

Antioxidant Capacity and Phytochemical Profile of *Punica granatum L.* Peel Extracts Using Different Solvent Extraction

Mohamad Rafi^{1,2*}, Laela Wulansari¹, Dewi A Septaningsih^{1,3}, Tsania F Purnomo⁴, Reza Auliatifani⁴, Khaydanur⁴, Auliya Ilmiawati², Wina Yulianti⁴, Nunuk K Nengsih¹, Irma H Suparto^{2,5}, Wisnu A Kusuma^{1,6}

¹Tropical Biopharmaca Research Center, Institute of Research and Community Services, IPB University, Jl Taman Kencana No. 3, Bogor 16128, Indonesia

²Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Jl Tanjung Kampus IPB Dramaga, Bogor 16680, Indonesia

³Advance Research Laboratory, Institute of Research and Community Services, IPB University, Jl Palem Raya Kampus IPB Dramaga, Bogor 16880, Indonesia

⁴Chemical Analyst Program, Vocational School, IPB University, Jl Kumbang No. 14 Kampus IPB Lodaya, Bogor 16128, Indonesia

⁵Primate Research Center, Institute of Research and Community Services, IPB University, Jl Lodaya II No. 5 Kampus IPB Lodaya, Bogor 16128, Indonesia

⁶Department of Computer Science, Faculty of Mathematics and Natural Sciences, IPB University, Jl Meranti Kampus IPB Dramaga, Bogor 16680, Indonesia

Article history:

Submission January 2020

Revised January 2021

Accepted April 2021

*Corresponding author:

E-mail: mra@apps.ipb.ac.id

ABSTRACT

Pomegranate is known to have valuable nutrients and contains various bioactive compounds found in the fruit's peel. The utilization of these bioactive compounds could be used as herbal medicines and supplements, such as antioxidants. This study aimed to determine the antioxidant capacity, phytochemical profile, and pomegranate peel extract grouping using different extracting solvents. The extraction solvents used were water, 70% ethanol, and ethanol pro analysis (p.a) grade. The antioxidant capacity of the three extracts was measured using DPPH and CUPRAC methods. We also determined the total phenolic and flavonoid levels and the TLC fingerprint analysis and FTIR spectrum of the pomegranate peel extracts. The 70% ethanol extract owned the largest antioxidant capacity. It was significantly different from the other two extracts with a value of 358.67 and 2981.59 $\mu\text{mol trolox/g}$ dried sample using the DPPH and CUPRAC methods, respectively. The three pomegranate peel extracts' total phenolic and flavonoid levels ranged from 287.26–1068.81 mg GAE/g dried sample and 0.24–0.75 mg QE/g dried sample. TLC fingerprint analysis of pomegranate peel extract yielded 2, 6, and 6 bands for water extract, 70% ethanol, and p.a ethanol, respectively. The three extracts can be grouped based on FTIR spectrum data using principal component analysis using three principal components with a total variance of 93%. The results obtained showed that using different extracting solvents provides different antioxidant capacities and phytochemical profiles.

Keywords: Antioxidant, FTIR spectra, *Punica granatum*, TLC fingerprint analysis, Principal component analysis

Introduction

Pomegranate (*Punica granatum L.*) is a plant that belongs to the Lythraceae family, easy to grow in almost all climates spread in subtropical

to tropical areas, from lowlands to highlands 1000 m above sea level. Several studies have reported that pomegranate has several biological activities,

How to cite:

Rafi M, Wulansari L, Septaningsih DA et al. (2021) Antioxidant Capacity and Phytochemical Profile of *Punica granatum L.* Peel Extracts Using Different Solvent Extraction. Journal of Tropical Life Science 11 (3): 375 – 382. doi: 10.11594/jtls.11.03.13.

such as antioxidants, antiviral, antidiabetic, anti-diarrheal, anticancer, antiproliferative activity, antitumor, antimicrobial, and anti-inflammatory [1-5]. Antioxidant compounds known could inhibit reactive oxygen species, reactive nitrogen species, and other free radicals, so the damage of normal cells, proteins, and fats can be prevented, preventing degenerative diseases [6].

