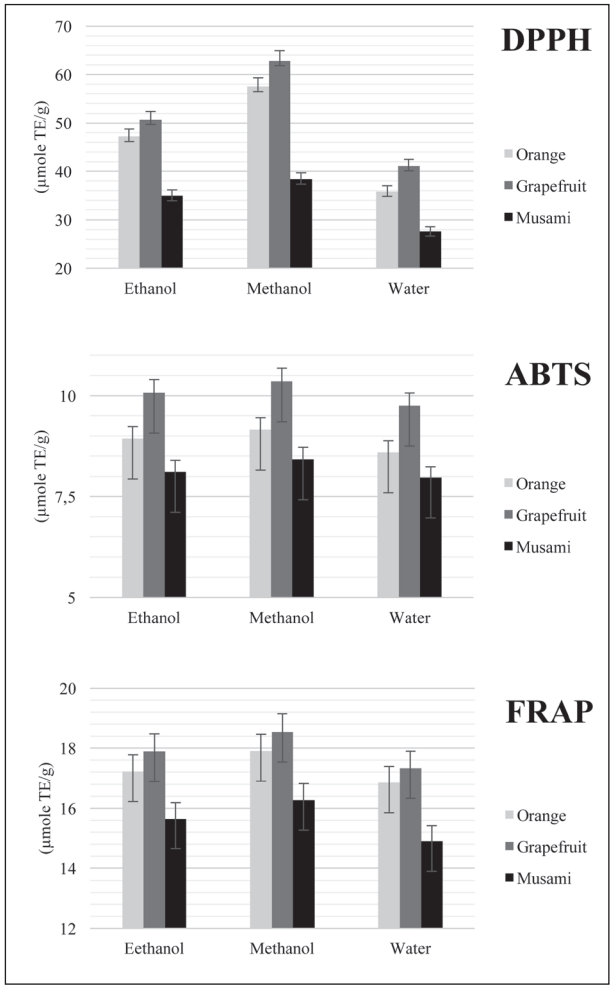

Figure 1. Antioxidant Activity of peel extracts of different citrus varieties using DPPH, ABTS and FRAPS assays

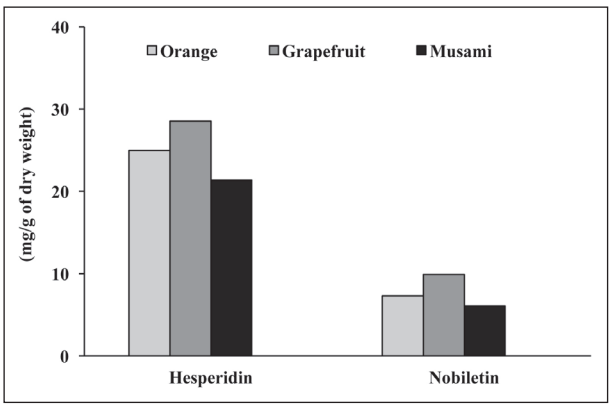

Figure 2. HPLC quantification of Hesperidin and Nobiletin (mg/g of dry weight)

amount of bioactive entities in citrus peel due to its organic and polar nature as compared to other two solvents.

The current findings were in line with the findings of Kim and Kim (27), who reported that the hesperidin quantification of citrus waste was 0.104±0.05 g/100 g. Moreover, Garcia-Castello *et al.* (28) suggested that by using different concentration of ethanol and water the hesperidin concentration in grapefruit peel varied from 0.23 to 0.74 mg/g. Previously, Inoue *et al.* (33) reported that the hesperidin and nobiletin content in mature citrus waste was 18.8±0.1 and 0.1±0.00 mg/g of citrus peel.

Conclusion

The phyto-constituents isolated from fruit by-products also have great impact on the prevention of oxidative stress. This can be proved by the in vitro analyses that had been carried out in this article. The results depicted that grapefruit peel has the maximum antioxidant potential among all the varieties.

