Print ISSN : 0972-8813
e-ISSN : 2582-2780

[Vol. 22(1) January-April 2024]

Pantnagar Journal of Research

(Formerly International Journal of Basic and Applied Agricultural Research ISSN : 2349-8765)

G.B. Pant University of Agriculture & Technology, Pantnagar

PANTNAGAR JOURNAL OF RESEARCH

Vol. 22(1) January-April 2024

CONTENTS

Comparative study of antioxidant potential of fresh peel from different citrus species

TARU NEGI[1], ANIL KUMAR[1*], ARCHANA GANGWAR[1], SATISH KUMAR SHARMA[1], ANURADHA DUTTA[2], NAVIN CHAND SHAHI[3], OM PRAKASH[4] and ASHUTOSH DUBEY[5]

[1]Department of Food Science and Technology, College of Agriculture, [2]Department of Foods and Nutrition, College of Community Sciences, [3]Department of PHPFE, College of Technology, [4]Department of Chemistry, [5]Department of Biochemistry, College of Basic Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar-263145(U.S. Nagar, Uttarakhand)
[*]Corresponding author's email id: anilkumargbpuat@gmail.com

ABSTRACT: Citrus peels contain high concentration of bioactive compounds, particularly phenols and flavonoids that have a positive impact on human health. In this study, phenol, flavonoid, and antioxidant potency composite index (APC Index) of fresh citrus peels (kinnow, orange, mosambi, hill lemon, and malta) were assessed. DPPH, FRAP, and ABTS assays were done to determine the APC Index of the peels. The study findings showed that, among fresh citrus peels, orange flavedo was found to contain the highest total phenols (15.11 mg GAE/g dw) whereas, kinnow flavedo had highest flavonoid (5.86 mg QCE/g dw). Highest APC Index was found in kinnow followed by orange, malta, hill lemon and mosambi. Therefore, it can be concluded from the present study that kinnow fresh peel had highest antioxidant potential as compared to other citrus peels taken in this study. It was also noted that the fresh peels of various citrus species have enormous source of bioactive compounds and high antioxidant capacity which has tremendous potential to be utilized in functional foods and as nutraceuticals.

Key words: Antioxidant potency composite index (APC index), bioactive compounds, citrus peel, flavonoid, phenol

Bioactive compounds (phenols, flavonoids etc) are secondary metabolites that are produced by plants in smaller quantities together with primary metabolites (carbohydrate, lipid, protein) and protect the plants from adverse conditions like drought. These secondary metabolites are known for health beneficial properties such as antiviral, antioxidant, antimicrobial and anti-inflammatory whose intake has been encouraged with the purpose to decrease cardiovascular diseases, hypertension, diabetes, and weight gain (Borgonovi *et al.,* 2022). Food industry wastes (such as pulp, peel, stem, and seeds) contain a higher amount of bioactive compounds than fruit juice. Fruit peels are one of the food industry by-products, which are commonly discarded during the consumption or manufacturing stage. Particularly, citrus peel waste (CPW) has immense economic value as it contains abundant amount of bioactive compounds (Sharma *et al.,* 2017). According to reports, around 75% of total production of citrus fruit is used as fresh, while the remaining 25% is used for the development of commercial products (Mahato *et al.,* 2019). The annual citrus waste generated in India is 7.8 million tons (Khan *et al.,* 2021). Consequently, the amount of citrus waste produced by processing enterprises is estimated to be over 60 million tons per year around the world (Mahato *et al.,* 2019). After processing, 50-60% of the fruit parts is mainly citrus waste (peels, seeds, and membrane residue) and 30-34% citrus peel waste (CPW) is generated which is known as a treasure of bioactive compounds (Rafiq *et al.,* 2018).

As already stated, bioactive compounds present in citrus peel are well known to have various health beneficial properties. Moreover, researchers discovered that flavonoids particularly, hesperidin, hesperetin, naringenin and essential oil of citrus fruit plays important role in preventing the coronavirus disease (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Citrus flavonoids, one of the bioactive compounds, prevent the binding of SARS-CoV-2 with angiotensin-converting enzyme 2 which is a cell membrane protein receptor for SARS-CoV-2 present in the host cell and therefore, inhibit the virus

replication (Alberca *et al.,* 2020, Bellavite and Donzelli 2020 and Agrawal *et al.,* 2021).