The demand for pomegranate for consumption worldwide is increasing due to its high nutritional value and antioxidant activity [7]. One of the pomegranate parts that has been known to have antioxidant activity is pomegranate skin [8]. Peel, inner lamellae, and consumed part are 38%, 10%, and 52% of the total fruit weight. Pomegranate peel has a higher antioxidant activity than flowers, leaves, and seeds [9]. This activity is due to the high content of phenolic compounds such as phenolic acids, flavonoids, and tannins, which are generally antioxidants in pomegranate skin [10]. Pomegranate has high amounts of protein, organic elements, and volatile compounds (alcohols, esters, and aldehydes) [11].

The value of pomegranate peel antioxidant capacity can be influenced by the number of antioxidant compounds extracted and several factors in the extraction process. The extraction time, temperature, concentration, and polarity of the extracting solvent used affect the extraction efficiency [12, 13]. Based on their chemical properties, various phytochemical compounds are extracted in solvents with different polarities. No single solvent can be relied on to extract all phytochemical compounds whose ends will affect the antioxidant capacity value generated in a sample [14].

Extraction of phytochemical compounds, especially like the phenolic group, using ethanol, methanol, glycerol, and their aqueous solution, are common and efficient as solvent extraction [15]. As we know, there is more attention in using water and ethanol as extraction solvents because of their safety [16]. From the previous studied, ethyl acetate, acetonitrile, and water were used to extract metabolites from pomegranate peel [17]. Therefore, this study evaluated and determined the extracting solvents (water, 70% ethanol, and ethanol p.a.) that provide high antioxidant compounds such as phenolics and total flavonoids from pomegranate peel extract. We also performed metabolite fingerprinting using thin-layer chromatography (TLC) and FTIR spectrum to see

the differences in each extract's phytochemical profile.

Material and Methods

Sample preparation and extraction

The pomegranate is separated between the peel and the fruit. The pomegranate peel is cleaned and then dried under the sun for one day. We found the dried pomegranate peel's moisture content about 6.71 ± 0.14 (mean \pm standard deviation, $n = 3$). Samples were ground using a disk mill FFC-15 (Shan Dong Ji Mo Disk Mill Machinery, China) and filtered to a size of 100 mesh. Dried pomegranate peel weighed about 200 grams and then extracted by maceration using three extracting solvents: water, 70% ethanol, and ethanol p.a. The ratio of the sample weight to the extracting solvent is 1:10 and macerated for 2×24 hours. The filtrate obtained was concentrated using a rotary evaporator R-100 (Buchi, Flawil, Switzerland) at room temperature.

Determination of total phenolics content

We used a procedure described in Indonesia Herbal Pharmacopeia 1st edition [18] to determine the total phenolics content. Pomegranate peel extract was weighed about 5 mg and dissolved with methanol in a 25 mL volumetric flask. The calibration curve was prepared by diluting gallic acid in 5 different concentrations as 10, 30, 50, 70, and 100 (mg/L) with methanol as a solvent. A total of 1 mL of the sample or standard solutions were put into a test tube, then added 5 mL of 7.5% Folin Ciocalteu reagent, homogenized with a vortex, then incubated in a dark room for 8 minutes. The incubated samples were added with 4 mL of 1% NaOH, homogenized by vortex, and re-incubated in a dark room for 1 hour. Measurements were made using a UV-Vis spectrophotometer U-2800 (Hitachi, Tokyo, Japan) at a wavelength of 730 nm. Total phenolics content in each extract was expressed in mg gallic acid equivalent (GAE)/g dried sample. We performed the total phenolics content in triplicate.

