ASIAN J NAT PROD BIOCHEM
Volume 20, Number 1, June 2022
Pages: 21-26

P-ISSN: 2775-4189
E-ISSN: 2775-4197
DOI: 10.13057/biofar/f200105

Extraction, purification, and quantification of hesperidin from the immature *Citrus grandis*/*maxima* fruit Nepal cultivar

HEMANTH KUMAR MANIKYAM[1,♥], PRATHIBHA TRIPATHI[2], SANDEEP BALVANT PATIL[3], JANARDAN LAMICHHANE[4], MVNL CHAITANYA[5], ABHINANDAN RAVSAHEB PATIL[6]

[1]Department of Chemistry, Faculty of Science, North East Frontier Technical University. Aalo, West Siang 791001, Arunachal Pradesh, India. Tel./Fax.: +91-3783-222-444, ♥email: phytochem2@gmail.com
[2]Ayurveda Campus, Tribhuvan University. Kirtipur Rd, Kirtipur 44618, Nepal
[3]Dr. Shivajirao Kadam College of Pharmacy. Sangli Rd, Maharashtra 416301, India
[4]Department of Biotechnology, Organic farming and Natural Product Research Centre, Kathmandu University. Kavre, Dhulikhel 45200, Nepal
[5]Chitkara College of Pharmacy, Chitkara University. Punjab 140417, India
[6]Department of Pharmaceutics, Sanjay Ghodawat University. Kolhapur, Maharashtra 416118, India

Manuscript received: 9 April 2022. Revision accepted: 26 June 2022.

Abstract. *Manikyam HK, Tripathi P, Patil SB, Lamichhane J, Chaitanya MVNL, Patil AR. 2022. Extraction, purification, and quantification of hesperidin from the immature* Citrus grandis/maxima *fruit Nepal cultivar. Asian J Nat Prod Biochem 20: 21-26.* Hesperidin, a flavanone group of flavonoids and a 7-O-rutinoside of hesperitin is abundantly present in citrus fruits. Pomelo or *Citrus grandis* (L.) Osbeck/ *C. maxima* (Burm.) Merr. is grown and cultivated in Nepal as seasonal edible fruit. Pomelo has flavanone and other chemical constituents. Flavanones like naringin, hesperidin, neohesperidin, and naringenin are present in the fruits of *C. grandis*. Many extraction techniques have been reported in the extraction and purification of hesperidin citrus fruits like sweet lemons, grapefruits, mandarins, etc. However, little information has been provided on hesperidin content in pomelo fruits. In order to estimate the quantity of hesperidin in the pomelo fruits of Nepal, we have first time made study. The immature pomelo fruits of size 2-7 cm were collected from the cultivation field and dried completely. The pulverized fruit powder with moisture content less than 15% was subjected to 5% methanolic acetic extraction and Dimethylformamide (DMF) solvent extraction in Soxhlet apparatus by refluxing at 90°C in 1:15 ration in three subsequent extractions of each 1 hour. The concentrates were crystalized at freezing temperatures in methanol and further purified by repeating the crystallization process. The white amorphous powder was subjected to RP-HPLC analysis method, chromatographic conditions 285 nm wavelength, C18 column 4.6 mm x 15 cm x 5 µm, flow rate 1.2 mL/minute with injection volume 10 µL run time of 30 minutes, column temperature 40°C. Hesperidin yields were 0.12 g/100 g with acetic acid methanol with a purity of 87% and 0.17 g/100 g in DMF extraction with a purity of 90% with recoveries of 87% in acetic acid methanol extraction and 90% in DMF extraction when compared with standard 93%. Further extraction does not yield any hesperidin content indicating a total of 0.15-0.17 g/100 g of hesperidin content in the whole immature fruit of *C. grandis/maxima*. Thus we found DMF and 5% methanolic acetic solvents as preferable solvents to extract hesperidin.

