

Extraction of flavonoids in pomelo peels using Box-Behnken response surface design and their biological activities

Truong Ngoc Quynh Phuong^{1,2}, Pham Van Hung^{1,2}, Nguyen Thi Lan Phi^{2,3*}

¹Department of Food Technology, International University, Vietnam National University - Ho Chi Minh City, Quarter 6, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam

²Vietnam National University - Ho Chi Minh City, Quarter 6, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam

³Department of Food Technology, University of Technology, Vietnam National University - Ho Chi Minh City, 268 Ly Thuong Kiet Street, Ward 14, District 10 Ho Chi Minh City, Vietnam

Received 9 January 2020; revised 20 March 2020; accepted 6 April 2020

Abstract:

The objectives of this study were to optimize the extraction conditions of flavonoids from the pomelo peel using the Box-Behnken response surface methodology and to investigate the chemical composition and biological activities of the extracts from three different pomelo species under optimized extraction conditions. Three extraction condition factors of were varied, including the solid-solvent ratio (1/15-1/45 g/ml), temperature (30-60°C), and time (20-60 min), which affected the efficiency of the total flavonoid content (TFC). The high R^2 coefficient of 0.93 indicated that the experimental data obtained in this study fit well to a second-order polynomial using multiple regression analysis. The maximum TFC extracted from the pomelo peel was 6.0 mg/g (dried basis, db), which was obtained using Derringer's desired function methodology under the following optimum conditions: a solid-solvent ratio of 1/44 g/ml, temperature of 30.7°C, and time of 34.6 min. The results also indicated that the Tan Trieu pomelo peel had the highest TFC and naringin concentration followed by the Nam Roi and Duong Hong pomelo peels, whereas the Nam Roi pomelo peel had a higher hesperidin concentration than the others. The extracts of the pomelo peels exhibited strong antioxidant activities with low IC_{50} values. However, these extracts showed only a slight effect on cancer cells, including lung cancer and breast cancer, when tested at 100 µg/ml.

Keywords: antioxidant, Box-Behnken design, extraction, flavonoids, pomelo.

Classification number: 3.1

1. Introduction

Pomelo (*Citrus grandis*), a member of the citrus family, is grown in many eastern countries including India, Vietnam, and Thailand. Pomelos have been used as fresh fruit or for juice processing with good quality and low price. However, after processing, their peel is a primary by-product that contributes to environmental pollution. Therefore, it is necessary to take full advantage of technology to develop other kinds of products from the peels [1]. In recent years, pomelo has attracted more attention from scientists because of their nutritional and antioxidant properties. In addition, pomelo peels and seeds were found to contain two natural compounds such as flavonoids and limonoids, respectively, which play an important role in living systems. Significantly, flavonoids have a wide range of biological effects, such as chelation of metal catalysts, inhibition of key enzymes in mitochondrial respiration, protection from heart disease,

and anti-inflammatory, anticancer, and antimicrobial activities [1, 2]. In the pomelo peels, flavonoids are present in three classes including flavanone, flavone, and flavanol compounds, in which flavanone is considered as the most abundant flavonoid in the pomelo peel (up to 80%). Naringin has been reported to be a predominant flavanone in the peel and edible portions of many varieties of pomelos [3-5], while a small concentration of hesperidin in a pomelo peel was also found [5, 6]. The naringin, hesperidin, and neohesperidin contents were found to be much higher in the peels than in the juice [5]. Naringin has the ability to prevent cancer by carcinogenesis suppression and induction of cell apoptosis, whereas neohesperidin contributes to the bitter taste in grapefruit and pomelo, and has the power to stimulate the immune system [2, 6-8]. Therefore, it is necessary to extract these flavonoids from the peels of the pomelo for food and pharmaceutical application.