Determination of total flavonoids content

Total flavonoids content was determined using a procedure described in Indonesia Herbal Pharmacopeia 1st edition [18]. The pomegranate peel extract was weighed about 0.3 g added with 1 ml of 0.5% hexamethylenetetramine, 20 mL acetone, and 20 mL of 25% HCl solution. The mixture was

then hydrolyzed by reflux for 30 minutes and was filtered, then put filtrate into a 100 mL volumetric flask, and added acetone up to 100 mL. A total of 20 mL of the diluted filtrate was taken and then put into a 250 mL separating funnel, 20 mL of distilled water was added and extracted twice. The ethyl acetate fraction was put into a 50 mL volumetric flask. The calibration curve was prepared by diluting quercetin in 5 different concentrations as 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 (mg/L) with methanol as solvent. A total of 10 mL of sample extract or standard solution was added with 1 mL of 2% AlCl_3 (dissolved by 5% glacial acetic acid in methanol). Measurements were made 30 minutes after adding AlCl_3 using a UV-Vis spectrophotometer at a wavelength of 425 nm. Total flavonoid content in each extract was expressed in mg quercetin equivalent (QE)/g dried sample, and we measured in three replication.

Determination of antioxidant capacity

DPPH radical scavenging activity

The pomegranate peel extracts' antioxidant capacity was measured using the DPPH method according to the procedure described by Salazar-Arandra et al. [19]. In 96 well plates, 40 μL of pomegranate peel extract solution was mixed with 250 μL of DPPH 125 μM solution (dissolved in ethanol). The solution was incubated for 30 minutes in a dark room at room temperature. Absorbance was measured using a microplate reader (Epoch-Biotek, Winooski, USA) at a wavelength of 515 nm using ethanol as the blank. Trolox solutions were prepared in 6 different concentrations from 25-150 μM with ethanol p.a. The antioxidant capacity was expressed in μmol trolox equivalent/g dried sample and measured in triplicate.

Cupric ion reducing antioxidant capacity

We followed a procedure described by Ozturk et al. [20] for measuring the cupric ion reducing antioxidant capacity (CUPRAC) of the pomegranate peel extracts. Approximately 40 μL of extract solution, 50 μL 10 mM CuCl_2 , 50 μL 7.5 mM neocuproine, and 60 μL $\text{NH}_4\text{CH}_3\text{COOH}$ (1 M, pH 7) were added to 96 well plates with a total volume of 200 μL . The mixture was incubated for one hour, and after that, the absorption was measured at a wavelength of 450 nm with a microplate reader (Epoch-Biotek, Winooski, USA). Trolox

solutions were prepared in a concentration range of 0-700 μM in ethanol p.a. The antioxidant capacity is expressed in μmol trolox equivalent/g dried sample with triplicate measurement.

TLC fingerprint analysis

Each extract was dissolved in methanol with a 10,000 $\mu\text{g}/\text{ml}$ concentration and spotted using a semiautomatic CAMAG Linomat 5 (Muttentz, Switzerland) TLC of 20 μm on a 10 \times 14 cm TLC plate. The eluent used is a mixture of acetone: methanol: toluene: water with a ratio of 4: 2: 4: 1 and saturated in a chamber for 30 minutes. After saturation, the TLC plate was eluted until it reached the finish line and was detected using UV-Vis at 254 nm and 366 nm using CAMAG Reprostar 3 (Muttentz, Switzerland). After directly detecting UV, the TLC plate was stained with 10% sulfuric acid in methanol by immersion. After that, the TLC plate was dried and heated for 10 minutes at 105°C. After heating, the plate obtained was detected again using UV-Vis with the same wavelength. We performed TLC fingerprint analysis in duplicate.

Measurement of FTIR spectra

Accurately weighed 2 mg of pomegranate peel extract, then mixed with 200 mg of potassium bromide with a mortar, then made pellets using manual compression. FTIR spectra were measured in the FTIR spectrophotometer Tensor 37 (Bruker, Ettlingen, Germany) equipped with a deuterated triglycine sulfate detector. FTIR spectra were recorded in the region of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans/min controlled by OPUS 4.2 software (Bruker, Ettlingen, Germany). FTIR spectra were stored as a data point table, and we measured for each extract five times.

