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# Determination of Total Polyphenol Content in Bali Local Red Pomegranate (*Punica granatum* L.) Peel Extract Using UV-Vis Spectrophotometry

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## ABSTRACT

Pomegranate (*Punica granatum* L.) peel is a major agro-industrial byproduct rich in bioactive polyphenols, yet studies on the local Balinese red pomegranate variety remain limited. This study aimed to determine the total polyphenol content and phytochemical profile of red pomegranate peel collected from Bali, Indonesia. The peels were dried, powdered, and extracted by maceration using 96% ethanol, followed by phytochemical screening and quantification using the Folin-Ciocalteu method with UV-Vis spectrophotometry. The extraction produced a yield of 25.78%, and screening confirmed the presence of major secondary metabolites, including flavonoids, tannins, saponins, and steroids/triterpenoids. The total polyphenol content was found to be 147.953 mg GAE/g, indicating a significant concentration of bioactive compounds. These findings demonstrate the strong antioxidant potential of this local variety. Consequently, the results support the valorization of Balinese red pomegranate peel waste. It is specifically recommended to utilize this extract as a functional ingredient in nutraceuticals or phytopharmaceuticals as a preventative measure against oxidative stress-related diseases.

**Keywords:** *Punica granatum L.*; polyphenol content; Folin-Ciocalteu; UV-Vis spectrophotometry; phytochemical screening.

## ABSTRAK

Kulit delima (Punica granatum L.) adalah produk sampingan agro-industri utama yang kaya akan senyawa bioaktif, terutama polifenol, yang semakin menarik minat untuk aplikasi makanan fungsional dan fitofarmasi. Namun, studi yang secara khusus menganalisis total kandungan polifenol dari kulit buah delima merah Bali lokal masih terbatas. Studi ini bertujuan untuk menentukan kandungan total polifenol dan melakukan skrining fitokimia pada kulit buah delima merah yang dikumpulkan dari Bali, Indonesia. Penelitian ini menggunakan desain laboratorium eksperimental. Kulit delima merah dikumpulkan dari Desa Jadi, Kabupaten Tabanan, Bali. Kulit buah delima dikeringkan, dihaluskan, dan diekstraksi dengan cara perendaman menggunakan etanol 96%. Skrining fitokimia dilakukan untuk mendeteksi metabolit sekunder utama. Kandungan total polifenol ditentukan menggunakan metode Folin-Ciocalteu dengan asam galat sebagai standar, diukur dengan spektrofotometri UV-Vis pada 765 nm. Semua analisis dilakukan dalam triplikat. Ekstraksi menghasilkan 25,78% ekstrak etanol kental. Skrining fitokimia mengkonfirmasi keberadaan flavonoid, tanin, saponin, dan steroid/triterpenoid. Kurva kalibrasi asam galat standar menunjukkan linearitas yang baik dengan koefisien korelasi ($R^2$) sebesar 0,9916. Kandungan total polifenol ditemukan sebesar 147,953 mg GAE/g pada konsentrasi 1.000 ppm dan 34,136 mg GAE/g pada 100 ppm. Temuan tersebut menunjukkan bahwa kulit delima merah Bali lokal mengandung senyawa polifenolik yang signifikan dan metabolit sekunder dengan potensi antioksidan. Ini mendukung potensinya untuk valorizasi sebagai sumber antioksidan alami untuk makanan fungsional, nutraceutical, atau aplikasi kemasan berkelanjutan. Studi lebih lanjut direkomendasikan untuk menilai aktivitas antioksidannya dan membandingkan variasi musiman serta varietas.

**Kata kunci:** *Punica granatum L.*; kandungan polifenol; Folin-Ciocalteu; spektrofotometri UV-Vis; skrining fitokimia.

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## INTRODUCTION

Pomegranate (*Punica granatum* L.), a plant from the Punicaceae family that thrives in tropical and subtropical climates, has been consumed by humans since ancient times. Pomegranate (*Punica granatum* L.) is a well-known member of the Punicaceae family, consisting of two species: Punica granatum (native to Iran and the Mediterranean region) and Punica protopunica (native to the Socotra archipelago). It is believed to originate from Iran and Afghanistan, but it can also be found in Central Asia, the Himalayas, the Middle East, the Southwestern United States, and the Mediterranean (1). The parts of the pomegranate, especially the seeds and arils, are often eaten fresh or used as industrial raw materials for juice, syrup, and even the highly valuable pomegranate seed oil. However, few people know that the skin of the pomegranate constitutes about 50% of the fruit and contains many bioactive compounds, especially polyphenols (2). Pomegranate peel is increasingly recognized not just as waste, but as a commercial source of bioactive compounds with myriad health benefits, including antioxidant and anti-inflammatory properties (3).

