Metabolite Bioactive Contents of Parkia timoriana (DC) Merr Seed Extracts in Different Solvent Polarities

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ABSTRACT

Although Parkia timoriana (DC). Merr seeds have popular folkloric ethnomedicinal in Indonesia, to treat colic, cholera, for the treatment of spasms during menstruation, and as a stomach-strengthening. This research focuses on finding metabolite bioactive contents in different solvent polarities, such as water, methanol, ethyl acetate, and hexane. The total phenol content was analyzed by the Follin-Ciocalteu method and found to be in the range of 43.82 to 137.42 mg GAE/g. Total flavonoid compounds were measured by the aluminum chloride colorimetric method and were obtained in the range of 20.42 to 45.90 mg QE/g. The alkaloid, saponin, tannin, terpenoid, and cardiac glycoside contents were measured by Spectrophotometry UV-Vis and found 16.34-48.90 mg CoE/g; 1.76-16.04 mg DE/g; 0.21-7.29 mg TA/g; 50.12-91.02 mg Linalool Equivalent/g and 7.24-36.53 mg DXE/g, respectively. The extracts were investigated for antioxidant activities by DPPH (2,2-diphenyl-1-picrylhydrazyl) free-radical and ABTS (2,2’ azinobis (3-ethylbenzene-thiazoline-6-sulfonic-acid) scavenging ability. The methanol extract was found to have the highest antioxidant activity with the value of IC\textsubscript{50} 28.13µg/ml for DPPH and 45.39 µg/ml for ABTS. Antimicrobial and antifungal activities were performed against at methanol extract Escherichia coli (24 mm) and Candida albicans (16 mm). Antidiabetic activities were in vitro assayed by α-amylase inhibition and α-glucoside inhibition. The methanol extract have IC\textsubscript{50} α-amylase inhibition (25.35 µg/ml), and IC\textsubscript{50} of α-glucoside inhibition (23.04 µg/ml). Overall, P. timoriana seed contains secondary metabolites which are good candidates as lead compounds for the development of potent drugs.

1. Introduction

World Health Organization (WHO) has confirmed that medicinal plants could be the best source for finding drugs (Gislene et al. 2000). The use of various medicinal plants to treat various diseases and to promote healthcare delivery system have received increasing attention. Various parts of plants such as seeds, flowers, stems, roots, seeds, fruit, and bark contain metabolite bioactive compounds as antioxidants, antidiabetics, antihyperlipidemics, antihyperglycemics, antibacterials, and antifungals (Sivakumar and Balasubramanian 2020; Suryanti et al. 2020). Antioxidants are capable of stabilizing or deactivating free radicals (Argaloo et al. 2004; Jaafar et al. 2017). Free radicals are highly reactive chemicals that can damage healthy cells and cause disorders, illnesses, and a variety of disorders (Mitra 2020). Cell destruction caused by free radicals is a major cause of aging, weakening of immune system and brain function, cancer, and heart disease. Antidiabetic, antihyperlipidemic, and antihyperglycemic effects of plant secondary metabolites are attributed to their abilities to restore the function of pancreatic tissues by increasing insulin output or inhibiting the intestinal absorption of glucose (Malviya et al. 2010). Medicinal plants also have been used as antimicrobial agents (Abioye et al. 2013; Das et al. 2010). Derived bioactive compounds from medicinal plants demonstrate safe...
antimicrobial and antifungal agents (Mickymaray 2019).

Indonesia has biodiversity medicinal plants which need to be fully explored for properly utilized. *P. timoriana* is a tropical multipurpose leguminous which belongs to the family Mimosaceae. In Indonesia, *P. timoriana* is known locally as Kedawung and its seed is widely used as an herb. *P. timoriana* seed has a popular folkloric ethnomedicinal for the treatment of colic, cholera, spasms during menstruation, and stomach strengthening (Sumarni et al. 2021). Moreover, Malaysians consumed *P. timoriana* pods to treat kidney disorders, urinary tract infections, hypertension, and headache (Angami et al. 2018). In spite of having long ethnomedicinal history, research on *P. timoriana* is very limited. Ethyl acetate and methanol crude extract of pods possess anti-diabetic compounds which inhibited -amylase and -glucosidase. (Sheikh et al. 2016). Methanol extracts of seeds of *P. timoriana* contain a higher amount of total phenolic and flavonoid content. It has antioxidant activity through the inhibition of free radicals DPPH and ABTS. Furthermore, it has antibacterial activity against *Bacillus pumilus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Ralte et al. 2022). This work studies metabolite bioactive contents of *P. timoriana* seeds in different solvent polarities and evaluate their antioxidant, -glucosidase, and -amylase inhibition, antidiabetic and antimicrobial activities.