Keywords: Acetic acid, *Citrus grandis/maxima*, dimethylformamide, hesperidin, methanol, Nepal cultivar, RP-HPLC UV method

INTRODUCTION

Citrus grandis (L.) Osbeck/ *C. maxima* (Burm.) Merr. commonly called pomelo (red pomelo), is an important member of the Rutaceae family, well known for its high morphological variability, nutritional and commercial values (Alam et al. 2014; Susandarini et al. 2020). It was found in Barbados and commercially grown in Morocco, Israel, Spain, South Africa, Brazil, USA, and some Asian countries (Jokic et al. 2019). In Nepal, it is called Bhogate and is eaten as seasonal fruit. In Nepal, they make Bhogate sadheko, a common salad eaten by local people. In India commonly called Chakotra. China is the major producer of pomelo, which is more than 60% of the world's cultivation (Man et al. 2015). Many bioactive compounds like flavanones, flavones, flavonols, and anthocyanin are abundantly present in the pomelo fruit. Chemical ingredients like limonoid glycones and aglycones, coumarins, naringin, hesperidin flavanones, etc. are the most abundant compounds in fruits of pomelo. Naringin is the most abundant flavanone present, around 295 to 377 mg/L in grapefruits and pomelo. many research studies have proved that the extracts of pomelo have a therapeutic effect on cancer, inflammation, diabetes, neurodegenerative and cardiovascular diseases with powerful antioxidant activity (Damon et al. 1987; Ma et al. 2008). It was studied that active components in the pomelo extract by using high-performance liquid chromatography (HPLC) had naringin (11.90 ± 0.21 mg/g dried extract), hesperidin (12.04 ± 0.12 mg/g dried extract), neohesperidin (25.4 ± 0.12 mg/g dried extract), and naringenin (9.20 ± 0.19 mg/g dried extract) (El-Shafae and El-Domiaty 2001; Lucker et al. 2002; Iglesias-Carres et al. 2019).

Hesperidin bio-flavanone, also known as hesperitin 7-rutinoside or 7-O-glycoside hesperitin (3,5,7 trihydroxyfavanone 7-rhamnoglucoside, C28H34O15). Figure 1, abundantly present in citrus fruits with a molecular weight of 610.57 with melting point 250-255°C and boiling point of 576.17°C, respectively. It is not soluble in water and highly soluble in alkaline solutions but

moderately soluble in propylene glycol and Poly ethylene glycol 400 (Kanaze et al. 2004). It is highly soluble in DMSO and DMF. Poorly soluble in solvents like ethanol, isopropanol, and methanol (when purified) (Pandey et al. 2015).

Hesperidin is generally considered safe for topical and systemic administrations with 2% in topical applications, it has shown no adverse cutaneous reactions in mice for 9 days of administration (Xiong et al. 2019). In addition, the oral formulation Daflon-500 mg containing 10% hesperidin with a daily dosage of 100 mg showed no adverse effects in rats (Razavi and Hosseinzadeh 2019).

Multiple therapeutic effects of hesperidin include anti-inflammatory, lipid-lowering, analgesic, antioxidant, and anticancer activities. Other than mentioned health benefits, hesperidin is well known to treat venous insufficiency, varicose veins, venous ulcers, and hemorrhoids (Paudyal and Haq 2008; Man et al. 2019). By stimulating the release of the appetite-regulating hormone cholecystokinin (CCK) in enteroendocrine STC-1 cells, both hesperitin and hesperidin help treat obesity by controlling appetite (Magwaza et al. 2015). In one of the studies on the neuroblastoma cell line, hesperidin has exerted a neuroprotective effect against rotenone by its antioxidant effect and maintaining mitochondrial functioning (Wu et al. 2007).

In recent studies, attempted extraction of hesperidin from peels of *Citrus reticulata* Blanco mandarin using deep eutectic solvents (DESs), a sustainable green extraction technique using choline chloride:acetamide (1:2) and water DESs had shown most efficient extraction of hesperidin 112.14 mg/g (Hendrickson and Kesterson 1954). Extraction of hesperidin by ultrasound assistance has shown that solvent has a profound effect on the yields, along with an increase in temperature and extraction time (Kim and Lim 2020). The highest flavonoid yields were achieved by 58.4% ethanol concentration at 80°C, 40 mL/g solvents to feed concentration for 30 minutes, and hesperidin yields of 66.6%. The extraction conditions for the highest flavonoid yields based on a response surface methodology were 80.3°C, 58.4% (ethanol concentration), 40 mL/g (solvent/feed), and 30 min (Tamilselvam et al. 2013). Ethanol extraction was found to be more effective when compared to methanol (yield 57.3%) and acetone (yield 37.7%) (Tamilselvam et al. 2013). Some studies showed that the yield of hesperidin was higher in 70% methanol than in 80% ethanol at 35°C from mandarin peels (Caengprasath et al. 2013), and *C. sinensis* pulp was higher in 90% methanol than 90% ethanol at 55°C (Kim and Lim 2020).