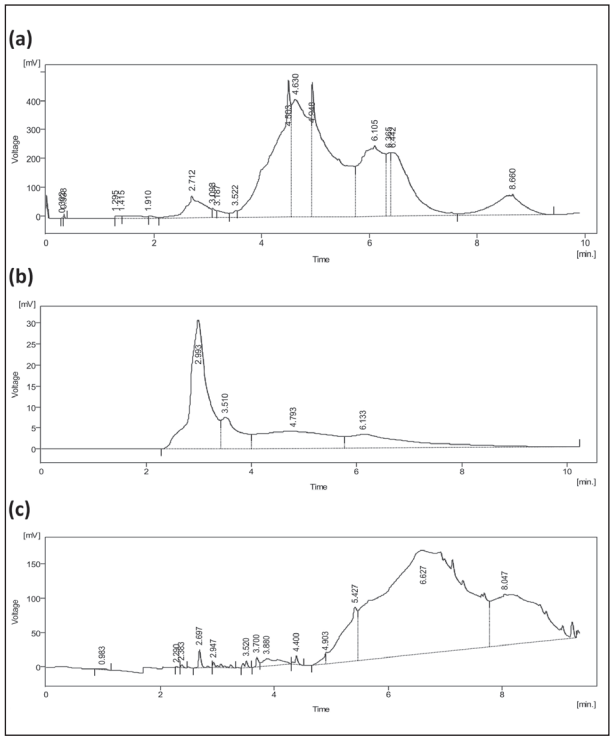

Figure 3A. HPLC chromatograms of nobiletin in (a) methanolic (b) ethanolic and (c) water extract of grapefruit peel

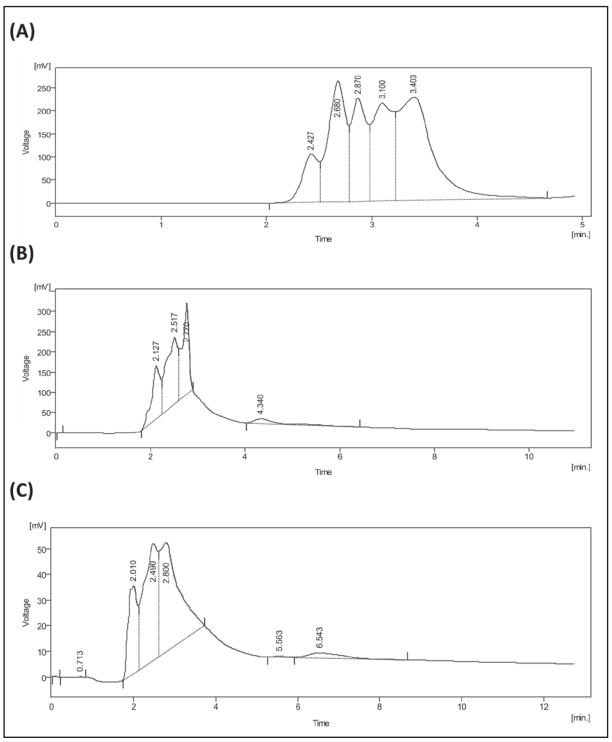

Figure 3B. HPLC chromatograph of hesperidin in (A) methanolic (B) ethanolic and (C) water extract of grapefruit peel

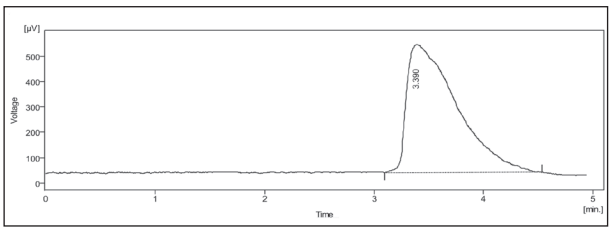

Figure 3C. HPLC chromatograph for nobiletin standard

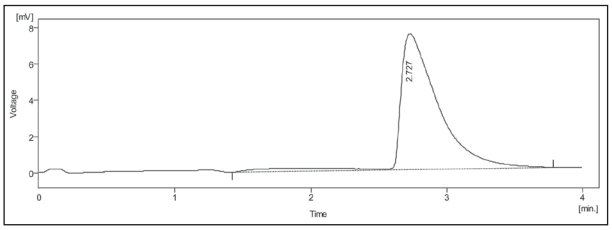

Figure 3.3. HPLC chromatograph for hesperidin standard

Similarly, the HPLC analyses grapefruit peel has stronger antioxidant assay as compared to oranges and musami peel.

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