2. Materials and Methods

2.1. Chemicals

The chemicals used in this study were dimethyl sulfoxide (DMSO), n-hexane, methanol, ethyl acetate, Potassium iodide, ferric chloride, hydrochloric acid (HCl), sulfuric acid (H₂SO₄), chloroform, glacial acetic acid, and media cultures (nutrient agar and nutrient broth), which obtained belonging to Merck. DPPH was obtained belonging to TCI (Tokyo Chemical Industries) Tokyo, Japan. Gallic acid was obtained belonging from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Microbial Culture Conditions

2.2.1. Bacterial

Bacteria livestock of *Bacillus subtilis* NBRC 3009, *Escherichia coli* NBRC 3301, *Staphylococcus aureus* NBRC 102135, *Pseudomonas aeruginosa* NBRC 3080, *Salmonella typhi* NBRC 14193, *Propionibacterium acne* NBRC 111530, and *Porphyromonas gingival* NBRC 115147 (NITE Biological Resources Center, NBRC; Chiba, Japan) taken from the Collection of Microbial Chemistry Laboratory, Department of Chemistry, Institut Ilmu Kesehatan Bhakti Wiyata Kediri, in 100-ml Erlenmeyer flasks, the colony was cultured into 60 ml of nutrient broth (NB, Merck, Darmstadt, Germany) medium. The cultivation was pre-incubated for 20 hours at 37°C with a shaker (Sariwati et al. 2017).

2.2.2. Fungal

Fungal livestock of *Candida albicans* NBRC 0197, *Aspergillus niger* NBRC 5376, *Aspergillus flavus* NBRC 4186, and *Aspergillus fumigatus* NBRC 4057 (NITE Biological Resources Center, NBRC; Chiba, Japan), which taken from the Collection of Microbial Chemistry Laboratory, Department of Chemistry, Institut Ilmu Kesehatan Bhakti Wiyata Kediri was cultured on 9-cm in a petri dish containing potato dextrose agar (PDA, Merck, Darmstadt, Germany) that had been cultivated at 37°C. The colony was injected inside 60 mL of nutrient broth (NB, Merck, Darmstadt, Germany) medium in 100-ml Erlenmeyer flasks. The cultivation was pre-incubated for 20 hours at 37°C with a shaker set to 180 rpm (Sariwati et al. 2018).

2.3. Plant Materials and Sample Preparation

Collected plant materials in Kediri, East Java, Indonesia. *P. timoriana* seeds were cleaned with purified water and chopped on a small scale before being drained overnight at room temperature and ground to a particle mass of 25 mesh using a grinder.

2.4. Extraction of *P. timoriana* Seeds

A 500 ml flask was filled with dried powder samples (20 g), which were then filled with 200 ml of (methanol, water, n-hexane, dichloromethane, and ethyl acetate) and securely wrapped in aluminum foil. Following extraction, the resipites were refined using Whatman No 1 filter paper and an orbital shaker at 180 rpm for 24 hours. To obtain drained extracts, the extracts were dried in a rotatory evaporator at a set temperature under the boiling point of the solvent (Santoso et al. 2021). The boiling point of n-hexane was 68°C, 100°C for water, 77°C for ethyl acetate, and 65°C for methanol. The solvent-free extracts were transferred to extraction vials and stored at 4°C for future use (Sariwati et al. 2019).

2.5. Preliminary Phytochemical Ccreening of *P. timoriana*

*P. timoriana* was phytochemically assessed for the presence of alkaloids, saponins, tannins, flavonoids, and triterpenoids by treating seeds with different extracts using a standard procedure (Amalia and Sariwati 2019).
2.6. Total Phenolic Contents

*P. timoriana* extracts (20 mg) were preserved in a solvent of 5 ml of 3% HCl in 60% methanol, and the involved alloy (100 µl) was added to 2 ml of aqueous sodium carbonate solvent. After 3 minutes, the alloy was treated with 100 µl of Follin-Ciocalteu phenol reagent. After 30 minutes of incubation, the absorbance at 750 nm was measured in comparison to a blank. In a similar procedure, gallic acid concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 mM were used as calibration curves and the results were reported as gallic acid equivalents (GAE) mg per gram of extract (Sariwati et al. 2019).

2.7. Total Alkaloid Content

5 ml of pH 4.7 phosphate buffer and 5 ml of BCG (Bromocresolgreen) solution were added to 1 ml of extract. The mixture was then vigorously shaken with chloroform before being collected in a 10 ml volumetric flask and diluted with chloroform. In the same manner, as previously described, a set of colchicine reference standard solutions was prepared. A UV–VISble spectrophotometer was used to measure the absorbance of test and standard solutions against the reagent blank at 470 nm. The total alkaloid content was measured in milligrams of colchicine equivalent per gram (mg CoE/g) (Umdale et al. 2021).