In the present study for the first time, for the first time, we are extracting hesperidin from immature fruits of the *C. grandis/maxima* (pomelo) cultivar of Nepal and quantifying it.

MATERIALS AND METHODS

Plant material preparation: *C. grandis/maxima* fruits were obtained from the local cultivation field Chisapani area, Kathmandu, Nepal, and immature fruits of size 2-7 cm see Figure 2, were dried till moisture content reached 15%. The 200 g of dried immature fruits of 2-7 cm were pulverized to 180 microns mesh size.

Extraction of hesperidin using acetic acid methanol solvent

Weight separately 100 g of dried immature fruit powder of 2-7 cm size and place it in Soxhlet apparatus with 5% acetic in methanol (1:15 feed/solvent) and continued extraction for 1 hour at 100°C with a circulation of solvent. Total 3 extractions were carried out at an interval of each 1 hour with 1:15 feed to solvent ratio consequently. The total extract is collected, filtered, and concentrated to 80% before chilling. The 80% reduced liquid concentrate was chilled at 5°C for a few hours and filtered. The precipitate of hesperidin is filtered and washed with pure methanol till white-colored amorphous powder appears and is further quantified.

Extraction of hesperidin with dimethylformamide

Weight separately 100 g of dried immature fruit powder of 2-7 cm size and place it in maceration apparatus in DMF (1:15 feed/solvent) and continued extraction stirring for 1 hour at 100°C with a solvent circulation. Total 3 extractions were carried out at an interval of each 1 hour with 1:15 feed to solvent ratio consequently. The extract is collected, filtered and concentrated to 80% before chilling. The 80% reduced liquid concentrate was chilled at 5°C for a few hours and filtered. The precipitate of hesperidin is filtered and washed with pure methanol till white-colored amorphous powder appears and is further quantified.

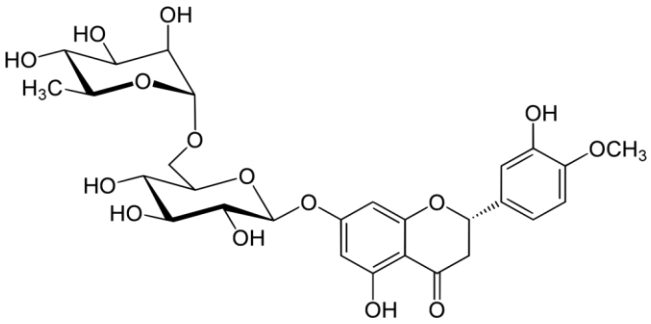

Figure 1. Structure of hesperidin

Figure 2. Immature fruit (2-7 cm) of *Citrus grandis*/*maxima*

Methods

Loss on drying

Accurately weigh 1.0~2.0 g of sample, and spread it on a dry flat weighing bottle previously weighed. Dry the bottle at 135 ± 2°C for 4 hours. Calculate loss on drying by the following formula, which should be less than 5.0%.

Loss on drying%= $(M1 - M2/M1 - M)$ X100%

Where:

M: Tare weight of the weighing bottle, g;

M1: Weight of the weighing bottle and sample before drying, g;

M2: Weight of the weighing bottle and sample after drying, g

Assay (RP-HPLC)

Chromatographic system

Chromatography system: HPLC with UV detector; Column: 4.6 mm x 15 cm x 5 µm packing C18; Wavelength: UV 284 nm; Flow rate: 1.2 mL/min; Column temperature: 40°C; Injection volume: 10 µL; Run time: 30 minutes

Mobile phase: Methanol: water: Glacial acetic Acid: acetonitrile= 28:66:6:2(V/V), diluent for sample preparation: dimethyl sulphoxide

Preparation of solution

Test solution: Accurately weigh 25 mg of sample to a 50 mL volumetric flask, dissolve and dilute to exactly 50 mL with dimethyl sulphoxide, and mix well.