Hence, this study was carried out to compare the antioxidant properties of bioactives compounds extracted from fresh flavedo (outer colored part) of different citrus fruit which may act as an active ingredient for various foods or pharmaceutical products intended for improving immunity in human population.

MATERIALS AND METHODS

Collection of materials

Citrus fruits of different types i.e., Orange (*Citrus sinensis*), Mosambi (*Citrus limetta*), Kinnow (*Citrus reticulata*), Hill Lemon (*Citrus pseudolimon*) and Malta (*Citrus sinensis*) were procured from the local market of Pantnagar, India and were brought to the Department of Food Science and Technology, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, India.

Preparation of extract

Extract of fresh citrus flavedo was prepared according to the method of Jin *et al.* (2022) with slight modification. Fresh citrus flavedo (1 g) of the different citrus fruits under consideration were weighed and crushed in a pestle mortar. Solvent (20 mL) of absolute ethanol was added in it and extraction by reflux method was performed at 80ºC for 2 h, filtered using a Whatman No. 1 filter paper and stored at 4ºC till further use.

Physiochemical parameters

Moisture content

The moisture content was estimated by drying the weighed sample up to a constant weight in hot air oven at 105 ± 2ÚÙC (Özcan *et al.*, 2021). The dried sample was then cooled to room temperature in desiccator prior to weighing. Moisture in terms of percentage was calculated as given below:

$$\text{Moisture (\%)} = \frac{(\text{Weight of fresh sample} - \text{Weight of dried sample})}{\text{Weight of fresh sample}} \times 100$$

Total Phenolic Content

Folin–Ciocalteu method (Singleton *et al.*, 1999) was used for the estimation of total phenolic content (TPC) from citrus peel flavedo with slight modification. All experiments were performed in triplicates. A total of 300 µL of prepared extract, 1700 µL of distilled water and 100 µL of Folin-Ciocalteu reagent (FCR) were vortexed. Following this, 300 µL of sodium carbonate (20 %) was added and mixed thoroughly. The mixture was kept for 60 min in a dark place and the absorbance was measured at 750 nm in a UV-Vis spectrophotometer. TPC was expressed in mg of gallic acid equivalent per gram of peel (mg GAE/g dry weight basis, dw).

Total Flavonoid Content

Total flavonoid content (TFC) was estimated following the method of Formagio *et al.* (2014) with slight modifications. All experiments were performed in triplicates. Extract (80 µL) was taken in a test tube, 0.42 mL of 95 % ethanol was added followed by addition of 0.025 mL aluminum chloride (10 %), 0.025 mL sodium acetate solution (1 M) and 0.7 mL distilled water. Solution was mixed and incubated for 40 min and the absorbance was measured at 415 nm. TFC was expressed in mg quercetin equivalents per gram of peel (mg QCE/g dw).

Antioxidant activity assays and Antioxidant potency composite index (APCI)

Antioxidant activity was measured using three different *in vitro* assays i.e., 2, 2-Diphenyl-1-picryhydrazyl (DPPH), 2,2-azinobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) and Ferric reducing antioxidant power (FRAP). Method for measuring antioxidant activity by DPPH method was adopted from Abu Bakar *et al.* (2009) with slight modifications. An aliquot of citrus flavedo extract (130 µL) mixed with 5 mL of DPPH (0.004 %) dissolved in methanol and incubated at room temperature for 30 min. The final absorbance was measured at 517 nm using the UV-visible spectrophotometer while methanol was taken as blank. ABTS *in vitro* antioxidant method was performed according to the method of Belwal *et al.* (2016). Absorbance was measured at 734 nm using

the UV-visible Spectrophotometer. For FRAP, the method of Singanusong *et al.* (2014) was followed. Absorbance was taken at 593 nm with the help of UV-visible spectrophotometer after incubating the sample in dark at 25 ± 2 °C for 30 min. Results were expressed in mg of BHT equivalent per gram of citrus peel (mg BHTE/g dw).

Antioxidant Potency Composite Index (APCI) of DPPH, ABTS and FRAP were calculated according to the method of Chen *et al.* (2021) using the following equation:

$$APCI = \frac{\text{measured value of assay (sample score)}}{\text{maximum value of assay (best score)}} \times 100$$

Further, the average of all APCI index (DPPH, ABTS, FRAP) was calculated for each fresh citrus flavedo which was considered as APCI.