Data analysis

The reported results were expressed as mean \pm standard deviation (SD) from a triplicate measurement. Analysis of variance (ANOVA) followed by Duncan's test was performed to compare data between aqueous, 70% ethanol, and ethanol p.a. extract. Significant differences were defined at the 5% level ($p < 0.05$). Clustering of pomegranate peel extracts was conducted using principal component analysis (PCA) with absorbance data from FTIR spectra as a variable. We used XLStat ver-

Table 1. Total phenolic, total flavonoid, and antioxidant capacity of pomegranate peel extracts (n = 3)

Sample extract	Total phenolic (mg GAE/g dried sample)	Total flavonoid (mg QE/g dried sample)	Antioxidant capacity (μ mol trolox equivalent/g dried sample)	
			CUPRAC	DPPH
Water	501.91 \pm 10.30 ^a	0.47 \pm 0.01 ^a	1065.12 \pm 4.75 ^a	152.50 \pm 2.11 ^a
70% ethanol	1068.81 \pm 40.12 ^b	0.75 \pm 0.02 ^b	2981.59 \pm 25.99 ^b	358.67 \pm 5.77 ^b
Ethanol p.a	287.26 \pm 16.13 ^c	0.25 \pm 0.02 ^c	839.79 \pm 8.33 ^c	108.11 \pm 0.92 ^c

The mean \pm SD within each measurement in the same column followed with different lowercase letters represent significant differences at $p < 0.05$.

GAE = gallic acid equivalent

QE = quercetin equivalent

sion 2019.4.1 (Addinsoft, New York, United States) for performing ANOVA and PCA.

Results and Discussions

Total phenolics and flavonoid content of pomegranate peel extracts

Phenolic compounds are known as chemical compound classes with antioxidant properties [21]. The phenolics content in a plant could be used to indicate high or low antioxidant activity. The total phenolic content is generally determined using the Folin Ciocalteu method, although the Folin Ciocalteu reagent can react with other oxidizable compounds so that it is not too specific [22]. The pomegranate peel's total phenol levels were obtained at 287.26-1068.81 mg GAE/g dried sample using water solvent, 70% ethanol, and ethanol p.a. solvent.

Flavonoids are one of the phenolic group compounds with a free radical scavenger activity and are usually contained in all parts of the plant. However, each extract's total flavonoids were not the most significant contributor to phenolic compounds' presence. This is because the total flavonoid analysis method using $AlCl_3$ as a complex-forming reagent only reacts to flavones and flavonols [23]. We found total flavonoid content in each pomegranate peel extract was 0.25-0.75 mg QE/g dried sample.

Based on the total phenolic and flavonoid content measurements in the extract (Table 1), 70% ethanol extract was higher than water and ethanol p.a extracts. A previous study from Li *et al.* [24] found that extraction of pomegranate peel using different solvents, i.e., methanol, ethanol, acetone, and their combinations gave different total phenolic and total flavonoid content. Shiban *et al.* [25] reported different total phenolic and total flavonoid content when pomegranate peel was extracted with 80% methanol, water, and

ether. This shows a difference in the extracting solvent's polarity, which will give a different amount of phenolics and flavonoids extracted. Differences in the amount of phenolics and flavonoids extracted appear because the polarity of each phenolic and flavonoid contained in pomegranate peel was varied. ANOVA followed by the Duncan test also confirmed this result that the three extraction solvents used had significantly different results in the total phenolic and flavonoid levels with $p < 0.05$.

Antioxidant capacity

In this paper, pomegranate peel extract's antioxidant capacity was determined using the DPPH and CUPRAC methods. The DPPH method is commonly used because it is suitable for determining both polar and nonpolar solvents' antioxidant capacity. While the CUPRAC method shows the extract's ability to reduce Cu metal, the results are proportional to the total amount of copper reduced by antioxidant compounds through electron transfer. The different methods used to determine antioxidant activity give unexpected results, are difficult to compare, and sometimes lead to mismatches. The antioxidant capacity is expressed in terms of trolox equivalent.

