Journal of Health Management and Pharmacy Exploration
https://shmpublisher.com/index.php/johmpe
p-ISSN: 2985-4814
e-ISSN: 2985-5543

Standardization of ethanol extracts from pomegranate fruit peels (*punica granatum l.*) using the reflux method

Wulandari[1*], Nuri Ardiantika[1]
[1] Department of Pharmacy, Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang, Indonesia

Article Info

Article history:

Received February 04, 2026
Revised February 10, 2026
Accepted February 11, 2026

Keywords:

Pomegranate peel
Extraction
Reflux
Standardization

ABSTRACT

Pomegranate peel (*Punica granatum* L.) is known to contain various active secondary metabolite compounds that have potential in the field of pharmacy. To ensure the quality, safety, and consistency of extracts as raw materials for traditional medicines, a standardization process is required that includes specific and nonspecific parameters. This study aims to standardize pomegranate peel extract and identify the active compounds it contains. Extraction was performed using the reflux method with 96% ethanol solvent. The specific parameters tested included organoleptic properties, water-soluble and ethanol-soluble extract levels, and identification of active compounds through phytochemical testing. The results showed that pomegranate peel extract met the quality standards for extracts with good specific and non-specific characteristics. The extract yield was 12.76%. The results of testing the non-specific parameters of water content, drying loss, ash content, and acid-insoluble ash content were 3.12%, 1.24%, 0.33%, and 0.19%, respectively. Specific standardization includes water-soluble extract content of 3.2% and ethanol-soluble extract content of 4.4%. Phytochemical testing of the extract showed the presence of active compounds such as alkaloids, flavonoids, saponins, tannins, quinones, and steroids.

Corresponding Author:
Wulandari,
Department of Pharmacy,
Sekolah Tinggi Ilmu Farmasi Yayasan Pharmasi Semarang,
Indonesia.
Email: wulwul001@gmail.com
DOI: https://doi.org/10.52465/johmpe.v4i1.655

1. Introduction

Pomegranate peel (*Punica Granatum* L.) is a waste product from pomegranate fruit utilization. Most people only eat the fruit, so the peel becomes waste that is rarely utilized. Pomegranate peel contains active compounds such as polyphenols, flavonoids, and tannins [1]. These compounds have beneficial pharmacological activities. Pharmacological effects need to be supported by standardization of natural products to ensure the quality of preparations.

Standardization of natural extracts is carried out to ensure the quality of traditional medicinal raw materials to be used as preparations and to ensure the reproducibility of the quality of preparations and their therapeutic effects. Standardization of extracts is carried out using two parameters, namely specific and non-specific parameters. Specific parameters include organoleptic properties (shape, smell, taste, and color), water-soluble extracts, ethanol-soluble extracts, and phytochemical content. Non-specific parameters include drying shrinkage, microbial contamination, ash content, acid-insoluble ash content, and heavy metal contamination [2]

Extraction is a basic process for separating active compounds from natural sources using appropriate solvents based on the principle of solubility. The extraction process can be carried out using hot or cold methods. Cold extraction methods include maceration, remaceration, and percolation, while hot methods include reflux, Soxhlet extraction, digestion, infusion, and decoction. The main difference between hot and cold extraction methods is the temperature used. The choice of method also depends on whether the active compound is heat-resistant or not [2].

Reflux is extraction with a solvent at its boiling point, for a certain period of time with a relatively constant amount of solvent and the presence of a reflux condenser. Extraction can take place efficiently and the compounds in the sample can be extracted more effectively by the solvent [3]. This study needs to be conducted to determine the quality or standardization of pomegranate peel ethanol extract obtained from the reflux method.

2. Method

Equipment and Materials

The equipment used in this study consisted of a reflux apparatus, glassware, crucibles, an oven, a crucible holder, porcelain dishes, a desiccator, a muffle furnace, a chamber, and thin-layer chromatography plates. The materials used in this study were pomegranate peel simplisia, 96% ethanol, Dragendrof, Mayer, chloroform, Mg powder, amyl alcohol, 30% ammonia, Na acetate, Stiasny reagent, sodium hydroxide, hydrochloric acid, 1% Fecl3, and anhydrous acetic acid.