With the emergence of functional food and phytopharmaceutical trends, there is growing interest in discovering bioactive compounds from agricultural waste, including fruit peels (4) (5). Pomegranate (Punica granatum L.) has been proven to possess promising anti-inflammatory and chronic disease properties. Furthermore, it has been found that pomegranate fruit possesses antifungal, insecticidal, antibacterial, anticoccidial, and molluscicidal qualities that combat microorganisms affecting plants and humans (6). Magangana et al. (2020) state that pomegranate peel contains many polyphenols, including punicalagin, punicalin, ellagic acid, gallic acid, and other flavonoids that perform antioxidant, anti-inflammatory, and anticancer functions. Flavonoids, phenolic acids, tannins, anthocyanidins, ellagic acid, quercetin, gallic acid, catechin, and vitamin C are all abundant in pomegranate peel. Vitamin C also acts as an antioxidant. They also improve skin health (7). However, the polyphenol content is highly variable depending on the variety, cultivation location, post-harvest processing methods, and extraction techniques used. This variation allows for more specific local research, especially in tropical regions like Bali, which has a unique agroecology.

New studies show that pomegranate peel has the potential to function as an antioxidant and help manage degenerative diseases. In clinical trials on adult patients, it was found that pomegranate peel extract supplements can reduce risk factors for metabolic syndrome, non-alcoholic fatty liver disease (NAFLD), dyslipidemia, and high blood pressure. There is a possibility that the use of pomegranate peel will encourage the development of herbal supplements related to the trend of evidence-based prevention of metabolic diseases (8). In previous research, conducted on the extract of white pomegranate peel using a 100 ppm concentration and gallic acid concentrations of 20, 40, 60, 80, and 100 ppm, the total polyphenol content in the white pomegranate peel was found to be 282.02 µg GAE/ml or equivalent to 0.28202 mg GAE/g (9).

However, the profile of secondary metabolite compounds in local varieties can be influenced by agroclimate, soil type, and different cultivation methods. Therefore, understanding the polyphenol levels in local varieties is very important. In molecular pharmacology research, it was found that pomegranate polyphenol compounds, such as punicalagin, work through the antioxidant pathway, the anti-inflammatory pathway, and the MAPK inhibition pathway. This is related to the cessation of oxidative stress and chronic inflammation in various degenerative diseases (10). Unfortunately, until now, scientific studies related to the total polyphenol content of the skin of the local Balinese red pomegranate variety are still very limited, even though Bali has an advantage in tropical horticultural commodities with export potential (11).

Based on the presentation, this research aims to determine the total polyphenol content in the skin of local Balinese red pomegranate fruit using the Folin-Ciocalteu method with UV-Vis Spectrophotometry detection. It is hoped that

the results of this research can serve as an initial database to support the innovation of value-added processed pomegranate skin products, while also strengthening the literature on the phytochemical profile of pomegranate varieties in Indonesia, particularly in Bali.

## MATERIALS AND METHODS

This research was designed as an experimental laboratory study using a quantitative approach. The study aimed to determine the total polyphenol content of red pomegranate (*Punica granatum L.*) peel extract using the Folin-Ciocalteu method with UV-Vis spectrophotometry. The research was conducted from November 2024 to January 2025 at the Pharmaceutical Biology Laboratory and Analytical Chemistry Laboratory, Institut Teknologi dan Kesehatan Bintang Persada Denpasar.

### Materials

The equipment used in this research included a UV-Vis Spectrophotometer (Genesys™ or equivalent), cuvettes, rotary evaporator, analytical balance, glass beakers, measuring flasks (10 mL, 100 mL), Erlenmeyer flasks, volumetric pipettes, drop pipettes, test tubes, filter paper (Whatman No. 1), glass funnels, blender, sieve (60–100 mesh), water bath, and oven. The materials used included fresh red pomegranate peels (*Punica granatum L.*) from Jadi Village, Tabanan Regency, Bali; 96% ethanol; Folin-Ciocalteu reagent; gallic acid standard; sodium carbonate ($Na_2CO_3$) 7.5% solution; concentrated and dilute hydrochloric acid (HCl); magnesium powder; ferric chloride ($FeCl_3$); chloroform; glacial acetic acid; sulfuric acid ($H_2SO_4$); Mayer's reagent; Dragendorff's reagent; and distilled water (aquadest).

### Methods

1. Sample Collection and Preparation

Red pomegranate fruits were collected purposively from Jadi Village, Kediri District, Tabanan Regency. The peels were separated, washed, and sun-dried for seven days, then oven-dried at 60°C for 48 hours until constant weight (12). The dried peels were ground using a blender and sieved with a 60-mesh sieve to obtain fine powder (13).