Reference solution: Accurately weigh 25 mg of a hesperidin reference standard to a 50 mL volumetric flask, dissolve and dilute to exactly 50 mL with dimethyl sulphoxide, and mix well.

Test procedure

Inject reference solution, test solution, and record the chromatograms. Calculate the assay of hesperidin according to a standard external method based on an anhydrous basis. The result of the assay should be not less than 90%.

Assay (%w/w) = $(A_T/A_R \text{ X } W_S/W_R) \text{ X P X } 100\%/1\text{-LOD}$

A_T : Main peak area in the chromatograms obtained with test solution;

A_R : Main peak area in the chromatograms obtained with reference solution;

W_S : Weight of sample, mg;

W_R : Weight of reference standard, mg;

P : Assay of reference standard, %;

LOD : Loss on drying of sample, %.

Method validation

P Parameters like linearity, precision, accuracy, and limits of detection (LOD) and quantification (LOQ) were developed to determine the hesperidin content by HPLC method. Calibration graphs for linearity were determined using standard solution preparation of hesperidin at different concentrations of 20, 60, and 100 µg/mL. The dilutions were injected in series, and the peak area was calculated for each concentration plotted against the peak area. By adding standard at three concentrations of 20, 60, and 100 µg/mL, percentage recovery was calculated for each concentration to determine accuracy. Hesperidin was injected three times on the same day and on 3 different days to determine intra-day and inter-day variations to determine system precision. Related standard deviation (RSD) was considered a precision measure in both cases. By determining the LOD and LOQ, sensitivity was evaluated. The amount of analyte which gives a peak with a signal-to-noise ratio of 3 was defined as LOD, and the lowest amount of analyte with a signal-to-noise ratio of 10 was considered LOQ. The International Conference on Harmonization (ICH) was considered to validate the method.

Quantification of hesperidin in concentrated methanolic acetic acid extract

The total methanolic acetic acid extracts thus collected after 3 subsequent extractions were combined and concentrated. The concentrated extract was then subjected to RP-HPLC chromatography as mentioned in the assay method for standard hesperidin, and the area of peak in correlation to the retention time of standard was analyzed, and the amount of hesperidin was calculated from the calibration plot obtained by a regression equation.

RESULTS AND DISCUSSION

Hesperidin a bioflavonoid present in all citrus fruits. It is also known as Hesperitin 7-rutinoside or 7-O-glycoside hesperitin (3, 5, 7 trihydroxyfavanone 7-rhamnoglucoside. It is a highly economically viable phytochemical in demand for the Pharmaceutical, Nutraceutical, and cosmeceutical industries. It is a precursor molecule to synthesize Diosmin, a molecule used to treat neurological problems. Extraction of hesperidin using economically viable methods is needed in the present scenario looking at the abundant availability of raw material resources and market demand. Most of the immature citrus fruits varying sizes of 2 mm- 3 mm contain 10-12% of hesperidin. Pomelo (*C. grandis/maxima*) is abundantly grown in Nepal as edible fruit. Pomelo fruit contains vitamin C, beta carotene, anthocyanin, hesperidin, and Naringin ingredients. Even though hesperidin content is found to be less in fruits of pomelo, we attempted to quantify hesperidin in immature dried fruits for the first time in the Nepal cultivar of pomelo. Many works have suggested using ethanol and methanol as solvents to extract hesperidin. In our present work, we have used two solvent methods like 5% acetic acid in methanol and dimethylformamide, to extract hesperidin. The pH and temperature directly affect the recovery of the extraction of hesperidin. In experiments conducted by Hendrickson and Kesterson Florida horticulture society in 1955, they found hesperidin poor solubility in high pH alcoholic solvents and high solubility in low pH alcoholic solvents, and also, the