Extraction

200 grams of crude drug powder was weighed and placed in a round-bottom flask with 96% ethanol solvent at a ratio of 1:5. It was then extracted using the reflux method for 2 hours. The filtrate obtained was evaporated using a rotary evaporator and then

concentrated using a water bath at a temperature of 60°C until a thick extract was obtained [3].

$$\text{Yield} = \frac{\text{Weight of extract}}{\text{Weight of simplicia}} \text{ x } 100\% \quad (1)$$

Non-Specific Standardization of Extract [2]

a. Moisture content of extract: Weigh 1 g of extract and place it in a moisture analyzer at a temperature of 105° for 15 minutes. Calculate the moisture content obtained using the following formula.

$$\text{Moisture content} = \frac{W-W1}{W} \times 100\% \quad (2)$$

Description:
W= Initial weight
W1 = Weight after drying

b. Drying shrinkage: The crucible is heated to 105°C for 15 minutes until the weight is constant. 1 gram of pomegranate peel extract is weighed, placed in the crucible, and spread evenly by shaking the crucible until it forms a 5-10 mm layer. Placed in an oven at 105°C until a constant weight is achieved. Dried in a desiccator for 5-10 minutes. The requirement is that the difference between each 2 weighings is no more than 0.5 mg (crucible) and 0.25% (extract). Drying shrinkage is calculated using the following formula.

$$\text{Drying shrinkage} = \text{Moisture content} = \frac{W-W1}{W} \times 100\% \quad (3)$$

Description:
W= Initial weight
W1 = Weight after drying/constant

c. Ash content: 1 g of pomegranate peel extract is placed in a constant crucible, then incinerated until the charcoal is completely burned in a muffle furnace at a temperature of 600°C. After that, it is cooled and the ash content obtained is calculated.

$$\text{Ash content (\%)} = \frac{W2-W0}{W1-WO} \times 100\% \quad (4)$$

Description:
W0: empty crucible weight
W1: crucible weight + extract before ignition
W2: crucible weight + ash after ignition

d. Acid-insoluble ash content: The ash obtained from determining the ash content is added to 25 ml of dilute H2SO4. The acid-insoluble portion is filtered using Whatman paper and placed in an oven at 105°C until a constant weight is achieved. The acid-insoluble ash content is calculated using the following formula.

$$\text{Acid-insoluble ash content (\%)} = \frac{W2-W1}{W0} \times 100\% \quad (5)$$

Description:
W0 : empty crucible weight
W1 : crucible weight + ash residue from initial calcination
W2 : crucible weight + acid-insoluble ash after recalcination

Specific Standardization [2]

a. Organoleptic: Observe the shape, smell, color, and taste of the extract obtained
b. Water-soluble extract content test: Weigh 1 g of pomegranate peel extract, dissolve it in 100 ml of water:chloroform (1:1) for 24 hours, place it in a measuring flask and shake it for 6 hours, then let it stand for 18 hours. Filter and evaporate 20 ml of the filtrate to dryness in a porcelain dish. Heat the residue at 105°C until the weight remains constant and calculate the water-soluble extract content using the following formula.

$$\text{Water-soluble extractive content (\%)} = \frac{\text{Final weight}}{\text{Sample weight}} \times \frac{\text{Solvent volume}}{\text{Filtrate volume}} \text{ x } 100\% \quad (6)$$

c. Ethanol soluble extract content test: Weigh 1 g of pomegranate peel extract and dissolve it in 100 ml of 70% ethanol for 24 hours. The extract is placed in a measuring flask and shaken for 6 hours, then left to stand for 18 hours. It is filtered and 20 ml of the filtrate is evaporated to dryness in a porcelain dish. The residue is heated at 105°C until the weight remains constant and the ethanol soluble extract content is calculated using the following formula.

$$\text{Ethanol-soluble extractive content (\%)} = \frac{\text{Final weight}}{\text{Sample weight}} \times \frac{\text{Solvent volume}}{\text{Filtrate volume}} \text{ x } 100\% \quad (7)$$

d. Identification of chemical compounds using color reactions and thin layer chromatography.