*Corresponding author: Email: lanphi@hcmut.edu.vn

Various methods have been applied to extract flavonoid compounds of plants based on the manipulation of the physical properties of solvents to reduce surface tension, increase the solute's solubility, promote the higher diffusion rate, and change in solvent polarity. Solid-liquid extraction or the solvent extraction method, one of the most widely used extraction methods, has been used to extract one or more solutes from a solid matrix by a liquid solvent and has widely applied in food industry to recover various products including sugars, teas, coffees, vegetable oils, and functional compounds. The yield of the solutes is also influenced by numerous factors including the preparation of the solid, diffusion rate, temperature, and solvent choices [9]. Several extraction methods have been reported for the extraction of flavonoids from pomelo peels such as conventional extraction, enzyme-assisted extraction, ultrasound-assisted extraction, microwave-assisted extraction, supercritical CO₂ extraction, and pressurized fluid extraction [10-13]. To the best of our knowledge, there is no report on the optimization of extraction conditions to obtain maximum TFC from pomelo peels. Therefore, the objectives of this study is to optimize extraction conditions such as solid-solvent ratio, extraction temperature, and extraction time to get the highest flavonoid from the pomelo's peel based on Box-Behnken response surface design and to determine the chemical composition and biological properties of the extracts from the Tan Trieu, Duong Hong, and Nam Roi pomelo varieties.

2. Materials and methods

2.1. Plant materials

Tan Trieu, Duong Hong, and Nam Roi pomelo (*Citrus grandis* Osbeck) varieties grown at different locations of Vietnam were used in this study. The pomelo fruits were carefully washed to remove soil particles and dust. Peels were taken out from the pulp and then cut into small pieces. Then, the peel pieces were dried using a freeze-drying machine and ground into fine powder. The pomelo peel powder, with a moisture content of 12-14%, was stored in a desiccator prior to the experiment.

2.2. Extraction method

The dried peel powder (1 g) was accurately weighed and mixed with ethanol under predetermined extraction conditions based on the Box-Behnken response surface design as described in the experimental design section below and in Table 1. After shaking the sample in a water bath for a certain temperature and time, the samples were centrifuged for 15 min at 4°C. The supernatant was kept while the residue was repeatedly extracted 3 times. Then, the supernatants were combined, evaporated, and diluted with methanol to yield 20 ml of crude extract before storing for analysis.

Table 1. The coded level of variables chosen for the experiments.

Variable	Coded	Range and level		
		-1	0	+1
Solid-solvent ratio (w/v)	X ₁	1/15	1/30	1/45
Incubation Temperature (°C)	X ₃	30	45	60
Incubation time (h)	X ₄	30	45	60

2.3. Experimental design

The total flavonoids of the pomelo peels were extracted with a solvent of 75% ethanol and optimized using the Box-Behnken design with three variables including solid-solvent ratio (1/15, 1/30 and 1/45), temperature (30, 45 and 60°C) and time (30, 45 and 60 min). The scientific basis behind the chosen testing levels was determined by screening experiments using the one-factor optimization method at which the total flavonoids were the highest (the details of these experiments are not shown in this paper).

Each independent variable was coded at three levels of -1, 0, and +1 (Table 1). The experimental design consisted of a total of 17 experiments with five centre points as shown in Table 2.

Table 2. Total flavonoids content of pomelo peel extracted using Box-Behnken design.

Trial no.	Solid-solvent ratio (w/v)	Temperature (°C)	Time (min)	TFC (mg/g)
1	1/15	45	20	4.31
2	1/30	45	40	5.81
3	1/30	60	60	4.15
4	1/30	45	40	5.17
5	1/30	30	60	4.28
6	1/45	30	40	6.00
7	1/15	45	60	3.55
8	1/45	60	40	5.26
9	1/45	45	20	4.94
10	1/30	30	20	5.21
11	1/15	60	40	4.81
12	1/30	45	40	5.81
13	1/45	45	60	5.11
14	1/30	60	20	4.67
15	1/30	45	40	5.42
16	1/15	30	40	4.94
17	1/30	45	40	5.32

2.4. Determination of total flavonoids content

The TFC was determined using the colorimetric method previously described by P.V. Hung, et al. (2008) [14] with a minor modification. The extract (0.5 ml) was mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride solution, and 0.1 ml of 1 M potassium acetate. Then, distilled water was added to adjust the volume to 10 ml. The tubes were thoroughly mixed and stood at ambient temperature in the dark for 30 min. The absorbance was measured at 415 nm against the reagent blank using a spectrophotometer (Genesys 10S UV-Vis, USA). Rutin was used as the standard and the TFC was expressed as micrograms rutin equivalent (RE) per gram of sample.

