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Phenolic and flavonoids compounds Punica Granatum L peel extracts obtained under different extraction conditions and their antioxidant properties

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Abstract. This study investigates the effects of maceration extraction conditions, including temperature variation, simplicia/solvent ratio, and extraction duration, on pomegranate peels' phytochemical content, yield, total phenolics, flavonoids, and antioxidant activity. Pomegranate peel is rich in phenolic and flavonoid compounds, which have been reported to have therapeutic properties against diseases such as liver inflammation and leukaemia cells, and as an antibacterial agent. Experimental results indicate that pomegranate peel extract contains active components, including phenols, flavonoids, alkaloids, steroid glycosides, saponins, and tannins. The study demonstrates that the optimum conditions for extracting pomegranate peels are a maceration temperature of 27°C for 30 minutes with a simplicia/solvent ratio of 1:3, resulting in a yield of 88.13%, total phenolic content of 12.47%, total flavonoid content of 4.78%, and an IC50 value of 46.05 ppm, which classifies it as a powerful antioxidant. The study highlights the importance of extraction conditions in obtaining optimal phytochemical content and antioxidant activity from pomegranate peels, which may have implications for their use in disease treatment and prevention.

Keywords: bio- Pomegranate peel, extraction, phenolic, flavonoid, antioxidant.

1. Introduction

Pomegranate (*Punica granatum* L.) belongs to the Punica genus and is easy to cultivate in exotic areas [1, 2]. Pomegranate is one of the most readily damaged fruits [3, 4]. Pomegranate peel accounts for almost 26-30% of the fruit's content. Pomegranate peel includes several active components, such as phenolic compounds, which are effective for treating a variety of diseases [5] including liver

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inflammation [6], leukemia cells [7], dysentery [8], lowering blood glucose levels [9] and antibacterial [10]. Phenolic compounds can help reduce oxidative damage and act as antioxidants [11]. There are three types of phenolic compounds: phenolic acids (hydroxybenzoic and hydroxyinnamic), polyphenols (hydrolysables and tannins), and flavonoids [12]. Milling, homogenization, and extraction are procedures used to extract active components from a natural substance. The extraction approach has various advantages, including a straightforward process that is inexpensive and does not harm the active components.

The selection of the extraction method as a starting point for the isolation and purification of the chemical components contained in the sample is a critical initial step in the extraction stage [13]. The extraction technique, extraction time, temperature, solvent type, and ratio of materials and solvents all have an impact on the quality of the extract [14, 15]. The maceration procedure, either cold or hot, is one of the most commonly utilized extraction methods. Flavonoid and phenolic compounds have heat-sensitive or thermolabile characteristics. This study aimed to determine how the maceration extraction conditions (temperature variation, simplicia/solvent ratio, and extraction duration) affected the phytochemical content, yield, total phenolics, flavonoids, and antioxidant activity in pomegranate peels.

2. Materials and methods

2.1. Materials

Pomegranates were purchased locally in Bogor, Indonesia. The pomegranate peel was separated, cleaned, and air-dried for two weeks in the shade. Pomegranate peel was crushed into fine powder before being stored in polypropylene pouches until use. Pomegranate peel simplicia with less than 10% moisture content was used as the test material. Distilled water, 100 mg/L gallic acid, (2,2-diphenyl-1-picrylhydrazyl) (DPPH), Folin-Ciocalteu reagent, sodium carbonate solution (Na_2CO_3), aluminium chloride 10%, potassium acetate 1 M, and ethanol 70% were the analytical grade chemicals.

2.2. Pomegranate peel extraction

Pomegranate peel simplicia was weighed at 25 g and macerated in distilled water at 27 and 50 degrees Celsius with weight ratios of simplicia to solvent of 1:2 and 1:3. The simplicia was macerated for 15 to 30 minutes before being extracted. The residue is macerated with the same solvent after the solvent has been separated. All liquid extract results were evaporated using a rotary evaporator and then dried at 70 °C to achieve a constant weight. The yield value of the obtained dry extract was then calculated. The obtained extract was then qualitatively tested. Phytochemical screening tests were performed to identify phenolic compounds, flavonoids, alkaloids, sterols, triterpenoids, steroid glycosides, saponins, and tannins [16].