Active compounds in the extract were identified using color reactions and TLC using appropriate reagents and eluents. Alkaloids were identified with 10 mL of 2% HCl, 10 mL of 10% ammonia, 20 mL of chloroform, phosphomolybdic acid LP, Wagner LP, Mayer LP, and Hager LP. The eluent was methanol:ammonia (100:1.5), with Dragendorff's reagent [4]. Flavonoids were identified with 0.1 g of magnesium powder, 1 mL of HCl, and 2 mL of amyl alcohol added to the sample [5]. The eluent used for flavonoid identification was butanol:acetic acid:water (6:1:2) and ammonia vapor spot [6]. Saponins were identified by shaking the sample with 10 mL of hot distilled water and 1 drop of hydrochloric acid. The eluent was chloroform: methanol: water (64:50:10) with anisaldehyde-sulfuric acid spot detection and heated on a hot plate at 100℃ [7]. Tannins were identified with 1% gelatin and 10% sodium chloride, toluene:acetone:formic acid (6:6:1) eluent, and 5% FeCl3 spot[8]. Quinones were identified by adding 10 mL of distilled water, boiling for 15 minutes, filtering while hot, adding 3 mL of filtrate and 1 mL of 1 N NaOH to form a red color. The eluent used was ethyl acetate : methanol : water (100 : 13.5 : 10), with a 10% KOH spot in methanol. Steroids/triterpenoids are identified using Liebermann-Burchard solution, anhydrous acetic acid and concentrated sulfuric acid, with toluene:chloroform:methanol as the eluent in a ratio of (40:40:10), showing anisaldehyde-sulfuric acid spots and heated on a hot plate at 100℃ [9].

3. Results and Discussion

Reflux is one of the hot extraction methods. The principle of this method is that the volatile solvent used in the extraction will evaporate at high temperatures, but will be cooled by a condenser so that the solvent, which was previously in vapor form, will condense in the condenser and return to the container containing the crude drug. The solvent will remain present throughout the reaction [10]. The extraction process using the reflux method is shown in Figure 1.

Figure 1. Reflux extraction process

Extraction is carried out with the aim of separating secondary metabolite content from the mixture using a suitable solvent. In this case, 96% ethanol is used. Ethanol is a universal solvent that can attract active compounds with various polarities, so it is expected that secondary metabolites in pomegranate peel extract can be maximally attracted to this solvent. The yield obtained from the extraction of pomegranate peel with 96% ethanol solvent was 12.76%. Research conducted by [11] on pomegranate peel ethanol extract using the maceration method produced a yield of 33.46%. Similar research conducted by [12] on pomegranate peel extract using the reflux method produced a yield of 18.83%. The results of the pomegranate peel ethanol extract are presented in Figure 2.

Figure 2. Ethanol extract of pomegranate peel

The yield is influenced by the solvent used for extraction and the choice of extraction method. The temperature and duration of heating during the extraction process can affect the concentration of the compounds obtained. In general, the solubility of the extracted active substance will increase with higher temperatures. However, the increase in

extraction temperature also needs to be considered, because excessively high temperatures can cause damage to the active compounds being processed. The extraction time also greatly affects the resulting compounds. The right extraction time will produce optimal compounds. Extraction times that are too long will cause the active compounds to hydrolyze, while extraction times that are too short will result in not all active compounds being extracted from the material.

The results of testing the non-specific parameters of pomegranate peel extract are presented in Table 1 below.

Table 1. Testing of non-specific parameters of ethanol extracts of pomegranate peel

Water content (%)	Drying shrinkage (%)	Ash content (%)	Acid-insoluble ash content (%)
3,12	1,24	0,33	0,19

In non-specific parameters, the water content of the extract was 3.12%. Similar research conducted by [13] on pomegranate fruit ethanol extract using the percolation extraction method produced a water content of 5.6%. Previous research on dried pomegranate peel extract showed a moisture content of 5.91% [14]. The purpose of determining the moisture content in the extract is to provide a minimum limit or range for the amount of water in the extract. If the moisture content is too high (>10%), the extract will be susceptible to mold growth and reduce the effectiveness of the active substances in the extract. The drying shrinkage parameter was determined to provide a maximum limit or range for the amount of compounds lost during the drying process. The drying shrinkage content was 1.24%. This figure is smaller than the water content of pomegranate ethanol extract using the percolation method, which is 3.16% [13].

The determination of total ash content aims to determine the mineral/metal content remaining after roasting. The ash content of pomegranate peel extract is 0.33%. This result meets the ash content requirement according to FHI, which is < 3.3%. The total ash content of pomegranate fruit ethanol extract using the percolation method is 6.80%. The ash content parameter can provide an overview of the internal and external mineral content originating from the initial process to the formation of the extract. A high ash content indicates that the extract contains high levels of metal contamination, and the acid-insoluble ash content is 0.19%. This figure is lower than that found in a study [13], which was 0.41%. Research on dried pomegranate peel extract contained an ash content of 3.41%. The addition of strong acid in testing the acid-insoluble ash content aims to remove silica and acid-insoluble substances. These non-specific parameters provide different results from previous studies because differences in extraction methods can affect various research parameters. In reflux, the water content and drying shrinkage were lower than in the percolation method because continuous heating during extraction caused free water and some bound water to evaporate or be released, and during extract concentration, the solvent was easier to evaporate. This results in a final extract that is drier and more stable. In testing ash content and acid-insoluble ash content, the reflux extraction method yields lower results than the percolation method because heating during reflux produces extracts that are more selective towards inorganic compounds, which contribute to ash content and acid-insoluble ash content.