2.5. HPLC analysis

Flavonoids in the citrus peels were analysed using high performance liquid chromatography (HPLC) according to the method of I.A. Ribeiro and M.H.L Ribeiro (2008) [15]. In detail, the extracts obtained at optimal extraction conditions were diluted with 20 ml of 0.02 M sodium acetate buffer (pH=4) and methanol (1:1). Then, the solutions were filtered through a 0.45 μ m membrane filter before injection into the HPLC system. The mobile phase, including acetonitrile (solvent A) and distilled water (solvent B), was used. The gradient elution was conducted as starting at 23% A in 8 min, 23-65% A in 7 min, 65-70% A in 5 min, 70% A - 23% A in 1 min, and completing the gradient at 23% A in 1 min. The elution was monitored at a flow rate of 1 ml/min and UV wavelength of 280 nm. Naringin (Product #N1376, Sigma), hesperidin (product #H5254), and HPLC grade acetonitrile (product #1000302500) and methanol (product #1060182500, Merck) were used in this study.

2.6. DPPH radical scavenging assay

The antioxidant activity of the extracts was determined according to the method of P.V. Hung, et al. (2008) [14]. The final concentration of the DPPH solution was 0.075 mM. A mixture of 3.9 ml of DPPH solution and 0.1 ml of the extract was mixed and kept in the dark at ambient temperature for 30 min. Then, the absorbance of the mixture was read at 515 nm. Methanol (0.1 ml) was used to replace the extract to mix with the DPPH solution and counted as the blank sample. The scavenging of DPPH was calculated according to the following equation:

$$\% \text{ DPPH scavenging} = \{[\text{Abs}_{(t=0)} - \text{Abs}_{(t=30)}] / \text{Abs}_{(t=0)}\} \times 100$$

where $\text{Abs}(t_0)$ is the absorbance of the DPPH radical and methanol solution at $t=0$ min and $\text{Abs}(t_{30})$ is absorbance of the DPPH radical and the extracts at $t=30$ min.

The results were reported as a half maximal inhibitory concentration (IC_{50}) value. A lower IC_{50} value represents stronger DPPH scavenging capacity. The IC_{50} value was determined from linear regression analysis using Microsoft Excel with data analysis add-in.

2.6. Cytotoxic activity on cancer cells

The colorimetric cytotoxicity assay reported by P. Skehan, et al. (1990) [16] was used to investigate the effects of the extracts on the survival of cancer cells including Hep-G2 (human hepatocellular carcinoma), LU-1 (human lung adenocarcinoma), and MCF-7 (human breast adenocarcinoma). By using SRB (sulforhodamine B) as a basic colorimetric method, the cytotoxicity of the compounds was investigated. These cancer cells were maintained in E'MEM (Dulbecco's Modified Eagle Medium) with the addition of L-glutamine, sodium pyruvate, NaHCO_3 , PSF (penicillin-streptomycin sulfate-fungizone), NAA (non-essential amino acids), and 10% BCS (bovine calf serum) before storage at 37°C in a 5% CO_2 incubator. A plate (96-well) was used to grow cells at 10^4 cells/well for Hep-G2 and MCF-7, and 7.5×10^3 cells/well for LU-1 in the growth medium. After 24 h of growth, these cells were incubated in the presence of the extracts with different concentrations in 48 h. Then, the total protein was maintained by using trichloroacetic acid (Sigma) 50% and dyed by SRB 0.2%. The standard was measured by ellipticine, vinblastine, or taxol dissolving DMSO. The result was read by an ELISA at 495-515 nm. The percentage of cell survival is determined by:

$$\% \text{ cell survival} = (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}}) / (\text{Abs}_{\text{DMSO}} - \text{Abs}_{\text{control}})$$

2.7. Statistical analysis

The data were analysed for multiple regression analysis and analysis of variance (ANOVA) to fit the mathematical modelling using Design Expert software (Version 11, Stat-Ease Inc., USA). After fitting the data to the models, the response surface and 3D contour were plotted and investigated.