2.3. Determination of total phenolics

Pomegranate peel extract samples were weighed at 450 mg before mixed with 200 mL of distilled water. The sample was heated for 15 minutes at 90 ºC before being cooled. After pipetting 0.5 mL of the produced sample solution, 4.5 mL of distilled water and 0.2 mL of Folin-Ciocalteu reagent were added, and the solution was homogenized. After 30 seconds, 0.5 mL of saturated Na_2CO_3 solution was added, followed by distilled water up to 10 mL, and the mixture was left to rest in a dark room for 1 hour. The samples were then examined with a visible spectrophotometer set to 765 nm and were taken thrice.

2.4. Determination of flavonoids content

Pomegranate peel extract samples weighing 50 mg each were diluted in 70% ethanol to form 50 mL. The sample was pipetted into a 10 mL measuring flask, and 3 mL of 70% ethanol, 0.2 mL of 10% aluminium chloride, and 0.2 mL of 1 M potassium acetate, along with distilled water, were added to form 10 mL. The standard solution was left to stand for 30 minutes after homogenization. The samples were then examined with a visible spectrophotometer at 418 nm and were taken thrice.

2.5. Antioxidant activity

The efficacy of antioxidants to scavenge DPPH free radicals was measured. By dissolving 4 mg of DPPH crystals in 100 ml of DPPH free radicals, a 0.1 mM DPPH solution was generated. Four milligrams of DPPH crystals were dissolved in 100 ml of 96% methanol to make a 0.1 mM DPPH solution. One millilitre of DPPH solution was mixed with 3 mL of methanol to make the control solution. Extract samples were separated into five concentration series: 40, 80, 120, 160, and 200 ppm. With these concentrations, extract samples of up to 3 ml were prepared, and 1 ml of DPPH was added. Samples were incubated for 30 minutes at 37 °C before being tested for DPPH absorption using a UV-Vis spectrophotometer at 517 nm.

3. Results and discussion methods

The maceration method was employed in this test, which entails soaking pomegranate rind in water as a solvent. Because flavonoids and phenolic compounds are vulnerable to heat, this method is best suited for extracting plant chemical components that are neither heat resistant nor thermolabile. Water is considered a solvent since it is inexpensive, readily available, stable, non-toxic, non-volatile, and non-flammable [17]. Water is also utilized as an extractor because it is safe for further consumption [18]. Water with hydroxyl groups will remove polar molecules, such as flavonoids and phenolic compounds, according to

the like dissolves like principle [19]. The results of extraction yield pomegranate peel can be seen in Figure 1.

Using the gravimetric method, the extract findings are evaporated to provide a solid value of 35%. This test aims to determine the amount of pomegranate peel extract contained in grams of extract. The entire value of the extract will influence its quality. According to the data in Figure 1, the more solvent used, the more the particle distribution in the solvent spreads, resulting in many extraction outcomes [20]. This demonstrates that polar chemicals predominate in the matrix of pomegranate peel and are highly soluble in water, making them easy to extract [21].

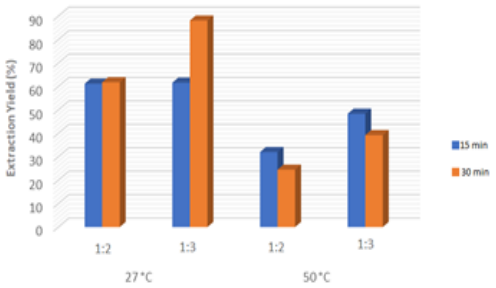

Fig. 1 Extraction yield pomegranate peel.

Furthermore, the longer the maceration duration, the higher the yield value because heat is transferred through convection and conduction [22]. This is because the contact time between the simplicia and the solvent will increase, allowing the solvent to better absorb the active ingredient [23]. Temperature increases can produce a rise in yield value. In this experiment, however, it is clear that there was a considerable decline in yield value between maceration at 50 °C. This is because a heating process happens at 50 °C, increasing the volume of water that evaporates.