Table 2 shows the antioxidant capacity values of the three pomegranate peel extracts. The antioxidant capacity of the three extracts using the DPPH method gave smaller results than the CUPRAC method. So, antioxidant compounds in pomegranate peel can work more specifically on the CUPRAC radical form. The difference in antioxidant activity level using the DPPH and CUPRAC methods because, in the CUPRAC method, the measurement was carried out at pH 7, close to physiological pH, thereby stimulating further antioxidants. Besides, it could be caused by the reactants used in the CUPRAC method are

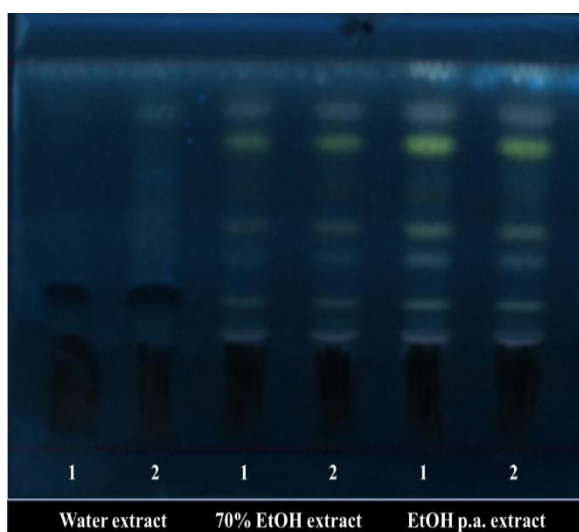


Figure 1. TLC fingerprint analysis of pomegranate peel extracts

Table 2. Detected bands and Rf value of TLC fingerprint of three pomegranate peel extracts

Rf	Extract					
	Water		70% Ethanol		Ethanol p.a.	
	1	2	1	2	1	2
0.27						
0.30			✓	✓	✓	✓
0.39			✓	✓	✓	✓
0.40	✓	✓				
0.52			✓	✓	✓	✓
0.58			✓	✓	✓	✓
0.80			✓	✓	✓	✓
0.89		✓	✓	✓	✓	✓

Note: ✓ = detected band

more stable reactants than the DPPH reactants [20].

Based on the measurement results, the highest antioxidant capacity is owned by 70% ethanol extract followed by water and ethanol extract p.a. The three extracts gave significantly different results based on ANOVA. This shows that 70% ethanol effectively extracts the optimum antioxidant compounds compared to water and ethanol p.a. This result is in line with the higher levels of total phenols and flavonoids in the 70% ethanol extract compared to the other two extracts. As is well known, phenolics or flavonoid compounds are generally antioxidants.

TLC profile of pomegranate peel extracts

TLC fingerprint analysis of pomegranate peel extracts was performed using eluent composition: water:acetone:methanol:toluene (1: 4: 2: 4) and 10% sulfuric acid in methanol as visualization reagent. The fingerprint chromatogram of pomegranate peel can be seen in Figure 1, with each band's Rf value shown in Table 2. The pomegranate peel extract's chromatogram profile shows that the water extract has a very different profile than the 70% ethanol and ethanol p.a. extract because it only detected two bands. The ethanol extract 70% and ethanol p.a gave a profile that was almost similar to each other, only with different intensities of the six bands detected. This indicates that the three extracting solvents provide different compositions and concentrations of metabolites extracted.

FTIR spectra and clustering of pomegranate peel extracts

Medicinal plant extract is a complex mixture of metabolites so that the FTIR spectra peak will result from the functional group present in the metabolites from the extract. Figure 2 shows the FTIR spectra of the three pomegranate peel extracts. Based on the FTIR spectra from the same experimental condition, the resulting absorption bands have spectral patterns similar to one another. The difference is only in the level of transmittance intensity, which is related to the concentration of metabolites extracted using the three types of solvents.

The three extracts have a characteristic peak at $\sim 3400 \text{ cm}^{-1}$ from the stretching vibration of the O-H functional group. A peak appeared at 2930 cm^{-1} , usually from the symmetric and asymmetric stretching vibration of methyl ($-\text{CH}_3$) and methylene ($-\text{CH}_2$). Strong absorption at $\sim 1720 \text{ cm}^{-1}$ is attributed to C=O; absorption at ~ 1620 and $\sim 1510 \text{ cm}^{-1}$ are assigned to C=C either from alkene or aromatic functional group. We also detected a peak at $\sim 1,060 \text{ cm}^{-1}$ is due to the C-OH stretching vibration, maybe from phenolics compounds.