2.8. Total Flavonoid Contents

The total flavonoid contents (TFC) of each extract were studied under the aluminum chloride colorimetry procedure proclaimed by John et al. 2014. In brief, the crude extracts (1 ml) and the calibration curve were processed by (20, 40, 60, 80, and 100 µg/ml) were alloyed while using 4 ml of demineralization water in a 10 ml volumetric flask. To the flask was poured 0.30 ml 5% sodium nitrite. The mixture was permitted to hold for 5 minutes and 0.3 ml 10% aluminum chloride was top-up thereafter. Beyond 5 minutes, 2 ml sodium hydroxide 1M was mixed and the volume was concocted to 10 ml by using demineralization water. The suspension was alloyed and absorbance was determined in comparison to the blank at 510 nm. Entire flavonoid compounds were then represented as milligram quercetin equivalent (QE) per 100 g dry weight (DW)(Sariwati et al. 2022).

2.9. Total Saponin Content

A 250 µl sample (1 mg/ml) was combined with 250 µl vanillin (8 g/100 ml ethanol) and topped off with 2.5 ml sulfuric acid (72 percent). The mixture was heated for 10 minutes at 60°C before being cooled for 5 minutes in an ice-water bath. A UV-vis spectrophotometer was used to measure the absorbance of the mixture at 544 nm. To create a calibration curve, diosgenin (5.7–71.4 mg/L) was used as the standard chemical. The results are given in milligrams of diosgenin equivalent per gram of sample (mg DE/g) (Chua et al. 2019).

2.10. Total Tannic Acid Content

A 1 ml reagent mixture was added to approximately 200 µl sample-extracted tannic acid standard solution (50-300 g/ml) (4% vanillin in methanol and 8% concentrated HCl in methanol, 1:1 ratio). After incubating for 20 minutes at room temperature, the color developed was measured at 500 nm. The results were expressed in mg TAE/g (Umdale et al. 2021).

2.11. Total Terpenoid Content

As an unknown sample, a 200 µl extract was taken. A standard curve was created using linalool (40 to 100 mg/ml). Each tube received 1.5 ml of chloroform, which was thoroughly mixed and allowed to rest for 3 minutes. Each tube received 100 µl of concentrated H₂SO₄ and was incubated for 10 minutes. Terpenoids precipitated as a dark brown precipitate. The precipitate was dissolved in 1.5 ml methanol after the supernatant was carefully decanted. Absorbance was measured in a spectrophotometer at 538 nm against methanol. The assay was repeated three times, and the concentration was expressed as mg linalool/g extract (Tandon and Gupta 2020).

2.12. Total Cardiac Glycoside

1 ml of extract solution was combined with 1 ml of freshly made Baljet’s reagent (95 ml of 1 percent picric acid was mixed with 5 ml of 10 percent sodium hydroxide solution). After 60 minutes at room temperature, the reaction mixture was diluted with purified water (2 ml), the absorbance at 482 nm was measured, and the content was calculated using a calibration curve of digoxin in the range of 5–50 mg/ml. The total cardiac glycoside content was expressed as mg digoxin equivalent per gram of extract (mg DYE/g extract) (Komonlakorn et al. 2020).

2.13. Antioxidant Activity

2.13.1. Antioxidant Activity (DPPH Radical Scavenging Activity)

The assortment suspension was arranged by mixing 24 mg DPPH by using 100 ml methanol, and measured absorbance at 517 nm applying a spectrophotometer. One milliliter of stock DPPH solution was blended with 33 µl of *P. timoriana* seeds extracts at diverse concentrations (10–100 µg/ml). After shaking the mixture to react, store it in the dark for 20 minutes at room temperature. The
control gallic acid (10-100 µg/ml) was exited as devoid of sample extracts with range concentration. The scavenging capacity was assessed bottom on the proportion DPPH radical scavenger as the further on the equation:

\[
\text{Inhibition radical scavenging (\%)} = \frac{[\text{Control absorbance} - \text{Sample absorbance}]}{\text{Control absorbance}} \times 100
\]

The IC\(_{50}\) values meaningful the concentrate of sample requisite to scratch 50% of the DPPH free radical were measured by systematizing IC\(_{50}\) = \(\frac{50 - a}{b}\),

the value of \(a\) and \(b\) was standardized response pattern by using a variable slope (Sariwati et al. 2019). The IC\(_{50}\) value was calculated using a linear regression equation between the percentage of inhibition and the concentration of the sample.