3.2. Phytochemical screening tests

The phytochemical analysis of pomegranate peel extract revealed the presence of phenols, flavonoids, alkaloids, sterol glycosides, saponins, and tannins. This is consistent with the findings of Pinnamaneni [24], who discovered that various extracts of pomegranate peels contain secondary metabolite compounds such as phenols, flavonoids, and tannins. Peltierine compounds in alkaloids, according to Wong, et al. [25], can act as anti-cancer agents. Tannins have antimicrobial and antiviral properties [26, 27]. The following ingredients are in flavonoids: prunin, catechin chrysin, cyanidin, apigenin, biochanin, glucoside, luteolin, and taxifolin [28]. Table 1 shows the results of the phytochemical screening test.

3.3. Total phenolics

To evaluate the total phenolic content of pomegranate peel extract, tests were performed using the Folin-Ciocalteu method [29]. This test employed Gallic acid as a positive control [30]. Pomegranate peel extract contains phenolic chemicals that react with the Folin-Ciocalteu reagent to generate a yellow colour, and the addition of Na_2CO_3 solution results in an alkaline environment. The phenolic compound's hydroxy group combines with folin to generate a blue molybdenum-tungsten complex. The higher the phenolic component, the more folin ions convert heteropoly acids (phosphomolybdate-phosphotungstate) into molybdenum-tungsten complexes, producing a deeper blue color. The phenolic acids, flavonoid complexes, and anthocyanins comprise the overall phenolic content [31]. The total phenolic test results can be seen in Figure 2.

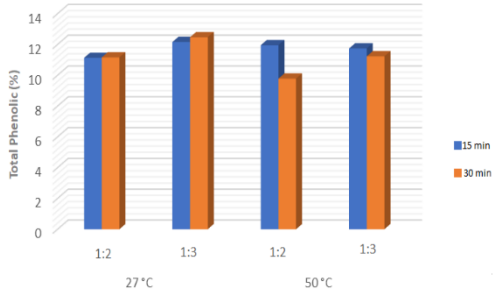

Fig. 2 The total phenolic on pomegranate peel extracts.

According to Figure 2, the specified condition variables, namely temperature, extraction time, and material/solvent ratio, have a substantial effect. Increasing the extraction temperature affects the total phenol content of pomegranate peel extract. At 50 °C, the total phenolic content of pomegranate peel extract decreased, with a minimum value of 9.78%. According to Miranda, et al. [32], phenolic compounds are harmed by using high temperatures for an extended period, causing the phenolic compounds to alter in terms of structure, converting these components into other compounds. Furthermore, phenolic compounds are heat-sensitive chemicals that allow hydrolysis and level drop at high temperatures. The creation of phenol complexes with other nutrients, such as protein, can also induce a decrease in phenolic compounds [33]. The most excellent total phenolic concentration was 12.47% at room temperature with a 1:3 simplicia-solvent ratio and a 30-minute extraction time. The longer the extraction time and the more solvent utilized, the higher the total phenolic content extracted. This is due to the growing dispersion of the particle distribution in the solvent, which expands the contact surface and attracts more phenolic chemicals.

Table 1 Phytochemical screening of pomegranate extract

Secondary metabolite	27 °C				50 °C			
	1:2		1:3		1:2		1:3	
	15 min	30 min	15 min	30 min	15 min	30 min	15 min	30 min
Phenol	+	+	+	+	+	+	+	+
Flavonoid	+	+	+	+	+	+	+	+
Alkaloid	+	+	+	+	+	+	+	+
Sterol	-	-	-	-	-	-	-	-
Triterpenoid	-	-	-	-	-	-	-	-
Steroid Glycosides	+	+	+	+	+	+	+	+
Saponin	+	+	+	+	+	+	+	+
Tannin	+	+	+	+	+	+	+	+

3.4. Total flavonoid

This test used The colourimetric approach to analyze total flavonoids [34]. The colorimetric approach employs the use of reagents such as 10% $AlCl_3$ and 1 M potassium acetate, with the role of the 10% $AlCl_3$ reagent being to generate a complex reaction between $AlCl_3$ with hydroxyl groups and flavonoid compound ketones [35]. $AlCl_3$ will generate a yellow complex molecule in an acidic environment by reacting with the ketone group on C4 and the OH group on C3 or C5. Flavonoids are commonly found in plants as glycosides such as quercetin-3-rutinoside or rutin [36]. The most frequent kind of quercetin glycoside is rutin, found in fruit peels, extracts, and vegetables. Figure 3 shows the findings for total flavonoid content.

