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Quantitative Analysis of Phytochemicals and Antioxidant Activity in Vitro Ethanol Extract of *Jengkol* Fruit Peel (*Archidendron pauciflorum* (Benth.) I.C.Nielsen)

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ABSTRACT

Jengkol (Archidendron pauciflorum (Benth.) I.C.Nielsen) is a widely consumed crop in Indonesia, while its fruit peel is generally considered an agricultural waste and underutilized. This study aimed to evaluate the phytochemical content and in vitro antioxidant activity of an ethanol extract of jengkol fruit peel. Phytochemical screening was performed to identify the presence of flavonoids, total polyphenols, and tannins, while antioxidant activity was assessed using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. All experiments were conducted in duplicate, and the results were expressed as mean ± standard deviation. Data were analyzed using descriptive statistical analysis and linear regression to determine IC_{50} values. Phytochemical analysis showed that the extract contained flavonoids (0.23%), total polyphenols (28.82%), and tannins (3.83%). The ethanol extract of jengkol fruit peel showed very strong in vitro antioxidant activity, with an IC_{50} value of 6.3 ppm. The high antioxidant activity is likely attributable to the presence of phenolic compounds, which contribute to free radical scavenging by donating electrons or hydrogen atoms. However, this study was limited to in vitro evaluation using a single antioxidant assay method. Further studies employing additional antioxidant assays and in vivo assays are needed to more comprehensively evaluate the potential of jengkol fruit peel extract as an antioxidant.

INTRODUCTION

Oxidative stress can affect disease development. Oxidative stress contributes to a wide range of pathological conditions and diseases, including cancer, neurological disorders, atherosclerosis, hypertension, ischemia or perfusion, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and asthma. Oxidative stress occurs when the balance between antioxidants and ROS (Reactive Oxygen Species) is disrupted, either due to reduced antioxidant levels or ROS accumulation. Higher ROS production in the body can alter DNA structure, leading to protein and lipid

modifications, activation of stress-inducing transcription factors, and production of pro-inflammatory and anti-inflammatory cytokines (Birben et al., 2012). Therefore, antioxidants are important to prevent oxidative stress.

Antioxidants are molecules that can interact with free radicals and prevent the formation of reactive oxygen species before they damage important molecules (Notas et al., 2006). Phytochemical screening is conducted to identify the presence of secondary metabolites such as flavonoids, saponins, and phenolic compounds, which play a role as natural antioxidants. Previous studies have reported that plant extracts contain various bioactive compounds that contribute to antioxidant activity (Rahmawati et al., 2024; Yang et al., 2025).

Polyphenols have a conjugated structure with one or more sugar residues that bind to hydroxyl groups or are direct bonds between sugars (polysaccharides and monosaccharides) to aromatic carbons (Pandey & Rizvi, 2009). Polyphenols function as hydrogen donors and dampen singlet oxygen (Kähkönen et al., 1999). Flavonoids are benzo-γ-pyrone derivatives consisting of phenolic rings and pyran rings.

Flavonoids can deliver electrons to free radicals, catalyze metal chelation, activate antioxidant enzymes, reduce alpha-tocopherol radicals, and inhibit oxidase (Heim et al., 2002). Tannins can chelate metal ions such as Fe(II) and inhibit the Fenton reaction, thereby inhibiting oxidation (Amarowicz, 2007). These antioxidant compounds can be found in various natural sources, including plant extracts.

Archidendron pauciflorum (Benth.) I.C. Nielsen is a tropical plant native to Southeast Asia, including Indonesia (Barceloux, 2009). This species has been widely used in traditional medicine, particularly its leaves and stems for treating various ailments and its leaf ash for wound healing. Additionally, its immature cotyledon seeds are reported to exhibit blood-purifying, antidiabetic, and diuretic properties (Bunawan et al., 2013).

The skin of the *jengkol* fruit is thrown away because it is considered waste. However, based on research by Kamilawati (2015) on ethnobotanical studies in Karangwangi Village, Cianjur Regency, the people in this village use distilled water from the skin of *jengkol* fruit (*A. pauciflorum*), which has been dried, as a medicine for diabetes mellitus. It is suspected to be due to the presence of several phytochemicals in the *jengkol* fruit skin. According to Ilma et al. (2016), *jengkol* fruit peel contains several phytochemicals, including alkaloids, flavonoids, polyphenols, tannins, saponins, monoterpenes, sesquiterpenes, steroids, and triterpenoids. The phytochemical compounds in *jengkol* fruit skin are thought to support the antioxidant activity of the

jengkol fruit peel extract, which can help cure several diseases.

