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Study of Antioxidant Activity, Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) of Ethanolic Extract Mango Peel (*Mangifera indica* L.)

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Abstract

Mango is one of the fruits rich in phenols and flavonoids, proven to have the ability to scavenge free radicals. The level of compounds in the sample can influence antioxidant capacity, including the total phenolic and flavonoid content. Peel of mango is a non-edible waste. This study aims to determine the total phenolic and flavonoid content and antioxidant activity of mango peel variety arum Manis, extracted by percolation using 70% ethanol. The total phenolic content was measured using Folin-Ciocalteu method, and flavonoid content was measured using $AlCl_3$. The absorbances were measured using UV-Vis spectrophotometry. The DPPH radical scavenging assay to assess antioxidant activity. The result reveals that the mango peel's ethanol extract had a total phenolic content of 19.31±0.41 mgGAE/g extract, a total flavonoid content of 22.06±1.74 mgQE/g extract, and an IC_{50} value of 766.444 ppm. This research showed that mango peel's antioxidant activity is weak compared to standard vitamin C (6.414 ppm). This is possibly due to the low level of metabolite content, including complexities of the phenolic and flavonoid types in the extract.

Keywords: mango peel, total phenolic content, total flavonoid content, antioxidant activity, percolation

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1 Introduction

The mango (*Mangifera indica* L.) is a well-known tropical fruit in Indonesia. Several mango varieties, such as golek, wirasangka, arummanis, dodor, and others, have unique characteristics and tastes [1], [2]. Mango is usually consumed directly, either raw, ripe, or processed, such as juice, salads, pudding, and mango sauce. Mango seeds and peel are non-edible waste and are thrown away.

Phytochemical compounds from each part of the plant may differ depending on external factors such as the variety and location of growth [3]. The mango plant's bark, leaves, roots, fruit, and flowers have been used in traditional medicine to cure numerous diseases. Mango bark and leaves are used for diarrhea, stomach disorders, liver disorders, urinary tract infections, diabetes, and coughs. Mango fruit flesh and seeds are used for dysentery and bleeding in the lungs and intestines [4].

The part of the mango fruit, especially the peel, is a non-edible waste. A study by Thambi et al. (2016) showed that the antioxidant effect of acetone extract mango peel powder has a potent free radical scavenging [5]. Aqyun et al. (2019) reported that the variety of mango gedong gincu has an antidiabetic activity [6]. The mango stems are active as renoprotective [7], and the mango peel is an antibacterial against MRSA bacteria [8]. Other parts of mango, including the leaves, stems, and fruits, also have the potential for antioxidants. Several natural substances, including polyphenols, phenolic acids, and flavonoids, have been identified as effective free radical scavengers [9], [10]. The flesh, peel, and fruit of mangoes are rich in fiber, vitamins C and A, amino acids, and polyphenols, including mangiferin, quercetin, kaempferin[11]. Several studies reported the value of TPF, TFC, or antioxidant activity of mango peels. Toyibah and Taswin [12], showed that the antioxidant activity of mango peel var. arum manis extracted by the infusa method has a higher IC_{50} value than the maceration methods. Another study by Noviyanty *et al* [13] determined the total flavonoid content of the ethanolic extract of mangga peel var. arum manis with the maceration method showing levels of 3.27%. Safitri *et al* [14] also reported that the difference in mango varieties, such as arum manis and manalagi, affected the total phenolic and total flavonoid levels even though extracted with the same method and solvent, which is maceration with 70% ethanol. Research on mango peel var.arum manis, extracted by percolation method, concerning total phenolic content, total flavonoids, and free radical scavenging activity by DPPH method has never been reported. This study aims to determine the antioxidant activity of the ethanolic extract of mango peel (Mangifera indica L. var. arum manis) using the DPPH method and its Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) levels. The extraction method is percolation with 70% ethanol.

2 Methods

2.1 Materials

Dried mango peel, 70% ethanol, Quercetin (Sigma-Aldrich), $AlCl_3$, sodium acetate anhydrous, gallic acid (Sigma-Aldrich), Folin Ciocalteu reagent, Na_2CO_3, DPPH (Merck), methanol, distilled water.

2.2 Instruments

Percolator, separating funnel, rotary evaporator, measuring flask tube, test tube, water bath, filler, cuvet, UV-Vis spectrophotometer

2.3 Sample preparation

A dried mango peel powder (100 g) is put into a beaker glass, 500 ml of 70% ethanol, and rest for 3 hours. After soaking, the mixture was put into the percolator and left for 24 hours. The percolation substrate drips down and collects.

At the top, it is maintained that there is always a layer of solvent. The percolation process was stopped when the extracted liquid was clear. The extracted liquid was evaporated until form a thick extract, and the yield was calculated [15].