Each pomegranate peel extract showed identical FTIR spectra, only different in their intensities, which is not easy to distinguish visually. Therefore, chemometric analysis, such as PCA was used to cluster the three extracts. The variable used is the absorbance data in the wavenumber range $1,800 - 400 \text{ cm}^{-1}$, the infrared spectrum's fi-

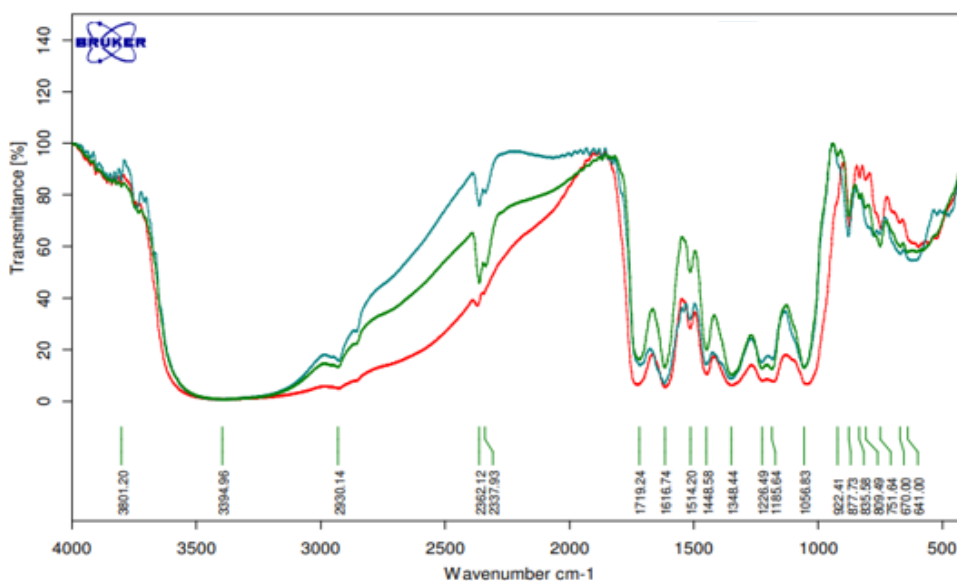


Figure 2. Representative FTIR spectra of three pomegranate peel extracts: water (blue line), 70% ethanol (red line), and ethanol p.a. (green line)

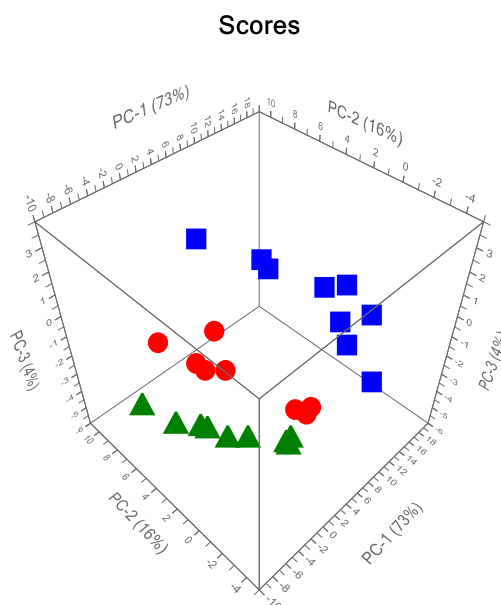


Figure 3. Principal component analysis score plot in three dimensional of pomegranate peel extracts: water (■), 70% EtOH (●), dan EtOH p.a (▲) extract.

ingerprint area. This fingerprint area is unique for each sample because it tends to have a complex spectrum due to various vibrations from the functional groups of a compound present in a sample.