Statistical analysis

Data was expressed as mean ± standard deviation of three replicates. One - way ANOVA with post-hoc Tukey's multiple comparison test was performed for phenol, flavonoid and antioxidant capacity analysis. The graphical representation of data was done using GraphPad Prism 8.0.1 version software.

RESULTS AND DISCUSSION

Moisture content

Fresh hill lemon peel contained high moisture content (80.76 ± 0.83 %) followed by kinnow (76.10 ± 0.56 %), malta (74.23 ± 0.23), orange (73.82 ± 0.91) and mousambi peel (62.56 ± 1.28). Like our study, Abd El-ghfar *et al.* (2016) also reported more moisture content of fresh lemon peel (81.23 %) as compared to orange peel (74.35 %). Özcan *et al.* (2021) noted 82.08 % of moisture content in fresh lemon peel which was similar to our findings.

Total Phenol Content and Total Flavonoid Content

Both phenol and flavonoid play a vital role in human health as they show antioxidant, antibacterial, anti-inflammatory, and anti-cancerous properties and, protect the human skin from UV rays (Tungmunnithum *et al.,* 2018). Orange showed the highest phenol content (15.11 ± 0.18 mg GAE/g dw) followed by kinnow (14.57 ± 0.41 mg GAE/g dw), malta (12.03 ± 0.99 mg GAE/g dw), mosambi (11.45 ± 0.87 mg GAE/g dw) and hill lemon (9.80 ± 0.24 mg GAE/g dw) (Fig. 1A). Statistically, total phenol content of orange and kinnow peel were found at par with each other. In case of TFC, kinnow showed

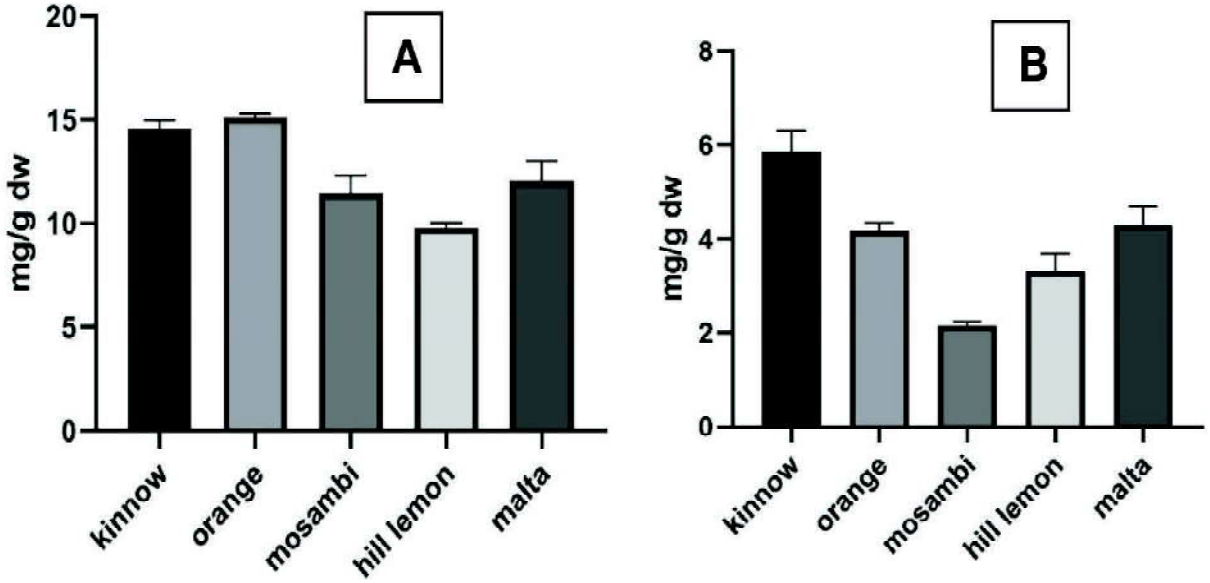

Fig. 1: (A) Total phenol content (mg GAE/g dw) (B) Total flavonoid content (mg QCE/g dw) in different fresh citrus flavedo, Error bars indicate the SD (n=3)

Table 1: Antioxidant activity (mg/g dw) and APCI with their ranking in different citrus fruits