2. Extraction

About 25 g of dried peel powder was macerated with 250 mL of 96% ethanol for 24 hours at room temperature (25–30°C) with manual stirring twice daily for 30 minutes. The filtrate was collected, and the residue was re-macerated three times to maximize extraction. The combined filtrates were concentrated using a rotary evaporator to obtain a viscous crude extract. The extract yield was calculated to determine extraction efficiency (14).

3. Phytochemical Screening

Qualitative screening was carried out to detect alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids using standard phytochemical reagents (Mayer's, Dragendorff's, $FeCl_3$, Mg-HCl, chloroform–acetic acid–$H_2SO_4$ combination).

4. Preparation of Standard and Sample Solutions

A 1,000 ppm gallic acid stock solution was prepared by dissolving 10 mg gallic acid in 10 mL ethanol. Working solutions of 40, 60, 80, 100, and 120 ppm were made by serial dilution. A stock extract solution (1,000 ppm) was prepared by dissolving 10 mg extract in 10 mL ethanol, then diluted to 100 ppm (15).

5. Determination of Total Polyphenol Content

For each standard and sample solution, 0.1 mL was pipetted and mixed with 1 mL Folin-Ciocalteu reagent, shaken, and incubated for 5 minutes. Then, 1 mL of 7.5% $Na_2CO_3$ solution was added, shaken until homogeneous, and incubated in the dark for 90 minutes. The absorbance was measured at 765 nm using the UV-Vis spectrophotometer. Each measurement was carried out in triplicate (15).

6. Sampling Method and Data Analysis Method

Purposive sampling was applied to ensure consistent fruit maturity and quality from a single source. The entire extraction and analysis procedure was repeated in triplicate to ensure reproducibility. Absorbance readings of the gallic acid standards were plotted to generate a calibration curve and a linear regression

equation. The total polyphenol content of the extract was calculated by inserting the sample's absorbance into the equation. Results were expressed as mg gallic acid equivalent (GAE) per gram of extract. The results were presented as mean ± standard deviation (SD). The coefficient of determination ($R^2$) was used to evaluate linearity, and t-tests ($\alpha = 0.05$) were conducted where needed to compare with previous studies (16).

## RESULTS AND DISCUSSIONS

### Results

The extraction of red pomegranate (*Punica granatum L.*) peel produced a viscous ethanol extract with a yield of 25.78%. The dried peel powder (147 g) produced 37.91 g of concentrated extract after maceration with 96% ethanol and evaporation using a rotary evaporator. Ethanol 96% was chosen as the solvent because it has been reported to effectively extract high levels of phenolic compounds from pomegranate peels compared to other solvents (17)

**Table 1.** Extract Yield of Red Pomegranate Peel.

| Sample (g) | Extract (g) | Yield (%) |
|---|---|---|
| 147 | 37.91 | 25.78 |

This yield complies with the standard extraction yield for pomegranate peel as referenced in the Indonesian Herbal Pharmacopoeia, which states that the extractive value should not be less than 13.7%.

The phytochemical screening supports the known chemical profile of pomegranate peel, which contains a high concentration of polyphenolic compounds. The presence of flavonoids and tannins is particularly significant, as these compounds are primarily responsible for the peel's antioxidant potential.

**Table 2.** Phytochemical Screening Results.

| Compound | Reagent | Results |
|---|---|---|
| Alkaloid | Dragendorff | (+) |
| Flavonoid | NaOH 10%, Mg-HCl | (+) |
| Saponin | Warm distilled water | (+) |
| Tannin | $FeCl_3$ 10% | (+) |
| Steroid/Triterpenoid | Chloroform–Acetic Acid–$H_2SO_4$ | (+) |

This finding is consistent with studies by Magangana et al. (2020) and Maphetu et al. (2022), who confirmed that the polyphenol content and profile can vary depending on variety and agroecological factors.

The total polyphenol content was determined using the Folin-Ciocalteu method with gallic acid as the standard. The standard calibration curve using gallic acid solutions at concentrations of 40, 60, 80, 100, and 120 ppm showed a good linearity with a correlation coefficient ($R^2$) of 0.9916. The linear regression equation obtained was:

$$y = 0.007x + 0.0153$$

where *y* is the absorbance and *x* is the concentration in ppm.