optimal temperature for extraction should be from 90°C to 100°C (Alam et al. 2014). Research shows hesperidin solubility in DMF and DMSO (30 mg/mL) and soluble in ethanol and methanol (~1 mg/mL). Two separate experiments were carried out to extract hesperidin using 5% acetic acid in methanol and DMF in 1:15 feed to the solvent ratio for 3 hours consecutively at 100°C while stirring, later the liquid extract was filtered and concentrated up to 80%. The concentrate chilled at 5 °C for a few hours. Thus, the precipitate was filtered and further purified by washing it with pure methanol. The obtained hesperidin was quantified using RP-HPLC UV analysis method, chromatographic conditions 285 nm wavelength, C18 column 4.6 mm x 15cm x 5 µm, flow rate 1.2 mL/minute with injection volume 10 µL run time of 30 minutes, column temperature 40°C. Hesperidin yields were 0.12 g/100g with acetic acid methanol with a purity of 87% and 0.17 g/100 g in DMF extraction with a purity of 90% with recoveries of 87% in acetic acid methanol extraction and 90% in DMF extraction when compared with standard 93% see Figure 3 and 4. Further extraction does not yield any hesperidin content indicating a total of 0.15-0.17 g/100 g of hesperidin content in the whole immature fruit of *C. grandis/maxima*. Thus we suggest using acetic acid methanol, methanol, or DMF as good solvents to extract high yields of hesperidin with good purity.

Method validation

Linearity, LOD, and LOQ

It was confirmed that the linearity of the method for concentrations ranging from 20 to 100 µg/mL of hesperidin. Good linear regressions observed with calibration curves y= 0.3456x + 0.1573 R^2= 0.9973 (Figure 5 and Table 1). The minimum amount of analyte plant extract LOD used for detection and LOQ was 6.66 µg/mL and 20.19 µg/mL, with a retention time of 7.45 minutes.

Precision and accuracy

The precision and accuracy tests were performed by injecting the hesperidin sample three times within the same and three consecutive days. Inter-day and Intra-day precision by RSD of the hesperidin peak areas ranged from 0.5-2.1, which is 2.5% under the limit as per ICH guidelines. By calculating recovery %, accuracy was determined, and for hesperidin, it ranged between 89.99-102%, and the method was proven accurate (Table 2).

Table 1. Calibration data for the proposed HPLC method

Concentration (µg/mL)	Area (10^5 mAU)
20	7.1
40	13.89
60	21.08
80	28.25
100	33.47
120	42.3

Table 2. Recovery and standard deviation data by HPLC

Compound	Amount added (µg/mL)	Amount recovered (µg/mL)[a]	Recovery (%)[a]	RSD (%)	
				Intra day	Inter day
Hesperidin	40	37.09 ± 0.26	92.75± 0.26	1.73	1.879
	80	81 ± 0.26	101.25 ± 0.26	2.12	0.156
	100	98.9 ± 1.69	98.9 ±1.69	0.65	0.48

Note: [a] Mean ± SD (n=3) mean the sample analyzed three times; [b] Samples were analyzed three times a day; [c] Sample were analyzed once a day over three consecutive days