Although *jengkol* peel is known to contain various phytochemicals with potential antioxidant activity, quantitative analysis of phytochemical content and its relationship with antioxidant activity in *jengkol* peel extract remains limited. Most early research on medicinal plants typically reports only qualitative phytochemical screening to detect secondary metabolites. At the same time, quantitative analysis and evaluation of their biological activity remain relatively limited (Panche et al., 2016).

Antioxidant activity is generally expressed in the IC_{50} value, which is the concentration of a sample that can inhibit 50% of free radicals, where the IC_{50} value <50 µg/mL is categorized as very strong, 50–100 µg/mL strong, 100–150 µg/mL moderate, and >150 µg/mL is classified as weak (Molyneux, 2004). Therefore, this study was conducted to quantitatively analyze the phytochemical content and antioxidant activity of the ethanol extract of *jengkol* fruit peel using the DPPH method, providing scientific information on its potential use as a natural antioxidant source.

RESEARCH METHODS

Study Design

This study employed an in vitro experimental design to analyze the phytochemical content and antioxidant activity of ethanol extract derived from *jengkol* fruit peel (*A. pauciflorum*). A descriptive approach was applied to present and interpret the results of phytochemical screening and antioxidant activity assays based on the obtained values.

Location

The research was conducted at the Biosystems Laboratory of the Biology Study Program and the Chemistry Application and Service Laboratory, both in the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Padjadjaran University.

Procedure and Data Collection

Manufacture of Jengkol Fruit Peel Ethanol Extract

Jengkol fruit peel (*A. pauciflorum*) is extracted using the maceration method with 70% ethanol. The skin of *jengkol* fruit (*A. pauciflorum*) is washed and dried, then blended into the form of simplicia powder. Simplicia powder is placed in a bottle, then 70% ethanol is added at a 1:10 (w/v) ratio, and soaking is carried out for 3 x 24 hours. The obtained maserat is then filtered and concentrated using a rotary evaporator at 400 °C (Khan et al., 2012) until a paste is obtained.

Phytochemical Test of Ethanol Extract of Jengkol Fruit Bark

The phytochemical content of *jengkol* fruit peel extract was quantitatively analyzed, including flavonoids, polyphenols, and tannins.

Flavonoid Test

The total flavonoid content of *jengkol* fruit peel extract is determined according to the method of Meda et al. (2005). A total of 2 mL of extract solution is placed in a test tube and then added to 2 mL of 2% AlCl3 dissolved in ethanol, then vortexed. The solution is incubated at room temperature for 30 minutes. The absorbance of the extract is measured on a UV-Vis spectrophotometer at 415 nm. Quercetin is used as a standard solution.

Polyphenol Test

Spectrophotometric methods determine the total polyphenol content. A total of 1 gram of sample was soaked in methanol up to a volume of 100 ml, then homogenized in a shaker for 1 hour at room temperature at 400 rpm. The solution is centrifuged at 5900 rpm. The sample supernatant was diluted to 50 µl with Folin-Ciocalteu reagent, then incubated at 450 °C for 15 minutes with 2 ml of 7.5% Na2CO3. The absorbance of the sample was measured with a spectrophotometer at 765 nm (Singleton et al., 1999). Gallic acid is used as a standard solution.

Tannin Test

The total tannin content was determined by spectrophotometry. A total of 1 gram of sample was added to 80 ml of aqueduct and then refluxed for 30 minutes. The solution is added to 5 ml of Folin-Denis reagent, 10 ml of Na2CO3 35%, diluted to 100 ml with water, and homogenized. The solution was left to sit for 30 minutes, and its absorbance was measured at 760 nm with a spectrophotometer (AOAC, 1954). Tannic acid is used as a standard solution.

In Vitro Antioxidant Activity Test of Jengkol Fruit Peel Ethanol Extract

The antioxidant activity in vitro of ethanol extract of *jengkol* fruit peel was measured using the 2.2-diphenyl-1-picrylhydrazyl (DPPH) method (Blois, 1958). Antioxidant activity was tested using the DPPH method with two repetitions (n = 2) for each sample concentration and the positive control (ascorbic acid). A total of 5 mg of sample was added to 5 ml of methanol, yielding a sample stock solution at 1000 ppm.

The stock solution is diluted with methanol to obtain concentrations of 0, 200, 400, 600, and 800 ppm. Each of these solutions is added to 1 ml of DPPH solution at a concentration of 4 x 10^-4 M and left for 30 minutes. The absorbance of the sample was measured at 517 nm using a spectrophotometer (Blois, 1958). The formula for the percentage of inhibition against DPPH radicals is as follows.

$$\%\ \text{inhibition} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100\%$$

After obtaining the inhibition percentage for each concentration, a linear regression is performed using the equation y

= A + Bx, where X is the concentration (µg/mL), and Y is the inhibition percentage (%). Antioxidant activity is expressed as the Inhibition Concentration of 50% (IC_{50}), which is the concentration of a sample that reduces DPPH radicals by 50%. The IC_{50} value is determined by linear regression of the curve.