Citrus peel	DPPH	DPPH index	ABTS	ABTS index	FRAP	FRAP index	Average	Ranking
Kinnow	60.41 ± 0.71	100.00	26.34 ± 1.32^{abc}	100.00	38.59 ± 4.87^{ab}	88.71	96.23	1
Orange	56.26 ± 0.45	93.13	22.71 ± 2.57^{adef}	86.22	41.33 ± 5.82^{ac}	95.01	91.45	2
Mosambi	45.29 ± 0.50	74.97	16.34 ± 2.25^{dgh}	62.01	19.98 ± 1.47^{d}	45.93	60.97	5
Hill Lemon	49.81 ± 0.41^{a}	82.46	19.83 ± 1.83^{begi}	75.26	26.39 ± 3.56^{d}	60.69	72.80	4
Malta	49.15 ± 1.03^{a}	81.36	22.45 ± 3.67^{efhi}	85.21	43.49 ± 2.48^{bc}	100	88.85	3

same lowercase letters show statistically non-significant difference within column, $p < 0.05$

highest TFC (5.86 ± 0.46 mg QCE/g dw) followed by malta (4.29 ± 0.99 mg QCE/g dw), orange (4.19 ± 0.16 mg QCE/g dw), hill lemon (3.32 ± 0.37 mg QCE/g dw) and mosambi (2.15 ± 0.10 mg QCE/g dw). Saleem *et al.* (2023) reported highest TPC (21.33 mg GAE/g) and TFC (2.02 mg QCE/g dw) in orange peel and lemon peel, respectively. Assefa *et al.* (2017) reported total flavonoid content of 2.1 mg/g dw in orange peel which was slightly less than our study. Different harvesting time, cultivar, extraction method, extract drying and geographical region are some of the important factors which may be responsible for the variations in the content of phenol and flavonoid in different studies (Rafiq *et al.,* 2019).

Antioxidant activity and Antioxidant potency composite index (APCI)

Antioxidant assay generally works on two principal modes of action i.e., hydrogen atom transfer (HAT) and single electron transfer (SET). DPPH and ABTS follow HAT mechanism whereas FRAP works on SET mechanism (Norma Francenia *et al.,* 2019, Siddeeg *et al.,* 2021). In DPPH assay, deep purple colour of DPPH is changed to pale yellow colour (2,2-diphenyl-1-picrylhydrazine) by the antioxidants present in sample whereas in ABTS assay, ABTS blue-green radical is generated that is reduced by antioxidants and changes its colour into pale blue (Kim *et al.,* 2023). On the contrary, FRAP assay examines the overall reducing ability of antioxidants present in the sample by estimating the reduction of ferric (III) ion to ferrous (II) ions (Li *et al.,* 2008). Proper selection of methods is necessary for the determination of antioxidant capacity, based on the reaction characteristics of the compounds, as there is no single method for determining antioxidant capacity that reflects the effect of all antioxidants present in a complex mixture of bioactive substances due to the different mechanisms of action and nature of antioxidant and oxidizing substances (Gómez-Urios *et al.,* 2023). Therefore, it is always preferable to check antioxidant activity of sample by more than one method. Table 1 shows the antioxidant capacity of citrus flavedo extract measured by different antioxidant assays i.e., DPPH, ABTS and FRAP assay. Kinnow flavedo showed the highest DPPH (60.41 mg/g dw) and ABTS (26.34 mg/g dw) antioxidant capacity as compared to other citrus flavedo which might be due to the presence of high phenol and flavonoid content. However, FRAP antioxidant capacity of orange flavedo (41.33 mg/g dw) was found at par with kinnow flavedo (38.59 mg/g dw). Chen *et al.* (2021) measured strong antioxidant capacity in mandarin and weakest in pummelo freeze-dried citrus peel powder extracted under ultrasonicated conditions with methanol.

All three assays i.e. DPPH, ABTS and FRAP gave different values of antioxidant capacities for citrus flavedo extract. Since a single method for antioxidant estimation may not be very reliable, APCI was determined to take into consideration the overall effect of almost all the active ingredients/ bioactive components contributing towards antioxidant activity. In this context, APCI took into consideration DPPH, ABTS and FRAP for depicting overall antioxidant potential of the samples. As shown in Table 1, the average for DPPH, ABTS and FRAP APCI (i.e. overall APCI) ranged between 60.97 to 96.23 for fresh citrus flavedo based on which different ranks were assigned to the samples. Considering this ranking system, fresh kinnow citrus flavedo was ranked first whereas mosambi ranked last, among all citrus flavedo peel. Chen *et al.* (2020) computed the APCI of 27 citrus cultivars peels of eight citrus species which ranged from 10.06 to 80.13 and the top six APC index was found under *Citrus reticulata* species. Kinnow mandarin that ranked first

in our study, also comes under *Citrus reticulata* species. Likewise, Chen *et al.* (2021) calculated APCI of 52 varieties including mandarin (27 cultivars), sweet orange (15 cultivars), pummelo (7 cultivars), lemon (2 species) and kumquat (1 cultivar)varied between 6.23 to 94.56 and mandarin showed the highest antioxidant capacity among all.