3. Results and discussion

3.1. Box-Behnken design analysis

The statistically designed experiments under different extraction conditions were carried out to investigate the combined effect of independent variables (solid-solvent ratio, extraction temperature and extraction time) on the

extraction yield of the TFC and the results are shown in Table 2. The results indicated that the TFC of the extracts from the pomelo peel varied with different extraction conditions. Table 3 showed the fitting of four high degree polynomial models (linear, interactive (2FI), quadratic, and cubic models) with the experimental data. The quadratic model had a maximum adjusted R^2 with low p-value. Therefore, this model was the most suitable for the designed experiments. The experimental data was analysed by ANOVA and the results in Table 4 indicated that the model was significant because the p-value of the model was less than 0.05. Moreover, the F-value was 0.6093 meaning that the lack of fit of the model was not significant as compared to pure error and the designed model was good.

Table 3. Sequential model fitting for the response.

Source	Sequential p-value	Lack of fit p-value	Adjusted R^2
Linear	0.095199	0.067263	0.232767
2FI	0.820918	0.041751	0.086334
Quadratic	0.001709	0.64349	0.831442
Cubic	0.64349		0.797545

Table 4. Analysis of variance (ANOVA) for Box-Behnken model.

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value
Model	6.22	9	0.6908	9.77	0.0033
Lack-of-fit	0.1552	3	0.0517	0.6093	0.6435
Pure error	0.3397	4	0.0849		
Corrected total	6.71	16			
R^2	0.9263				
adj R^2	0.8314				
C.V.%	6.71%				

3.2. Fitting of second order polynomial equation

The relationship between the three factors with the response is given by the following equation in terms of coded factors:

$$\text{TFC (mg/g)} = 5.51 + 0.4625X_1 - 0.1925X_2 - 0.2550X_3 - 0.1525X_1X_2 + 0.2325X_1X_3 + 0.1025X_2X_3 - 0.1768X_1^2 - 0.0768X_2^2 - 0.8517X_3^2$$

where X_1 is the solid-solvent ratio, X_2 is the extraction temperature, and X_3 is the extraction time.

The results indicated that the TFC of the extracts from the pomelo peels were not only dependent on all factors (X_1 , X_2 , and X_3), but also dependent on the relationship between the two independent factors (X_1X_2 , X_1X_3 , and X_2X_3).

3.3. Effect of independent variables on the TFC

Three factors including the solid-solvent ratio, extraction temperature, and time at three different levels were used to investigate the influence of the independent variables in the TFC. The 3D response surface and contour plots were developed from the model by maintaining two factors as constant while varying the third to illustrate the optimum conditions (Fig. 1).

Effect of solid-solvent ratio: Figs. 1B and 1C showed that the TFC increased with increasing solid-solvent ratio from 1:15 to 1:44 (g/ml). The difference in concentration between the cell tissue of the pomelo peel and the solvent improved the efficiency of extraction because of an increased mass transfer rate. The liquid circulation and turbulence produced by cavitation increased the contact surface between the solvent and target compounds by permitting greater penetration of solvent into the sample matrix [17].

Effect of extraction temperature: The effects of extraction temperature on the TFC under the Box-Behnken design can be observed in Figs. 1A and 1C. The results indicated that temperature did not affect TFC at solid-solvent ratio of 1:15 g/ml while it did affect the TFC at higher solid-solvent ratios. As a result, the TFC reached its highest level when the temperature was 30°C and the solid-solvent ratio was