Before doing PCA, the FTIR spectra of all pomegranate peel extracts were preprocessed with SNV and detrending. The pretreatment serves to reduce data variations that did not affect analytical information. The pretreatment also aims to avoid problems due to baseline shear and increase the

coincided spectrum resolution (data information improvement) [26]. PCA clustering using the three extracts is shown in Figure 3. Based on the plot of the three-dimensional PCA scores obtained, the three extracts can be clustered according to their respective groups. The clustering results showed a total PC of 93%, which can explain 93% of the total variance. The PCA results showed that the three extracts had different compositions and concentrations of extracted metabolites.

Conclusion

The 70% ethanol extract of pomegranate peel has the highest antioxidant capacity than water and ethanol p.a. extract based on measurement results using the DPPH and CUPRAC methods. This is in line with the high levels of total phenolics and flavonoids in the 70% ethanol extract. TLC fingerprint analysis showed that the water extract had very different chromatogram profiles than the 70% ethanol extract and ethanol p.a. The two ethanol extracts in this study showed the same TLC fingerprint profile differed in the intensity of the resulting band color. Similar observation was seen in the FTIR spectra showing that the spectral profiles were almost similar for the three extracts, only different in their intensity levels. PCA clustering succeeded in classifying the three extracts with a total PC of 93% of the total variance. Thus, it can be concluded that the three extracts provide different phytochemical profiles, causing different values of antioxidant capacity.

Acknowledgement

The authors gratefully acknowledged the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia through *Penelitian Unggulan Dasar Perguruan Tinggi* Research Grant for this research (No: 4162/IT3.L1/PN/2019).

References

- Sajjad W, Sohail M, Ali B et al. (2015) Antibacterial activity of *Punica granatum* peel extract, *Mycopath* 13 (2): 105 – 111.
- Shaygannia E, Bahmani M, Zamanzad B et al. (2016) A review study on *Punica granatum* L. *Journal of Evidence-Based Integrative Medicine* 21(3): 221 – 227. doi: 10.1177/2156587215598039.
- Bassiri-Jahromi S (2018) *Punica granatum* (pomegranate) activity in health promotion and cancer prevention. *Oncology Reviews* 12 (1): 345 – 351. doi: 10.4081/oncol.2018.345.
- Saeed M, Naveed M, Bibi J et al. (2018) The promising pharmacological effects and therapeutic/medical application of *Punica granatum* L. (pomegranate) as a functional food in humans and animals. *Recent Patents on Inflammation and Allergy Drug Discovery* 12 (1): 24 – 38. doi: 10.2174/1872213X12666180221154713.
- Morittu VM, Mastellone V, Tundis R et al. (2020) Antioxidant, biochemical, and in-life effects of *Punica granatum* L. natural juice vs. clarified juice by polyvinylidene fluoride membrane. *Foods* 9 (2): 242 – 253. doi: 10.3390/foods9020242.
- Sharifi-Rad J, Sharifi-Rad M, Salehi B et al. (2018) In vitro and in vivo assessment of free radical scavenging and antioxidant activities of *Veronica persica* Poir. *Cellular and Molecular Biology* 64: 57 – 64. doi: 10.14715/cmb/2018.64.8.9.
- Mditshwa A, Fawole OA, Al-Said F et al. (2013) Phytochemical content, antioxidant capacity and physicochemical properties of pomegranate grown in different microclimates in South Africa. *South African Journal of Plant and Soil* 30 (2): 81 – 90. doi: 10.1080/02571862.2013.802033
- Venkataramanamma D, Aruna P, Singh RP (2016) Standardization of the conditions for extraction of polyphenols from pomegranate peel. *Journal of Food Science and Technology* 53 (5): 2497 – 2503. doi: 10.1007/s13197-016-2222-z.
- Ehteshami S, Abdollahi F, Ramezani A et al. (2020) Maintenance of quality and bioactive compounds in pomegranate fruit (*Punica granatum* L.) by combined application of organic acids and chitosan edible coating. *Journal of Food Biochemistry* 44 (9): e13393. doi: 10.1111/jfbc.13393.
- Abid M, Yaich H, Cheikhrouhou S et al. (2017) Antioxidant properties and phenolic profile characterization by LC–MS/MS of selected Tunisian pomegranate peels. *Journal of Food Science Technology* 54 (9): 2890 – 2901. doi: 10.1007/s13197-017-2727-0.
- Escarcega GH, Sánchez-Chávez E, Álvarez SP et al. (2020) Determination of antioxidant phenolic, nutritional quality and volatiles in pomegranates (*Punica granatum* L.) cultivated in Mexico. *International Journal of Food Properties* 23 (1): 979 – 991. doi: 10.1080/10942912.2020.1760879.
- Tan MC, Tan CP, Ho CW (2013) Effects of extraction solvent system, time and temperature on total phenolic content of henna (*Lawsonia inermis*) stems. *International Food Research Journal* 20 (6): 3117 – 3123.
- Rafi M, Meitary N, Septaningsih DA et al. (2020) Phytochemical profile and antioxidant activity of *Guazuma ulmifolia* leaves extracts using different solvent extraction. *Indonesian Journal of Pharmacy* 31 (3): 171 – 180. doi: 10.22146/ijp.598.
- Nawaz H, Shad MA, Rehman N et al. (2020) Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Brazilian Journal of Pharmaceutical Sciences* 56: e17129. doi: 10.1590/s2175-97902019000417129.
- Zlabur JS, Žutic I, Radman S et al. (2020) Effect of different green extraction methods and solvents on bioactive components of chamomile (*Matricaria chamomilla* L.) flowers. *Molecules* 25 (4): 810 – 826. doi: 10.3390/molecules25040810.
- Badan Pengawas Obat dan Makanan Republik Indonesia [BPOM RI] (2019) Persyaratan Keamanan dan Mutu Obat Tradisional. Jakarta, BPOM RI.
- Fourati M, Smaoui S, Hlima BH et al. (2020) Variability in phytochemical contents and biological potential of pomegranate (*Punica granatum*) peel extracts: toward a new opportunity for minced beef meat preservation. *Journal of Food Quality* Volume 2020, Article ID 8853196. doi: 10.1155/2020/8853196
- Departemen Kesehatan Republik Indonesia [Depkes RI] (2008) Farmakope Herbal Indonesia. 1st edition. Jakarta, Depkes RI.