CONCLUSION

The results of this study showed that fresh citrus flavedo contains high concentration of flavonoid and phenolic content. Orange peel was found to contain a higher quantity of phenol content whereas kinnow peel had high flavonoid content, among all citrus peels. In DPPH and ABTS assay, kinnow flavedo showed higher antioxidant capacity that might be due to presence of sufficient TPC and TFC content in kinnow peel while in FRAP assay, orange flavedo showed the highest value. With respect to APCI, kinnow was ranked first followed by orange, malta, hill lemon and mosambi flavedo. Hence, extract from fresh citrus flavedo could be preferred for the utilization in functional food and nutraceuticals as they showed high phytochemical content as well as antioxidant capacity.

REFERENCES

Abd El-ghfar, MHA., Ibrahim, HM., Hassan, IM., Abdel Fattah, AA. and Mahmoud, MH. (2016). Peels of Lemon and Orange as Value-Added Ingredients: Chemical and Antioxidant Properties. *International Journal of Current Microbiology and Applied Science*, 5(12): 777-794.

Abu Bakar, M. F., Mohamed, M., Rahmat, A., and Fry, J. (2009). Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissimus*). *Food Chemistry*, 113(2): 479–483.

Agrawal, P.K., Agrawal, C. and Blunden, G. (2021). Pharmacological Significance of Hesperidin and Hesperetin, Two Citrus Flavonoids, as Promising Antiviral Compounds for Prophylaxis Against and Combating COVID-19. *Natural Product Communications*,16:1-15.

Alberca, R.W., Teixeira, F. M. E., Beserra, D. R., de Oliveira, E. A., Andrade, M. M. S., Pietrobon, A. J. and Sato, M. N. (2020). Perspective: The Potential Effects of Naringenin in COVID-19. *Frontiers in Immunology,*11: 570919.

Assefa, A.D., Saini, R.K. and Young-Soo (2017). Fatty acids, tocopherols, phenolic and antioxidant properties of six citrus fruit species: a comparative study. *Journal of Food Measurement and Characterization*. doi:10.1007/s11694-017-9546-x

Bellavite, P. and Donzelli, A. (2020). Hesperidin and SARS-CoV-2: New Light on the Healthy Function of Citrus Fruits. *Antioxidants (Basel),* 9:742.

Belwal, T., Dhyani, P., Bhatt, I. D., Rawal, R. S. & Pande, V. (2016). Optimization extraction conditions for improving phenolic content and antioxidant activity in Berberis asiatica fruits using response surface methodology (RSM). *Food Chemistry*, 207:115-24.

Borgonovi, T. F., Virgolin, L. B., Janzantti, N. S., Casarotti, S. N. and Penna, A. L. B. (2022). Fruit bioactive compounds: Effect on lactic acid bacteria and on intestinal microbiota. *Food Research Internationa*l, 161:111809.

Chen, Q., Wang, D., Tan, C., Hu, Y., Sundararajan, B. and Zhou, Z. (2020). Profiling of Flavonoid and Antioxidant Activity of Fruit Tissues from 27 Chinese Local Citrus Cultivars. *Plant*s, 9:196.

Chen, Y., Pan, H., Hao, S., Pan, D., Wang, G. and Yu, W. (2021). Evaluation of phenolic composition and antioxidant properties of different varieties of Chinese citrus. *Food Chemistry*, 364:130413.

Formagio ASN, Volobuff CRF, Santiago M, Cardoso CAL, Vieira MDC, Valdevina Pereira Z. (2014). Evaluation of Antioxidant Activity, Total Flavonoids, Tannins and Phenolic Compounds in Psychotria Leaf Extracts. *Antioxidants*, 3(4):745-757.