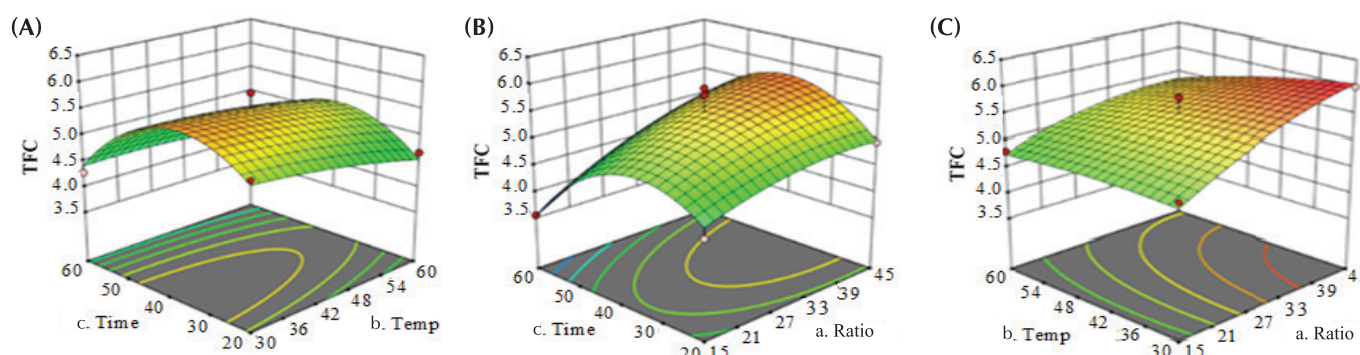


Fig. 1. Response surface plots representing the effect of extraction conditions on the TFC. (A) The solid-solvent ratio; (B) Extraction temperature; (C) Time at three different levels.

1:44 g/ml. As a result, the 30°C temperature was the ideal temperature for extraction of TFC from the pomelo peels in this study.

Effect of extraction time: The effects of extraction time on the TFC under the Box-Behnken design are shown in Figs. 1A and 1B. The TFC increased with increasing extraction time from 20 to 45 min. At a higher solid-solvent ratio, the TFC reduced upon lengthening the extraction time. The highest TFC of the pomelo peels was obtained with an extraction time of 34.6 min. Further increasing extraction time could cause the loss of solvent by vaporization.

3.4. Optimization and verification of model

The extraction conditions were optimized to get the maximum extractive TFC by employing Derringer's desired function methodology. From the model, the highest TFC was predicted to be 6.0 mg/g under the following optimized conditions: the solid-solvent ratio of 1/44 g/ml, extraction temperature of 30.7°C, and extraction time of 34.6 min.

For confirmation, triplicate extractions of TFC from pomelo peels were carried out under the optimum conditions stated above. The average TFC was 6.0±0.1 mg/g peels, which is not significantly different from the predicted results of the model. Thus, the model obtained in this study was significant. The TFC of the pomelo peels extracted under the optimal conditions in this study was significantly higher than that obtained by the conventional enzyme and ultrasound-assisted extraction methods reported by P.V. Hung, et al. (2020) [18].

3.5. Flavonoid concentration in pomelo's peels

The TFCs of the peel extracts from different pomelo varieties are given in Table 5. The TFC varied among the different varieties of pomelo ($p < 0.05$), which ranged from 4.3±0.1 to 6.0±0.1 mg/g peels. The lowest TFC was presented in the Duong Hong pomelo peels (4.3±0.1 mg/g peels), whereas the highest TFC was 6.0±0.1 mg/g peels from the Tan Trieu pomelo peels. The difference in the TFC among the different pomelo varieties might be due their chemical composition, maturity at harvest, soil and water qualities, and the condition of the post-harvest method [19].

Table 5. Total flavonoid, naringin and hesperidin concentrations of pomelo peels' extracts under optimized condition (mg/g).

Sample	Concentration (mg/g, db)		
	Total flavonoids	Naringin	Hesperidin
Tan Trieu pomelo	6.0±0.1 ^b	2.2±0.2 ^c	0.36±0.1 ^b
Duong Hong pomelo	4.3±0.1 ^a	1.6±0.1 ^b	0.18±0.2 ^a
Nam Roi pomelo	4.5±0.1 ^a	0.2±0.2 ^a	0.69±0.3 ^c

a, b, c: different letters in the same column are significantly different ($p < 0.05$).