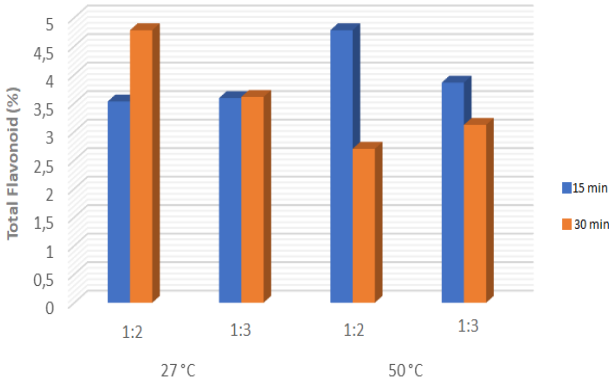

Fig. 3. The total flavonoid on pomegranate peel extracts.

3.5. Antioxidant activity

The DPPH method was used to test antioxidant levels with ascorbic acid as a reference [37]. DPPH radicals are persistent organic free radicals that can be absorbed in 517 nm with a colour change from purple to yellow [38]. The DPPH technique detects free radicals at low concentrations with high sensitivity [39]. According to de Sousa, et al. [40] and Sartinah, et al. [41], the antioxidant value and phenolic content in the extract have a synergistic relationship.

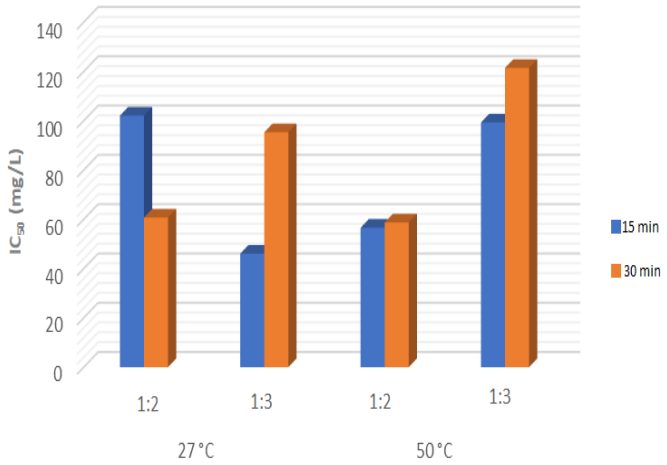

Fig. 4 Antioxidant activity test on pomegranate peel extracts.

Antioxidant characteristics are primarily connected to their redox capabilities, which allow them to function as radical scavengers, singlet oxygen quenchers, and hydrogen sources. Antioxidants primarily function by transferring single electrons and hydrogen atoms. Reducing power and DPPH tests use a single electron transfer pathway and are commonly used to assess the antioxidant activity of natural products [42]. Figure 4 depicts the results of the DPPH method antioxidant activity test on pomegranate peel. A linear graph of the regression curve was used to calculate antiradical activity (IC_{50}) [43, 44]. The extract with the highest IC_{50} value was made at a 1:3 ratio and macerated at 27 °C for 30 minutes. The IC_{50} value is the number of antioxidants required to reduce the DPPH concentration by 50%, and it is inversely proportional to the antioxidant capacity [45, 46].

4. Conclusion

In conclusion, the results of this study indicate that pomegranate peel is a rich source of bioactive compounds with potential therapeutic applications. The maceration extraction method was found to be an effective means of extracting these compounds, with the optimal conditions being a temperature of 27°C, a

simplicia/solvent ratio of 1:3, and an extraction duration of 30 minutes. These conditions resulted in a pomegranate peel extract with high yields of phenolics, flavonoids, and antioxidants, which exhibited potent antioxidant activity with an IC_{50} value of 46.05 ppm. These findings highlight the importance of careful selection and optimization of extraction conditions to maximize the yield and quality of bioactive compounds from natural sources. Further studies are needed to explore the potential therapeutic applications of pomegranate peel extract and its active components.

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