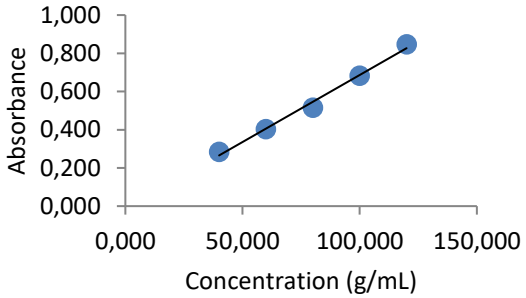


**Figure 1. Gallic Acid Calibration Curve**

**Table 3.** Total Polyphenol Content.

| Concentration (ppm) | Absorbance (Mean ± SD) | Total Polyphenol (mg GAE/g) |
|---|---|---|
| 1,000 | 1.023 ± 0.002 | 147.953 |
| 100 | 0.224 ± 0.001 | 34.136 |

Based on the absorbance values, the total polyphenol content of the red pomegranate peel extract was found to be 147.953 mg GAE/g at a concentration of 1,000 ppm and 34.136 mg GAE/g at 100 ppm. These results were consistent across three replications, indicating good repeatability.

### Discussions

The extraction yield of 25.78% indicates that the ethanol maceration method

used was efficient for recovering bioactive compounds from red pomegranate peel. This value complies with the minimum extractive value for pomegranate peel specified in the Indonesian Herbal Pharmacopoeia. The slightly lower yield compared to Wijayanti's study (39.25% for white pomegranate peel) (14) may be due to differences in the variety, agroecological conditions, or extraction parameters such as solvent volume and duration.

The phytochemical screening confirmed the presence of flavonoids, tannins, saponins, and steroids/triterpenoids, which are well-known for their contribution to antioxidant capacity (2) (18). This result aligns with Magangana et al., who reported that pomegranate peel is rich in punicalagin, ellagic acid, gallic acid, catechins, and tannins (1). The presence of these compounds is significant because they act through multiple bioactivity pathways, including scavenging free radicals and modulating inflammatory responses (19).

The total polyphenol content of the red pomegranate peel extract was determined to be 147.953 mg GAE/g, which demonstrates that the local Bali variety has a comparable polyphenol level to other reported varieties (20). For instance, Eghbali et al. (2021) noted that pomegranate peels from Iran and the Mediterranean typically contain between 100 and 250 mg GAE/g, depending on extraction conditions (1). This supports the potential of the local variety as a valuable source of natural antioxidants. The polyphenol content of the Bali variety was also compared with findings from other Indonesian regions, for example, Nuryanti and Dini (2018) analyzed pomegranate peels from South Sulawesi, which provides a broader perspective on the phytochemical potential of local Indonesian varieties (21). A relevant comparison can be made with Rafsanjani (2024), who analyzed the total polyphenol content of white pomegranate peel using the Folin-Ciocalteu method and UV-Vis spectrophotometry. That study found that the white pomegranate peel contained 282 µg GAE/mL (15). The difference in results between this study and Rafsanjani's work may be attributed to several factors, including the variety of pomegranate used (red vs. white), plant growth conditions, extraction method, and the sample concentration analyzed. Additionally, the use of different measurement units (mg GAE/g vs. µg GAE/mL) affects direct comparison and highlights the need for standardization in reporting results to ensure accurate benchmarking.

The linear regression coefficient ($R^2$ = 0.9916) indicates that the Folin-Ciocalteu method provided reliable quantification. However, it is recognized that this method is non-specific and may react with other reducing substances present in plant matrices (22). Therefore, further studies using more selective methods, such as High-Performance Liquid Chromatography (HPLC), are recommended to precisely identify and quantify individual polyphenolic compounds. In line with recent findings by Barghchi et al., supplementation with pomegranate peel extract has clinical relevance for managing metabolic syndrome and non-alcoholic fatty liver disease (NAFLD) (8). Thus, valorization of local pomegranate peel as a functional ingredient could contribute to the development of evidence-based herbal supplements (23). The potential of pomegranate peel is not limited to extraction but also extends to the development of functional flours, as shown by Gullon et al. (2020), who demonstrated its strong antibacterial and antioxidant properties in food applications (24). To further strengthen the functional claim, future studies should perform in vitro antioxidant assays such as DPPH or FRAP (25) and assess seasonal or varietal differences that may affect the polyphenol profile (10).

## CONCLUSIONS

This study confirmed that the red pomegranate (*Punica granatum L.*) peel from Bali contains significant levels of polyphenolic compounds, as evidenced by a total polyphenol content of 147.953 mg GAE/g using the Folin-Ciocalteu method and UV-Vis spectrophotometry. The extraction yield of 25.78% and the positive results of phytochemical screening for flavonoids, tannins, saponins, and steroids/triterpenoids demonstrate that the local red pomegranate peel has potential as a natural antioxidant source.

The results highlight that varietal and agroecological differences, as well as extraction and measurement methods, may influence the total polyphenol content

compared to other studies. Therefore, the Bali red pomegranate peel can be further developed for functional food, nutraceutical, or eco-friendly applications. Future research should include more selective analytical methods, antioxidant activity assays, and comparative studies on different varieties and seasonal harvests.

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