Data Analyze

The data were presented as mean ± standard deviation and analyzed descriptively to compare the antioxidant activity of *jengkol* fruit peel extract with the positive control (ascorbic acid). A regression curve was constructed to estimate the IC_{50} values. The validity of the curve was evaluated using the coefficient of determination (R^2).

RESULTS AND DISCUSSION

Archidendron pauciflorum (Benth.) I.C.Nielsen) belongs to the Fabaceae family, with a tree height of 18-25 meters. The leaves are double-pinned, reaching up to 25 cm in length. The stems are smooth and gray. The fruit of this tree is crescent-shaped, round, dark purple in color, 20-25 cm long by 4-5 cm wide, and one *jengkol* fruit contains 3-9 seeds. The crushed fruit will produce a sulfur smell. This species is native to tropical regions, namely Southeast Asia, including Malaysia, Myanmar, Southern Thailand, and Indonesia (Barceloux, 2009).

Parts of *A. pauciflorum* have been widely utilized in the health field. Crushed leaves and stems are traditionally used to treat toothache, gum pain, chest pain, and skin diseases, while ash from burned young leaves is applied as a wound remedy. In addition, immature cotyledon seeds are reported to function as blood purifiers, exhibit antidiabetic properties, and stimulate urine production (Bunawan et al., 2013).

The phytochemical content of *jengkol* peel extract measured included total flavonoids, polyphenols, and tannins. The percentage of phytochemicals is calculated by comparing the sample with the standard solution used. The measurement results are shown in **Table 1.**

The results of the phytochemical test (**Table 1**) show that the ethanol extract of *jengkol* fruit peel has a higher polyphenol content than flavonoids and tannins. This is consistent with the study by Muslim et al. (2012), which reported that the ethanol extract of *jengkol* fruit peel (*Pithecellobium jiringa*) contains a total polyphenol content of 143.95 mg/g (14.3%). The total flavonoid content in the extract was much lower, at 2.21 mg/g (0.22%).

Table 1. Phytochemical levels of ethanol extract of *jengkol* fruit peel

No.	Phytochemical Parameters	Rate (%)
1	Flavonoids	0,23
2	Polyphenols	28,82
3	Tannins	3,83

Table 2. IC_{50} value of *jengkol* fruit peel ethanol extract

Concentration (ppm)	% Inhibition	IC_{50} (ppm)
0	0	6.35 ± 0.07
2	15.38 ± 0.69	
4	32.32 ± 0.21	
6	47.95 ± 0.54	
8	62.51 ± 1.32	

Table 3. IC_{50} ascorbic acid value

Concentration (ppm)	% Inhibition	IC_{50} (ppm)
0	0	5.25 ± 0.07
1	10.15 ± 0.51	
3	28.82 ± 0.69	
5	47.76 ± 1.25	
7	66.88 ± 0.79	

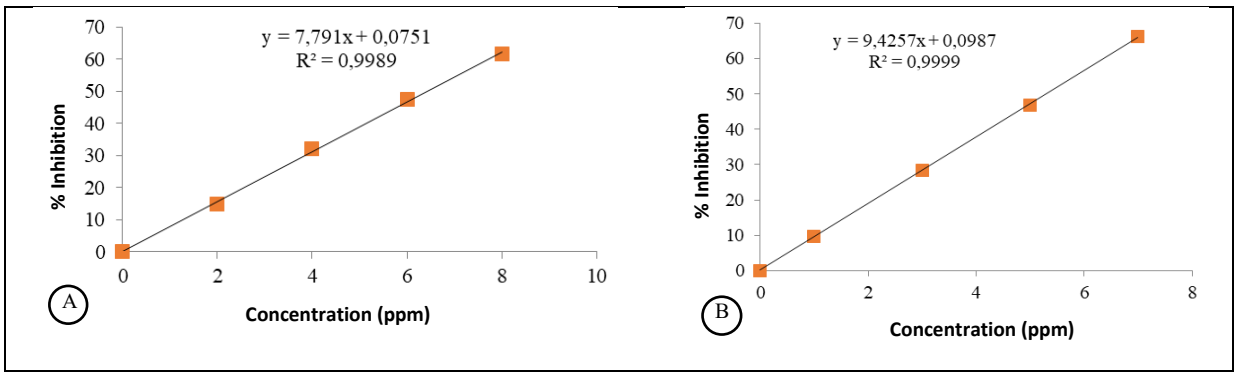

Figure 1. DPPH inhibition curves. A. *Jengkol* fruit peel extract; B. Ascorbic acid

Several studies have shown that extracts from Fabaceae plants contain high levels of polyphenols. Examples include *Genista quadriflora* (Lrhorfi et al., 2016) and *Retama monosperma* (Belmothekar & Harche, 2014). Other species with notable polyphenol content are *Galega officinalis* and *Astragalus glycyphyllos* (Kiselova et al., 2006).