Gómez-Urios, C., Viñas-Ospino, A., Puchades-

Colera, P., Blesa, J., López-Malo, D., Frígola, A. and Esteve, M.J. (2023). Choline chloride-based natural deep eutectic solvents for the extraction and stability of phenolic compounds, ascorbic acid, and antioxidant capacity from Citrus sinensis peel. *LWT-Food Science and Technology,*177:114595.

Jin, T., YU, M., CAO, M. and Zhu, X. (2022). Optimization of mechanochemical-assisted extraction of hesperidin from Pericarpium Citri Reticulatae. *Food Science and Technology*. 42:e79821.

Khan, U. M., Sameen, A., Aadil, R. M., Shahid, M., Sezen, S., Zarrabi, A., Ozdemir, B., Sevindik, M., Kaplan, D. N., Selamoglu, Z., Ydyrys, A., Anitha, T., Kumar, M., Sharifi-Rad, J. and Butnariu, M. (2021). Citrus Genus and Its Waste Utilization: A Review on Health-Promoting Activities and Industrial Application. *Evidence-Based Complementary and Alternative Medicine,*2021: 2488804.

Kim, J.-W., Ko, H. C., Jang, M.-G., Han, S. H., Kim, H. J. and Kim, S.-J. (2023). Phytochemical content and antioxidant activity in eight citrus cultivars grown in Jeju Island according to harvest time. *International Journal of Food Properties*, 26:14-23.

Li, H.B., Wong, C.C., Cheng, K.W. and Chen, F. (2008). Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. *LWT-Food Science and Technology*, 41:385-390.

Mahato, N., Sinha, M., Sharma, K., Koteswararao, R. and Cho, M. H. (2019). Modern Extraction and Purification Techniques for Obtaining High Purity Food-Grade Bioactive Compounds and Value-Added Co-Products from Citrus Wastes. *Foods*, 8:523.

Norma Francenia, S.-S., Raúl, S.-C., Claudia, V.-C. and Beatriz, H.-C. (2019). Antioxidant Compounds and Their Antioxidant Mechanism. In: Antioxidants (edited by EMAD, S.). Pp. Ch. 2. Rijeka: IntechOpen.

Özcan, MM., Ghafoor, K., Al Juhaimi, F., Uslu, N., Babiker, EE., Mohamed Ahmed, IA. and Almusallam, IA. (2021). Influence of drying techniques on bioactive properties, phenolic compounds and fatty acid compositions of dried lemon and orange peel powders. *Journal of Food Science and Technology,* 58(1):147-158.

Saleem. S., Arjumand, I.D., Asnuzilawati, A., Mahmood, A., Muhammad, A., Numan, Y., and Muhammad, M (2023). Investigation of antioxidant and antibacterial effects of citrus fruits peel extracts using different extracting agents: Phytochemical analysis with in silico studies. *Heliyon,* 9(4): e15433.

Singleton, VL., Orthofer, R. and Lamuela-Raventos, RM. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Method Enzymol.,* 299:152–178.

Rafiq, S., Kaul, R., Sofi, S. A., Bashir, N., Nazir, F. and Ahmad Nayik, G. (2018). Citrus peel as a source of functional ingredient: A review. *Journal of the Saudi Society of Agricultural Sciences,*17:351-358.

Rafiq, S., Singh, B. and Gat, Y. (2019). Effect of different drying techniques on chemical composition, color and antioxidant properties of kinnow (*Citrus reticulata*) peel. *Journal of Food Science and Technology,*56:2458-2466.

Sharma, K., Mahato, N., Cho, M. H. and Lee, Y. R. (2017). Converting citrus wastes into value-added products: Economic and environmently friendly approaches. *Nutrition,*34:29-46.

Siddeeg, A., AlKehayez, N. M., Abu-Hiamed, H. A., Al-Sanea, E. A. and Al-Farga, A. M. (2021). Mode of action and determination of antioxidant activity in the dietary sources: An overview. *Saudi Journal of Biological Science*s, 28:1633-1644.

Singanusong, R., Nipornram, S., Tochampa, W. and Rattanatraiwong, P. (2014). Low Power Ultrasound-Assisted Extraction of Phenolic Compounds from Mandarin (Citrus reticulata Blanco cv. Sainampueng) and Lime (Citrus aurantifolia) Peels and the Antioxidant. *Food Analytical*

Methods, 8:1112–1123.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A. and Yangsabai, A. (2018). Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines (Basel)*, 5:93.

Received: March 01, 2024
Accepted: April 02, 2024