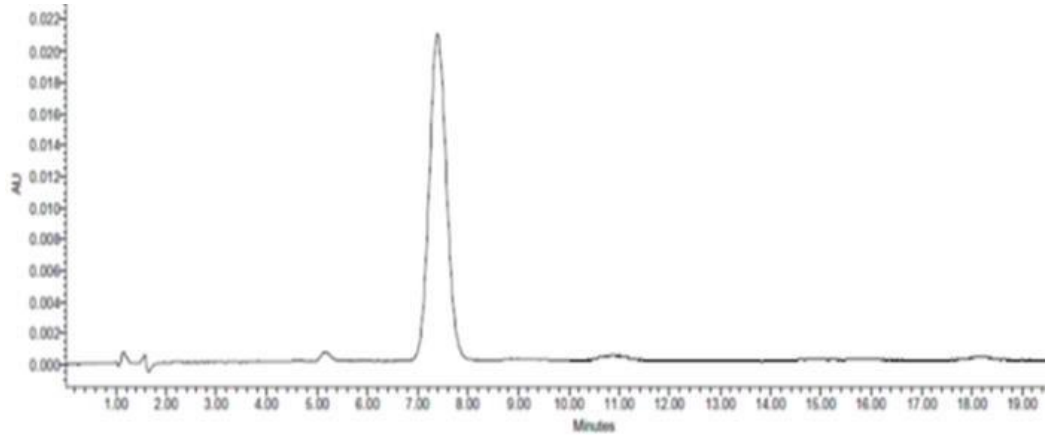

Figure 3. Chromatogram of hesperidin standard 93%

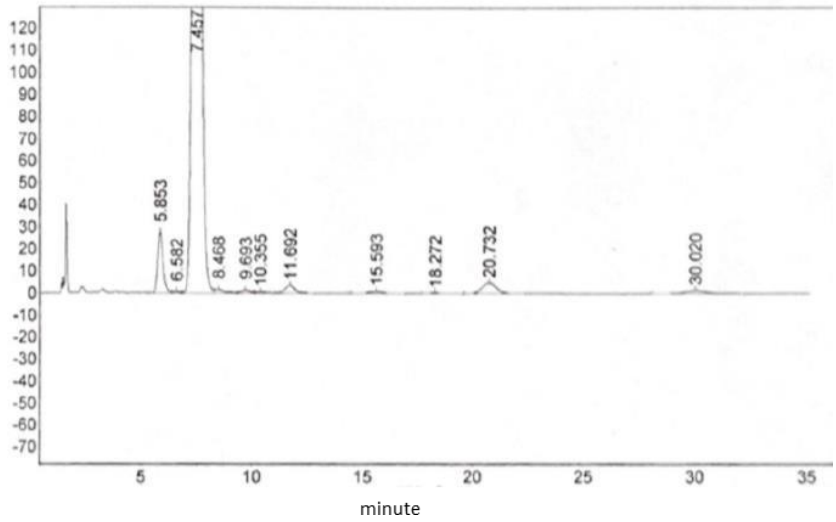

Figure 4. Chromatogram of hesperidin (90% pure) extracted from dried whole immature fruit of *C. grandis*/*maxima*