2.4 Determination of Total Phenolic Content

Total Phenolic content was determined by modifying the Folin-Ciocalteu method and using gallic acid as the standard [16]. The sample solution was made at 1,0 mg/mL with distilled water as a solvent. 0.1 ml of the sample solution was measured and put in a 10.0 ml measuring flask, and 7.9 ml of distilled water and 0.5 ml of Folin-ciaocalteu reagent, allowed to stand for 15 minutes, then 1.5 mL of Na_2CO_3 solution and incubated for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer at 760 nm. Gallic acid standards were prepared for samples with 60 -140 ppm concentrations. The total phenolic content in the ethanol extract was calculated equivalently to the gallic acid/gram sample (mg GAE/gram extract). This test was three replicated.

2.5 Determination of Total Flavonoid Content

The colorimetric approach with modified [14] determined the total flavonoid content. The concentration of the sample solution was 10,0 mg/mL, and then pipetted 0.5 mL, 1.5 mL methanol, 0.1 mL $AlCl_3$ solution, sodium acetate anhydrous 0.1 mL of 20%, and 2.8 mL of distilled water added. The mixed solution is put in a test tube and then homogenized. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 415 nm after 30 minutes of incubation. The concentration series of quercetin as standard is between 60–120 ppm. The total flavonoid content of the extract was expressed as mg quercetin equivalents (mg QE/gram of extract). This assay was conducted in triplicate.

2.6 Determination of DPPH radical scavenging activity

The antioxidant activity was determined using DPPH methods with modification [17]. 4.0 mL of 0.07 mM DPPH in a test tube. In the test tube, 200 µL of extract solution was added with a concentration of 0.3 mg/mL, 0.4 µg/mL, 0.5 mg/mL, 0.6 mg/mL, and 0.7 mg/mL. The mixture was homogenized and kept in a dark place for 30 minutes. The positive control was vitamin C with a 2-10 ppm concentration. At a wavelength of 517 nm, the absorbance of the solution and blank was measured, and the percentage of scavenging activity was estimated using the following formula equation 1.

$$\text{scavenging activity (\%)} = \frac{\text{Blank absorbance} - \text{sample absorbance}}{\text{Blank absorbance}} \times 100\% \quad \text{(Equation 1)}$$

Based on the percentage of scavenging activity data, the regression equation was determined from the concentration to the percentage of scavenging activity, and then the value of 50% inhibition (IC50) was calculated. The 50% inhibition (IC_{50}) describes the sample concentration that can scavenge 50% of DPPH free radicals.

3 Results and Discussions

The extraction method in this study was percolation because the process is simple and faster than maceration. Several studies reported that percolation yield was higher than other methods [18], [19]. Percolation is one of the cold extraction methods that can prevent degradation or damage to compounds. The extraction process in this percolation method is effective because the sample always flows with fresh solvent. This percolation method is more suitable for extracts containing thermolabile phytochemical compounds or compounds that are not heat stable [15]. The yield value indicates the amount of extract produced in units of a percent (%) by weight. The yield of dried mango peel extract was 38.0%, obtained from the extract weight of 38 g, and the simplisia was 100 g. The characteristic of the extract is a brown color and a distinctive odor. The higher the yield value indicates, the more extract is produced, and possibly more bioactive compounds are extracted [20][21].

The solvent used for extraction in this study is 70% ethanol. Similarly, research by [22] reported that 70 % of ethanol showed a higher phenolic content than 50% and 96% ethanol [22]. It may be that the complex phenolic in the

extract is more soluble in ethanol 70 % than others. In order to increase the solubility of phenol, the polarity of the solvent is crucial. Ethanol is an organic solvent suitable for extracting many secondary metabolites from plants; it is also cheap and low toxicity [23], [24].

The extracts were measured for total phenolic and flavonoid content and the radical scavenging activity using DPPH. The results of TPC, TFC, and antioxidant activity of mango peel ethanolic extract are presented in Table 1.

Table 1. Total phenolic content, total flavonoids of ethanolic extract of mango peel (*Mangifera indica* L. var Arum Manis).

Samples	TPC (mgGAE / g extract)	TFC (mgQE / g extract)
Replication 1	19.78	23.28
Replication 2	19.11	22.83
Replication 3	19.04	20.07
	19.31 ± 0.41	22.06 ± 1.74

The linear equation of gallic acid as a standard curve of TPC is y= 0,00402 x + 0.0095. The total phenolic content of mango peel extract, as shown in Table 1, was 19.31 mgGAE/g. Similar research conducted by [25] revealed that the total phenol content in mangoes of several varietals in India ranged from 48.9 to 63.0 mgGAE/g extract. Compared to Umamahesh's research, the total phenolic content in this study was lower; differences can influence this in varieties and growth places, which affect both the types and levels of secondary metabolites. According to [26], the total phenolic content of mango peel in nine varieties ranged from 462.2 – 4071 mgGAE/100 g fresh weight, which is quite high [26]. Phenolic compounds reported in mango peel include mangiferin, iso mangiferin gallate, mangiferin gallate, caffeic acid, ferulic acid, and gallic acid [27].