In the present study, the concentration of two flavonoids in pomelo peels including naringin and hesperidin was identified using the HPLC and are given in Table 5. The naringin concentration in the Tan Trieu pomelo was the highest (2.2±0.2 mg/g peels) followed by the Duong Hong pomelo peel (1.6±0.1 mg/g peel) and the Nam Roi pomelo peel (0.2±0.2 mg/g peel). The highest hesperidin concentration was 0.69±0.3 mg/g found in the Nam Roi pomelo peel, followed by the Tan Trieu and Duong Hong pomelo peels. The results found in this study were consistent with the results reported by G.H. Xu, et al. (2008) [20], which stated that the hesperidin concentration in the pomelo peels was 1.77 mg/g DW under the same extraction conditions. However, hesperidin could not be found in several of the pomelo types from China. It has been shown that flavonoids are not equally distributed among the different types and parts of the citrus fruit. H.D. Jang, et al. (2010) [21] reported that the essential oil of the Buntan peel contained a higher TFC than those from the extracts of fruit pulp with different solvents. The greatest TFC among the different parts of the fruits ranked as follows: peel>pulp>juice [3].

3.6. DPPH radical scavenging activities of pomelo peel extracts

The antioxidant capacities of the pomelo peel extracts measured by the DPPH scavenging activity and expressed as IC_{50} values are given in Table 6. The IC_{50} value was calculated as the amount of extract required to inhibit 50% of the DPPH radical. The lower the IC_{50} value is, the higher the antioxidant activity of the extract. The IC_{50} values of the citrus peel extracts ranged from 1.08 to 4.51 µg/ml, which is significantly higher than that of the Trolox (0.65±0.04 µg/ml). These results indicate that the Tan Trieu pomelo peel extract exhibited the highest antioxidant activity (IC_{50} =1.08±0.04 µg/ml), followed by the Nam Roi pomelo peel extract (IC_{50} =3.09±0.01 µg/ml) and Duong Hong pomelo peel extract (IC_{50} =4.51±0.03 µg/ml). Previous studies [22] revealed that the IC_{50} of ethanolic extracts from the peels of *C. hystrix* from Boyolali - Central Java, Indonesia were 16.7 µg/ml. The present study [23] also indicated that the peels of the pomelo varieties in Vietnam had a higher antioxidant capacity than other locations.

Table 6. DPPH radical scavenging (IC_{50} value) of pomelo peels' extracts.

Sample	IC_{50} value (µg/ml)
Trolox	0.65±0.04 ^a
Tan Trieu pomelo	1.08±0.04 ^b
Duong Hong pomelo	4.51±0.03 ^d
Nam Roi pomelo	3.09±0.01 ^c

a, b, c: different letters in the same column are significantly different ($p < 0.05$).

3.7. Cytotoxic activity on cancer cells of peel's extracts

Table 7. Anticancer activity of pomelo peels' extracts.

Sample	Initial concentration (µg/ml)	CS value (%)		
		Hep-G2	LU-1	MCF-7
Standard (+)	5	4.34±0.42 ^a	1.13±0.44 ^a	2.53±0.06 ^a
Tan Trieu	100	98.94±0.95 ^b	99.24±1.06 ^c	80.44±1.92 ^c
Duong Hong	100	97.42±1.13 ^b	83.77±1.51 ^b	99.54±0.62 ^d
Nam Roi	100	99.04±0.79 ^b	85.42±0.52 ^b	77.11±2.06 ^b

^{a, b, c}: different letters in the same column are significantly different ($p < 0.05$).

The anticancer activities of the pomelo peel extracts are shown in Table 7. The results indicate that the pomelo peels did not affect Hep-G2 (human hepatocellular carcinoma). The percentage of dead cells after treatment was 1.06% (Tan Trieu pomelo peel extract), 2.58% (Duong Hong pomelo peel extract), and 0.96% (Nam Roi pomelo peel extract). The Tan Trieu pomelo peel extract also had no effect on Lung cancer cells (LU-1), in which 99.24% of the cancer cells survived. The Duong Hong and Nam Roi pomelo peel extracts slightly affected the LU-1 cells with a total percentage of dead cancer cells being 16.23% and 14.58%, respectively. In addition, the highest percentage dead of breast cancer cells (MCF-7) was 22.89% when treated with the Nam Roi pomelo peel extract, followed by the Tan Trieu pomelo peel extract (19.56%) and Duong Hong pomelo peel extract (0.46%). The previous study [24] also reported that the essential oils of lemon and grapefruit peels exhibited a weak cytotoxicity toward human prostate (PC-3), lung (A549), and breast (MCF-7) tumour cell lines.