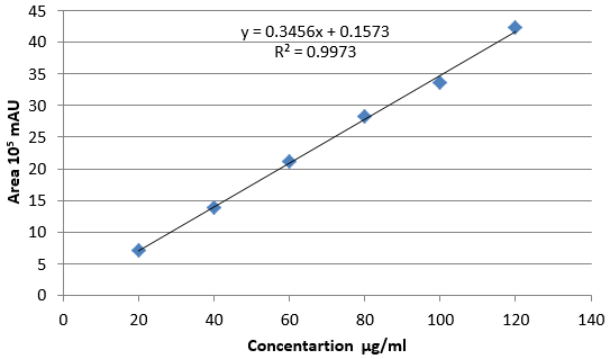

Figure 5. Linearity of hesperidin standard

Discussion

Hesperidin flavanone glycoside is usually found in citrus fruits, and its aglycone is called hesperitin. Plants produce it as a defense mechanism. Different extraction techniques were used earlier to isolate hesperidin with significant yields from different varieties of citrus fruits. It has been found that classical solvent extraction of hesperidin is preferred. The highest flavonoid yield was achieved by 58.4% ethanol concentration at 80°C, 40 mL/g solvents to feed concentration for 30 minutes, and hesperidin yields of 66.6%. The extraction conditions for the highest flavonoid yields based on a response surface methodology were 80.3°C, 58.4% (ethanol concentration), 40 mL/g (solvent/feed), and 30 min (Tamilselvam et al. 2013). Ethanol extraction was found to be more effective when compared to methanol (yield 57.3%) and acetone (yield 37.7%) (Tamilselvam et al. 2013). Some studies showed that the yield of hesperidin was higher in 70% methanol than in 80% ethanol at 35°C from mandarin peels (Caengprasath et al. 2013) and *C. sinensis* pulp was higher in 90% methanol than 90% ethanol at 55°C (Kim and Lim 2020). In our present work, we first time tried to extract hesperidin and quantify it using DMF and 5% methanolic acetic acid as solvents from the whole dried immature fruit of *C. grandis*/*maxima* of the Nepal cultivar variety. Two separate experiments were carried out to extract hesperidin using 5% acetic acid in methanol and DMF in 1:15 feed to the solvent ratio for 3 hours consecutively at 100°C while stirring, later the liquid extract was filtered and concentrated up to 80%. The concentrate chilled at 5°C for a few hours. Thus, the precipitate was filtered and further purified by washing it with pure methanol. The obtained hesperidin was quantified using RP-HPLC UV analysis method, chromatographic conditions 285 nm wavelength, C18 column 4.6 mm x 15 cm x5 µm, flow rate 1.2 mL/minute with injection volume 10 µL run time of 30 minutes, column temperature 40°C. Hesperidin yields were 0.09 g/100 g with acetic acid methanol with a purity of 87% and 0.17 g/100 g in DMF extraction with a purity of 90% with recoveries of 87% in acetic acid methanol extraction and 90% in DMF extraction when compared with standard 93% see Figures 3 and 4. It was confirmed that the linearity of the method for concentrations ranging from 20 to 100 µg/mL of hesperidin. Good linear regressions observed with calibration curves y= 0.3456x + 0.1573, R^2= 0.9973 (Figure 5 and Table 1). The minimum amount of analyte plant extract LOD used for detection and LOQ was 6.66 µg/mL and 20.19 µg/mL, with a retention time of 7.45 minutes. The precision and accuracy tests were performed by injecting the hesperidin sample three times within the same and three consecutive days. Inter-day and Intra-day precision by RSD of the hesperidin peak areas ranged from 0.5-2.1, which is 2.5% under the limit as per ICH guidelines. By calculating recover %, accuracy was determined, and for hesperidin, it ranged between 89.99-102%, and the method was proven accurate (Table 2).

Further extraction does not yield any hesperidin content indicating a total of 0.15-0.17 g/100 g of hesperidin content in the whole immature fruit of *C. grandis/maxima*. Thus, we suggest using acetic acid methanol, methanol, or DMF as good solvents to extract high yields of hesperidin with good purity.

REFERENCES

Alam MA, Subhan N, Rahman MM, Uddin SJ, Reza HM, Sarker SD. 2014. Effect of Citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. Adv Nutr 5 (4): 404-417. DOI: 10.3945/an.113.005603.

Caengprasath N, Ngamukote S, Mäkynen K, Adisakwattana S. 2013. The protective effects of pomelo extract (*Citrus grandis* L. Osbeck) against fructose-mediated protein oxidation and glycation. EXCLI J 12: 491-502.

Damon M, Flandre O, Michel F, Perdrix L, Labrid C, de Paulet AC. 1987. Effect of chronic treatment with a purified flavonoid fraction on inflammatory granuloma in the rat. Study of prostaglandin E2 and F2α and thromboxane B2 release and histological changes. Drug Res 37 (10): 1149-1153.

El-Shafae AM, El-Domiaty MM. 2001. Improved LC methods for the determination of diosmin and/or hesperidin in plant extracts and pharmaceutical formulations. J Pharm Biomed Anal 26 (4): 539-545. DOI: 10.1016/s0731-7085(01)00476-9.

Hendrickson R, Kesterson JW. 1954. Recovery of Citrus Glucosides. Proc Fla State Hort Soc 67: 199-203.

Iglesias-Carres L, Mas-Capdevila A, Bravo FI, Aragonès G, Muguerza B, Arola-Arnal A. 2019. Optimization of a polyphenol extraction method for sweet orange pulp (*Citrus sinensis* L.) to identify phenolic compounds consumed from sweet oranges. PLoS One 14 (1): e0211267. DOI: 10.1371/journal.pone.0211267.

Jokic S, Safranko S, Jakovljevic M, Cikoš A-M, Kajic N, Kolarevic F, Babic J, Molnar M. 2019. Sustainable green procedure for extraction of hesperidin from selected Croatian mandarin peels. Processes 7: 469. DOI: 10.3390/pr7070469.