The total flavonoid content of mango peel extract was 22.06 mgQE/g. It is calculated based on the linear regression of the standard curve, y = 0.0031 x – 0.0157. In addition to the total phenolic value, the TFC value is influenced by several things, such as mango varieties, solvents used, and the selection of extraction methods. Therefore, the results of the TFC analysis may differ. Research by Safitri et al., 2023 [14] reported that the TFC from mango extract variety Arum Manis is 4,4071 mgQE/g lower than Manalagi 7,6601 mgQE/g. Their extraction method was maceration. Based on our results, the TFC values of mango extracts extracted by percolation showed higher values even in the same mango variety. It concluded that extraction methods affect the flavonoid content of the extract [28].

Flavonoid is one of the secondary metabolites that contribute as antioxidants. The flavonoid comprises 15 carbon atom skeletons and a C6-C3-C6 ring (A, B, and C) [29]. The hydroxyl group at C-3'-C-4' with the ortho position affects the antioxidant ability of the flavonoids [30], [31]. This research uses quercetin as a standard because it is widely contained in mango peel and seeds. Some flavonoid compounds in mango peel include quercetin, quercetin 3-arabinoside, quercetin 3-rhamnoside, rhamnetin-3-O-galactoside and kaempferol [32], [33].

The antioxidant activity of an extract can be measured using the DPPH radical scavenging method. This approach is easy, simple, quick, sensitive, and less samples. The percentage of inhibition expresses the radical scavenging activity. The IC_{50} value was calculated based on the linear regression equation obtained by plotting the concentration of samples with the inhibition percentage, as presented in Figure 1 below. The IC_{50} represents the sample concentration required to inhibit 50% of DPPH free radicals. The smaller the IC_{50} value, the higher the ability to scavenge free radicals.

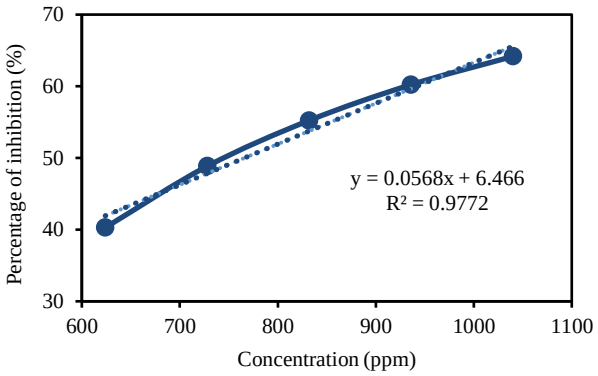

Figure 1. Graph of the relation between percentage inhibition and concentration

Based on Figure 1, the linear equation obtained is y = 0.0568 x + 6.466, and it can be concluded that as the concentration of the sample increases, the percentage of inhibition increases. According to the calculation of the $IC_{50,}$ the value of the arummanis mango peel extract is 766.444 ppm. The IC_{50} of Vitamin C is 6.414 ppm, calculated by regression linear y=7.8078x–0.0859. The criteria for antioxidants are: IC_{50} values <50 ppm are very strong, and 50-100 is a strong category. 100-150 is moderate, and if >150 ppm, it is weak [34]. Our results indicate that the antioxidant activity of arum manis mango peel is categorized as weak. It could be because the phenolic or flavonoid levels in the extract are low, affecting antioxidant activities. Another factor that may cause a weak antioxidant capacity is that flavonoids are in a state of binding to glycoside groups, where the glycosides reduce their activity [35].

The strength of the antioxidant activity of a sample can be associated with the presence of secondary metabolites extracted during the extraction process. Phenolics or flavonoids are secondary metabolites that are widely present in plants and are known to play a role in antioxidant activity. Phenolic compounds are reported to have antioxidant activity due to their oxidation-reducing properties. Furthermore, flavonoids can counteract free radicals by providing hydrogen atoms to free radicals [36]. There is a correlation between both groups of compounds and antioxidant activities, but the scavenging effect of the extract is not restricted to phenolics and flavonoids only. This activity may also derive from other secondary metabolites in the extract, such as essential oils, carotenoids, vitamins, or tannins [37]–[39].

Indeed, many plants have varying antioxidant capacities, and it is unclear which component is most responsible. However, many factors affect the results, including differences in the variety of samples, the extraction method and the levels of metabolites in the samples. Generally, the antioxidant activity increases with the amount of the compounds contained in the sample, but various methods of antioxidant activity analysis also showed different results. Rohmah [40] reported that the antioxidant activity of *Zingiber zerumbet* (L.) Roscoeex was determined using DPPH, FIC, FRAP, and ABTS methods, resulting in different IC_{50} values.

4 Conclusions

The results of this study suggest that the ethanol extract of mango peel (*Mangifera indica* L. var Arum manis) contains a total phenolic content 19.31±0.41 mgGAE/g extracts, total flavonoid content 22.06±1.74 mgQE/g extracts, and an IC_{50} value is 766.444 ppm.

5 Declarations

5.1 Acknowledgment

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5.3 Authors Contributions

The names of the authors listed in this journal contributed to this research.

5.4 Conflict of Interest

The authors declare no conflict of interest

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