The phenolic content obtained during extraction depends on the solvent used and its polarity. Medini et al. (2014) reported that *Limonium delicatum* extract showed different total polyphenols, flavonoids, and tannins across solvents, and that each solvent was able to extract different types of phytochemicals. According to Suzuki et al. (2002), ethanol and methanol, which have a water content of 40-80%, are more efficient at extracting polyphenol compounds than water or pure ethanol or methanol.

The antioxidant activity of *jengkol* fruit peel extract (*A. pauciflorum*) was carried out in vitro using the DPPH method. Based on the measurement results, the IC50 values of *jengkol* fruit peel extract (**Table 2**) and ascorbic acid (**Table 3**) were obtained for comparison. The IC_{50} value was determined through linear regression analysis of the percent inhibition comparison curve to the concentration of ethanol extract of *jengkol* fruit peel and to ascorbic acid (**Figure 1**).

The in vitro DPPH (2,2-diphenyl-1-picrylhydrazyl) assay measures antioxidant activity based on the ability of compounds to donate electrons or hydrogen atoms to stabilize DPPH radicals (Blois, 1958). Antioxidant activity is expressed as IC_{50},

defined as the concentration required to inhibit 50% of DPPH radicals. Lower IC_{50} values indicate stronger antioxidant activity (Molyneux, 2004).

Based on the results of the study, the ethanol extract of *jengkol* fruit peel had an IC_{50} value of 6.3 ppm (**Table 2**), while ascorbic acid had an IC_{50} value of 5.2 ppm (**Table 3**). The extract is considered a very strong antioxidant if it has an IC_{50} value < 50 ppm, a strong antioxidant if 50 < IC_{50} < 100 ppm, and a weak antioxidant if IC_{50}> 100 ppm (Kuete & Efferth, 2010). *Jengkol* peel ethanol extract and ascorbic acid have an IC_{50} < 50 ppm value, so *jengkol* peel ethanol extract is included in the category of very strong antioxidants, in the same category as ascorbic acid.

Based on the results of phytochemical tests and DPPH antioxidant activity tests, *jengkol* fruit peel ethanol extract has strong antioxidant potential, supported by its polyphenol, flavonoid, and tannin content. The results of the study by Rebaya et al. (2014) showed that the phytochemical content in the ethanol extract *of Halimium halimifolium* leaves, namely flavonoids, phenols, and tannins, was directly proportional to antioxidant activity (IC_{50}) measured by the in vitro DPPH method.

Medicinal plants with antioxidant activity can help reduce the formation of free radicals and increase endogenous antioxidant levels (Katalinic et al., 2006). The presence of phenolic compounds in plant extracts plays an important role in neutralizing free radicals through a radical-capture mechanism that involves donating electrons or hydrogen atoms from the phenolic group, thereby stabilizing free radicals and decreasing their reactivity (Platzer et al., 2022). However, the antioxidant activity obtained from in vitro testing has not fully represented a complex biological condition.

In vitro antioxidant activity testing has methodological limitations that may affect the results obtained. One factor that can cause bias in the DPPH test is the reaction conditions, such as the concentration of DPPH reagents, incubation time, and the wavelength of absorbance measurements. Variations in these conditions can affect the level of DPPH radical reduction by antioxidant compounds, resulting in different inhibition values (Molyneux, 2004).

In addition, the type of solvent used in the extraction process can affect the composition of the bioactive compounds extracted, as phenolic compounds and flavonoids differ in their solubility in certain solvents (Shahidi & Ambigaipalan, 2015). Another factor that can also affect the results is the limited number of test replications, so the variation in the resulting data may not fully represent the biological variation of the sample. Therefore, the results of this study

need to be interpreted as a preliminary description of the potential antioxidant activity of *jengkol* fruit peel extract. Further research is needed with a more comprehensive approach, including the use of multiple antioxidant testing methods, better to understand the antioxidant potential of *jengkol* peel extract.

CONCLUSION

Ethanol extract of *jengkol* fruit peel (*A. pauciflorum*) has a flavonoid content of 0.23%, polyphenols 28.82%, and tannins of 3.83%. It has the potential to be a very strong antioxidant in vitro with an IC_{50} value of 6.3 ppm. The results of this study can serve as a scientific basis for evaluating the potential of *jengkol* fruit peel as a natural antioxidant alternative. Further research is needed to identify and isolate key bioactive compounds and evaluate their activity using in vivo assays.

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