Kanaze FI, Kokkalou E, Georgarakis M, Niopas I. 2004. A validated solid-phase extraction HPLC method for the simultaneous determination of the citrus flavanone aglycones hesperetin and naringenin in urine. J Pharm Biomed Anal 36 (1): 175-181. DOI: 10.1016/j.jpba.2004.05.015.

Kim DS, Lim SB. 2020. Extraction of flavanones from immature Citrus unshiu pomace: Process optimization and antioxidant evaluation. Sci Rep 10: 19950. DOI: 10.1038/s41598-020-76965-8.

Lucker J, Tamer MK, Schwab W, Verstappen FWA, Plas LHWV, Bouwmesster HJ, Verhoeven HA. 2002. Monoterpene biosynthesis in lemon (*Citrus lemon*). Eur J Biochem 269: 3160-3171. DOI: 10.1046/j.1432-1033.2002.02985.x.

Ma Y, Ye X, Hao Y, Xu G, Xu G, Liu D. 2008. Ultrasound-assisted extraction of hesperidin from Penggan (*Citrus reticulata*) peel. Ultrason Sonochem 15 (3): 227-232. DOI: 10.1016/j.ultsonch.2007.03.006.

Magwaza LS, Opara UL, Cronje PJ, Landahl S, Ortiz JO, Terry LA. 2015. Rapid methods for extracting and quantifying phenolic compounds in citrus rinds. Food Sci Nutr 4 (1): 4-10. DOI: 10.1002/fsn3.210.

Man G, Mauro TM, Y. Zhai Y, Kim PL, Cheung C, Hupe M, Crumrine D, Elias PM, Man M-Q. 2015. Topical hesperidin enhances epidermal function in an aged murine model. J Invest Dermatol 135: 1184-1187. DOI: 10.1038/jid.2014.486.

Man M-Q, Yang B, Elias PM. 2019. Benefits of hesperidin for cutaneous functions. Evid-Based Complement Alternat Med 2019: 1-9. DOI: 10.1155/2019/2676307.

Pandey RP, Gurung RB, Sohng JK. 2015. Dietary sources, bioavailability and biological activities of naringenin and its derivatives. In: Stacks NM (eds). Apigenin and Naringenin: Natural Sources, Pharmacology and Role in Cancer Prevention. Nova Science Publishers, Hauppauge, New York.

Paudyal KP, Haq N. 2008. Variation of pomelo (*Citrus grandis* (L.) Osbeck) in Nepal and participatory selection of strains for further improvement. Agrofor Syst 72: 195-204. DOI: 10.1007/s10457-007-9088-z.

Razavi BM, Hosseinzadeh H. 2019. A Review of the Effects of *Citrus grandis/maxima* (Grapefruit) and Its Flavonoids, Naringin, and Naringenin in Metabolic Syndrome, Bioactive Food as Dietary Interventions for Diabetes. (Second Edition). Academic Press. DOI: 10.1016/B978-0-12-813822-9.00034-5.

Susandarini R, Subandiyah S, Daryono BS, Rugayah. 2020. Microsatellite polymorphism for molecular characterization of pomelo (*Citrus maxima*) accessions from Indonesia. Biodiversitas 21: 2390-2395. DOI: 10.13057/biodiv/d210608.

Tamilselvam K, Braidy N, Manivasagam T, Essa MM, Prasad NR, Karthikeyan S, Thenmozhi AJ, Selvaraju S, Guillemin GJ. 2013. Neuroprotective effects of hesperidin, a plant flavanone, on rotenone-induced oxidative stress and apoptosis in a cellular model for Parkinson's disease. Oxid Med Cell Longev 2013: 102741. DOI: 10.1155/2013/102741.

Wu T, Guan Y, Ya J. 2007. Determination of flavonoids and ascorbic acid in grapefruit peel and juice by capillary electrophoresis with electrochemical detection. Food Chem 100 (4): 1573-1579. DOI: 10.1016/j.foodchem.2005.12.042.

Xiong H, Wang J, Ran Q, Lou G, Peng C, Gan Q, Hu J, Sun J, Yao R, Huang Q. 2019. Hesperidin: A therapeutic agent for obesity. Drug Des Devel Ther 13: 3855-3866. DOI: 10.2147/DDDT.S227499.