19. Salazar-Arandra R, Perez-Lopez LA, Lopez-Arroyo J et al. (2011) Antimicrobial and antioxidant activities of plants from Northeast of Mexico. Evidence-Based Complementary and Alternative Medicine Volume 2011, Article ID 536139. doi: 10.1093/ecam/nep127.
20. Öztürk M, Ermin MD, Kivrak S et al. (2011) In vitro antioxidant, anticholinesterase and antimicrobial activity studies on three *Agaricus* species with fatty acid compositions and iron contents: a comparative study on three most edible mushrooms. Food and Chemical Toxicology 49 (6): 1353 – 1360. doi: 10.1016/j.fct.2011.03.019.
21. Haq M, Sani W, Hossain AB et al. (2011) Total phenolic contents, antioxidant and antimicrobial activities of *Bruquieria gymnorrhiza*. Journal of Medical Plants Research 5 (17): 4112 – 4118.
22. Escarpa A, González MC (2001) Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatographic and spectrophotometric methods. Analytica Chimica Acta 427 (1): 119 – 127. doi: 10.1016/S0003-2670(00)01188-0.
23. Chang CC, Yang MH, Wen HM et al. (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 10 (3): 178 – 182. doi: 10.38212/2224-6614.2748.
24. Li Y, Guo C, Yang J et al. (2006) Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chemistry 96 (2): 254 – 260. doi: 10.1016/j.foodchem.2005.02.033.
25. Shibani MS, Al-Otaibi MM, Al-Zoreky NS (2012) Antioxidant activity of pomegranate (*Punica granatum* L.) fruit peels. Food and Nutrition Sciences 3 (7), Article ID 20499. doi :10.4236/fns.2012.37131
26. Purwakusumah ED, Nurcholis W, Rafi M et al. (2014) Identifikasi dan autentikasi jahe merah menggunakan kombinasi spektroskopi FTIR dan kemometrik. Agritech 34 (1): 82 – 87. doi: 10.22146/agritech.9526.