4. Conclusions

The TFC of pomelo peel extracts were successfully maximized using the Box-Behnken design and response surface analysis. The results indicated that the solid-solvent ratio, extraction temperature, and extraction time were the most important factors that affect TFC values. After analysis, the quadratic models within the studied experimental range of the various process variables were used to maximize the TFC. As a result, the optimized TFC of the pomelo peel was 6.0 mg/g peels under the optimized extraction conditions, just as predicted. All pomelo peel extracts possessed strong antioxidant activities with a low IC_{50} . However, the pomelo peel extracts showed weak or moderate anticancer activities when treated with 100 µg/ml of the extract.

CRedit author statement

Truong Ngoc Quynh Phuong, Pham Van Hung, Nguyen Thi Lan Phi: Conceptualisation, Methodology, Software, Resources, Writing - Review and Editing, Validation.

ACKNOWLEDGEMENTS

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106-NN.02-2016.72.

COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

- [1] J.B. Harborne, C. William (2000), "Advances in flavonoids research since 1992", *Photochem.*, **55**(6), pp.481-504, DOI: 10.1016/S0031-9422(00)00235-1.
- [2] M.K.N. Chidambara, J. Kim, A. Vikram, et al. (2012), "Differential inhibition of human colon cancer cells by structurally similar flavonoids of citrus", *Food Chem.*, **132**(1), pp.27-34, DOI: 10.1016/j.foodchem.2011.10.014.
- [3] Y. Nogata, K. Sakamoto, H. Shiratsuchi, et al. (2006), "Flavonoid composition of fruit tissues of citrus species", *Biosci. Biotechnol. Biochem.*, **70**(1), pp.178-192, DOI: 10.1271/bbb.70.178.
- [4] M. Zhang, H. Nan, Y. Wang, et al. (2011), "The flavonoid composition of flavedo and juice from the pummelo cultivar (*Citrus grandis* L. Osbeck) and the grapefruit cultivar (*Citrus paradisi*) from China", *Food Chem.*, **129**(4), pp.1530-1536, DOI: 10.3390/molecules19117314.
- [5] Y.C. Wang, Y.C. Chuang, Y.H. Ku (2007), "Quantitation of bioactive compounds in citrus fruits cultivated in Taiwan", *Food Chem.*, **102**(4), pp.1163-1171, DOI: 10.1016/j.foodchem.2006.06.057.
- [6] M.A. Alam, N. Subhan, M.M. Rahman, et al. (2014), "Effect of citrus flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action", *Adv. Nutr.*, **5**(4), pp.404-417, DOI: 10.3945/an.113.005603.
- [7] O.B. Garcia, J. Castillo (2008), "Update on uses and properties of citrus flavonoids: New findings in anticancer, cardiovascular, and anti-inflammatory activity", *J. Agric. Food Chem.*, **56**(15), pp.6185-6205, DOI: 10.1021/jf8006568.
- [8] I. Erlund (2004), "Review of the flavonoids, quercetin, hesperidin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology", *Nutr. Res.*, **24**(10), pp.851-874, DOI: 10.1016/j.nutres.2004.07.005.
- [9] D.V. Dandekar, G.K. Jayaprakasha, B.S. Patil (2008), "Hydrostatic extraction of bioactive limonin from sour orange (*Citrus aurantium* L.) seeds", *Food Chem.*, **109**(3), pp.515-520, DOI: 10.1016/j.foodchem.2007.12.071.
- [10] S.M. Jeong, S.Y. Kim, D.R. Kim, et al. (2004), "Effect of heat treatment on the antioxidant activity of extracts", *J. Agric. Food Chem.*, **52**(11), pp.3389-3393, DOI: 10.1021/jf049899k.