The specific parameter testing of pomegranate peel ethanol extract conducted in this study included organoleptic testing, water-soluble extract content, ethanol-soluble extract content, and chemical compound identification using color reactions and thin-layer chromatography. The organoleptic results of the extract obtained were dark brown in color, with a distinctive sharp smell and bitter taste. The water-soluble extract content was 3.2%. This test was conducted to determine the amount of content in the material that could be absorbed by the solvent, namely water. Meanwhile, the ethanol-soluble extract content was 4.4%. This parameter aimed to determine the content of compounds in the sample that were soluble in ethanol solvent. Compared to water-soluble extract, the ethanol-soluble extract is greater. This indicates that more active compounds contained in pomegranate peel are attracted to ethanol than water. The results of the water-soluble and ethanol-soluble extract content tests in this study are lower than those in study [13], which were 14.33% and 70.5%, respectively. Pomegranate peel contains tannins, polyphenols, flavonoids, and ellagitannins, which are easily oxidized by heating, resulting in lower water and ethanol extract yields in reflux. In addition, the ratio of solvent and contact time between the sample and solvent in both methods also affects the results. In reflux, the contact time is faster than in percolation, resulting in less than optimal water and ethanol solubility of the compounds.

The identification test of active compounds in pomegranate peel ethanol extract using the color reaction/precipitation method is presented in Table 2.

Table 2. Results of Color Identification of Ethanol Extracts from Pomegranate Peels

No	Compound Group	Result
1	Alkaloid	+
2	Flavonoid	+
3	Saponin	+
4	Tannin	+
5	Quinone	+
6	Steroid	+

In addition to identification by color reaction, pomegranate peel ethanol extract was also identified by thin-layer chromatography. Chromatography is a separation technique based on differences in polarity. In thin-layer chromatography, the stationary phase used is silica gel plate. Meanwhile, the mobile phase is adjusted to the type of sample to be separated. The closer the polarity between the sample and the eluent, the more the sample will be carried by the mobile phase and will move faster. Factors that affect the Rf value are temperature, humidity, stationary phase particle size, and spot volume [15]. The results of thin-layer chromatography of pomegranate peel ethanol extract are presented in Table 3.

Table 3. Results of ethanol extract identification of pomegranate fruit peel

No	Compound Group	Result
1	Alkaloid	Brownish yellow spot. Rf1: 0,86 Rf2: 0,94
2	Flavonoid	No spot formed
3	Saponin	Purple spot.. Rf: 0,75
4	Tannin	Dark blue spot. Rf1: 0,74 Rf2: 0,88
5	Quinone	Reddish brown spot.. Rf1: 0,74 Rf2 : 0,87
6	Steroid	Dark purple spot Rf1 : 0,48 Rf2 : 0,55 Rf3 : 0,60 Rf 4: 0,75

From the results of thin layer chromatography identification of pomegranate peel ethanol extract, it was found to contain alkaloids, saponins, tannins, quinones, and steroids.

4. Conclusion

Standardization testing of pomegranate peel ethanol extract using the reflux extraction method, consisting of non-specific standardization testing of water content, drying loss, ash content, and acid-insoluble ash content, yielded the following results: 3.12%; 1.24%; 0.33%; and 0.19%, respectively. Specific standardization includes water-soluble extract content of 3.2%, ethanol-soluble extract content of 4.4%, and the presence of active compounds including alkaloids, flavonoids, saponins, tannins, quinones, and steroids.

REFERENCES

[1] S. Anusha, S. Singh, and G. Ahmad, "Bioactive compounds from pomegranate peels - Biological properties , structure – function relationships , health benefits and food applications – A comprehensive review," *Journal of Functional Foods*, vol. 116, no. March, p. 106132, 2024, doi: 10.1016/j.jff.2024.106132.

[2] Departemen Kesehatan RI, "Parameter Standar Umum Ekstrak Tanaman Obat," 2000.

[3] M. Djaeni, N. Ariani, R. Hidayat, and F. D. Utari, "Ekstraksi Antosianin dari Kelopak Bunga Rosella (Hibiscus sabdariffa L.) Berbantu Ultrasonik: Tinjauan Aktivitas Antioksidan Ultrasonic Aided Anthocyanin Extraction of Hibiscus sabdariffa L. Flower Petal: Antioxidant Activity," *Jurnal Aplikasi Teknologi Pangan*, vol. 6, no. 3, p. 71, 2017.