- [11] Y.Q. Ma, J.C. Chen, D.H. Liu, et al. (2009), "Simultaneous extraction of phenolic compounds of citrus peel extracts: effect of ultrasound", *Ultrason. Sonochem.*, **16**(1), pp.57-62, DOI: 10.1016/j.ultrason.2008.04.012.
- [12] E.M.G. Castello, A.D.R. Lopez, L. Mayor, et al. (2015), "Optimization of conventional and ultrasound assisted extraction of flavonoids from grapefruit (*Citrus paradisi* L.) solid wastes", *LWT-Food Sci. Technol.*, **64**(2), pp.1114-1122, DOI: 10.1016/j.lwt.2015.07.024.
- [13] N. M'hiri, I. Ioannou, M. Ghoul, et al. (2014), "Extraction methods of citrus peel phenolic compounds", *Food Rev. Int.*, **30**(4), pp.265-290, DOI: 10.1080/87559129.2014.924139.
- [14] P.V. Hung, N. Morita (2008), "Distribution of phenolic compounds in the graded flours milled from whole buckwheat grains and their antioxidant capacity", *Food Chem.*, **109**(2), pp.325-331.
- [15] I.A. Ribeiro, M.H.L. Ribeiro (2008), "Naringin and naringenin determination and control in grapefruit juice by a validated HPLC method", *Food Control*, **19**(4), pp.432-438, DOI: 10.1016/j.foodchem.2007.12.060.
- [16] P. Skehan, R. Storeng, D. Scudiero, et al. (1990), "New colorimetric cytotoxicity assay for anticancer drug screening", *J. Natl. Cancer Inst.*, **82**(13), pp.1107-1112, DOI: 10.1093/jnci/82.13.1107.
- [17] M. Romdhane, C. Gourdon (2002), "Investigation in solid-liquid extraction: Influence of ultrasound", *Chem. Eng. J.*, **87**(1), pp.11-19, DOI: 10.1016/S1385-8947(01)00206-6.
- [18] P.V. Hung, N.H.Y. Nhi, L.Y. Ting, et al. (2020), "Chemical composition and biological activities of extracts from pomelo peel by-products under enzyme and ultrasound-assisted extractions", *J. Chem.*, **2020**, DOI: 10.1155/2020/1043251.
- [19] C. Shahzad (2011), "Total phenolic content and antioxidant capacity of extracts obtained from 6 important fruit residues", *Food Res. Int.*, **44**(1), pp.391-396.
- [20] G.H. Xu, H.S. Oberoi, D.S. Uppal, et al. (2008), "Minerals, phenolic compounds, and antioxidant capacity of citrus peel extract by hot water", *J. Food Chem.*, **73**(1), pp.11-17, DOI: 10.1016/j.foodres.2010.10.001.
- [21] H.D. Jang, K.S. Chang, T.C. Chang, et al. (2010), "Antioxidant potentials of buntan pummelo (*Citrus grandis* Osbeck.) and its ethanolic and acetic fermentation products", *Food Chem.*, **118**(3), pp.554-558, DOI: 10.1016/j.foodchem.2009.05.020.
- [22] I. Fidrianny, P.I. Sari, K.R. Wirasutisna (2015), "Antioxidant activities in various peel extracts of four varieties rambutan (*Nephelium lappaceum*) using DPPH, FRAP assays", *Int. J. Pharmacog. Phytochem. Res.*, **7**(2), pp.280-285.
- [23] W. Xi, B. Fang, Q. Zhao, et al. (2014), "Flavonoid composition and antioxidant activities of Chinese local pummelo (*Citrus grandis* Osbeck.) varieties", *Food Chem.*, **161**, pp.230-238, DOI: 10.1016/j.foodchem.2014.04.001.
- [24] Y. Zu, H. Yu, L. Liang, et al. (2010), "Activities of ten essential oils towards propionibacterium acnes and PC-3, A-549 and MCF-7 cancer cells", *Molecules*, **15**(5), pp.3200-3210, DOI: 10.3390/molecules15053200.