[4] A. Mu' awwanah and M. Ulfa, "Uji Aktivitas Antioksida Fraksi N-heksan Ekstrak Etanol Daun Karika (Carica pubescens) Dan Identifikasi Senyawa Alkaloid Dan Flavonoid," *Prosiding Seminar Nasional Peluang Herbal Sebagai Alternatif Medicine*, no. 2012, pp. 118–124, 2015.

[5] M. R. R. Rahardhian *et al.*, "Exploring the α-Amylase Inhibitory Potential of Peronema canescens Jack: An In Vitro and In Silico Study," *Hayati Journal of Biosciences*, vol. 32, no. 3, pp. 712–726, 2025, doi: 10.4308/hjb.32.3.712-726.

[6] H. Firnando, N. U. Purwanti, and R. Susanti, "Penetapan Kadar Total Fenolik dan Flavonoid Fraksi Butanol dan Fraksi Air Dari Ekstrak Etanol Rimpang Acorus Sp.," *Jurnal Mahsiswa Farmasi Fakultas Kedokteran*, vol. 4, no. 1, pp. 1–13, 2019.

[7] A. Fahrurroji and H. Riza, "Karakterisasi Ekstrak Etanol Buah Citrus amblycarpa (L), Citrus aurantifolia (S.), dan Citrus sinensis (O.)," *Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia*, vol. 7, no. 2, p. 100, 2020, doi: 10.20473/jfiki.v7i22020.100-113.
[8] E. Hanani, *Analisis Fitokimia*. Jakarta: Buku Kedokteran EGC, 2015.
[9] I. Fajriaty, H. I H, Andres, and R. Setyaningrum, "Skrining Fitokimia Lapis Titpis Dari Ekstrak Etanol Daun Bintangur (Calophyllum soulattri Burm . F .)," *Jurnal Pendidikan Informatika dan Sains*, vol. 7, no. 1, pp. 54–67, 2018.
[10] L. S. Chua, N. A. Latiff, and M. Mohamad, "Reflux extraction and cleanup process by column chromatography for high yield of andrographolide enriched extract," *Journal of Applied Research on Medicinal and Aromatic Plants*, vol. 3, no. 2, pp. 64–70, 2016.
[11] S. Septiani, E. N. Dewi, and I. Wijayanti, "AKTIVITAS ANTIBAKTERI EKSTRAK LAMUN (Cymodocea rotundata) TERHADAP BAKTERI Staphylococcus aureus DAN Escherichia coli (Antibacterial Activities of Seagrass Extracts (Cymodocea rotundata) Against Staphylococcus aureus and Escherichia coli)," *SAINTEK PERIKANAN : Indonesian Journal of Fisheries Science and Technology*, vol. 13, no. 1, p. 1, 2017, doi: 10.14710/ijfst.13.1.1-6.
[12] N. S. N. Ningsih and K. N. Rachmadani, Atika Lailatul Wahyusi, "Ekstraksi Senyawa Flavonoid Dari Kulit Ari Buah Delima (Punica Granatum L.)," vol. 10, no. 1, pp. 8–11, 2025.
[13] A. A. Deanggi, T. Saptawati, and Ovikariani, "Penetapan Parameter Spesifik dan Non Spesifik Ekstrak Buah Delima Merah (Punica granatum L)," *Proceeding*, vol. 2, no. 1, pp. 89–99, 2023.
[14] J. H. Wattimenaa, F. L. Darsonoa, L. S. Hermanua, F. Farmasi, U. Katolik, and W. Mandala, "Formulasi Ekstrak Kering Kulit Buah Delima (Punica granatum L .) Sebagai Masker Wajah dalam Bentuk Peel-Off Gel The Formulation of Promegranate Peel Fruit (Punica granatum L .) in Peel – Off Face Mask Gel," vol. 7, no. 1, pp. 74–80, 2020.
[15] S. I. Lestari and B. Santoso, "Analisis Kromatografi Lapis Tipis (Klt) Dan Aktivitas Penangkapan Radikal Bebas (Prb) Ekstrak Etanol Lempuyang Emprit (Zingiber Americans) Hasil Maserasi Sekali Dan Maserasi Berulang," *Biomedika*, vol. 13, no. 1, pp. 76–82, 2021, doi: 10.23917/biomedika.v13i1.11439.