### 2.14. Antidiabetic

#### 2.14.1. Alfa Amylase Inhibition

The 3,5-dinitrosalicylic acid (DNSA) method was used for the \(\alpha\)-amylase inhibition assay. *P. timoriana* seed extract was dissolved in a minimum amount of 10% DMSO and then in buffer ((Na\(_2\)HPO\(_4\)/NaCl,PO\(_4\) (0.02 M), NaCl (0.006 M) at pH 6.9, to give concentrations ranging from 10 to 1000 µg/ml. A 200 µl \(\alpha\)-amylase solution (2 units/ml) was mixed with a 200 µl extract at one concentration (20-100 µg/ml) and incubated at 30°C for 10 minutes. Following that, 200 µl of starch solution (1% in water (w/v)) was added to each tube and incubated for 3 minutes. The reaction was terminated by the addition of 200 µl DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 ml of 2 M NaOH and 20 ml of 96 mM of 3,5-dinitrosalicylic acid solution) and boiling for 10 minutes in an 85–90°C water bath. The mixture was cooled to room temperature and diluted with 5 ml of distilled water before being measured with a UV-Visible spectrophotometer at 540 nm. By replacing the plant extract with 200 µl of buffer, a blank with 100% enzyme activity was created. In the absence of the enzyme solution, a blank reaction was prepared using the plant extract at each concentration. The positive control acarbose (20-100 µg/ml) was exited as devoid of sample extracts. The \(\alpha\)-amylase inhibitory activity was expressed as a percentage inhibition and was calculated using the equation given below: The graph was used to calculate the IC\(_{50}\) values by plotting the percent \(\alpha\)-amylase inhibition against the extract concentration (Wickramaratne et al. 2016).

\[
\text{\(\alpha\)-amylase inhibition (\%)} = \frac{[\text{Control absorbance} - \text{Sample absorbance}]}{\text{Control absorbance}} \times 100
\]

The IC\(_{50}\) values meaningful the concentrate of sample requisite to scratch 50% of the \(\alpha\)-amylase inhibition were measured by systematizing IC\(_{50}\) = \(\frac{50 - a}{b}\), the value of \(a\) and \(b\) was standardized response pattern by using a variable slope (Sariwati et al. 2019). The IC\(_{50}\) value to indicate antioxidant capacity was calculated by linear regression equation between the percentage of inhibition and the concentration of the sample.

#### 2.14.2. Alfa Glucosidase Inhibition

In 0.1 M phosphate buffer with a pH of 6.8, a stock solution of the \(\alpha\)-glucosidase enzyme (0.5 U/ml) was prepared and diluted to the required
concentration in the same buffer for the assay. Alpha-glucosidase inhibitory activity was measured spectrophotometrically with a 96-well microplate reader using nitrophenyl-D-glucopyranoside (PNPG) as a substrate according to the preincubation procedure described above (Kumar et al. 2013; Sheikh et al. 2016). Briefly, 25 µl of enzyme solution (0.5 U/ml of α-glucosidase in 0.1 M phosphate buffer, pH 6.8) was mixed with 25 µl (20-100 µg/ml) of the test sample and incubated for 10 minutes at 37°C. The mixture was then incubated for 30 minutes at 37°C with 25 µl of PNPG solution (0.5 mM PNPG in 0.1 M phosphate buffer, pH 6.8). The reaction was stopped by adding 100 µl of 0.2 M Na₂CO₃ solution, and the absorbance was measured at 405 nm using a multi-well plate reader. The positive control was acarbose (20-100 µg/ml), while the negative control was the uninhibited enzyme (DMSO control). The test was performed in three separate experiments (Sheikh et al. 2016).

\[
\text{α-glucosydase inhibition} = \frac{\text{[Control absorbance - Sample absorbance]}}{\text{Control absorbance}} \times 100
\]

The IC₅₀ values meaningful the concentration of sample requisite to scratch 50% of the ABTS free radical were measured by systematizing IC₅₀ = \frac{50 - a}{b} value of a and b was standardized response pattern by using a variable slope (Sariwati et al. 2019). The IC₅₀ value to indicate antioxidant capacity was calculated by linear regression equation between the percentage of inhibition and the concentration of the sample.

2.15. Antimicrobial Activity
2.15.1. Antibacterial

The antibacterial screening was carried out using the agar disk diffusion method with the width of restriction areas calculated using P. timoriana extract against various bacterial strains (Bacillus subtilis NBRC 3009, E. coli NBRC 3301, Salmonella typhi NBRC 14193, Propyphromonas gingival NBRC 115147 as gram-negative bacteria, Staphylococcus aureus NBRC 102135, Propionibacterium acne NBRC 111530 and Pseudomonas aeruginosa NBRC 3080 as gram-positive bacteria. Individual Petri plates were filled with sterilized nutrient agar (NA), which was then inoculated with a 100 µl suspension of the investigated bacteria. 5 mm discs of Whatman filter paper were prepared and were adsorbed in a 100 µl extract solution DMSO (10 mg/ml) (Sariwati et al. 2019). Ampicillin (10 mg/ml) was commonly used as a positive control. For maximum bacterial growth, the plates were cultured at 37°C for 24 hours. The antibacterial capacity was determined by measuring the width (mm) of restriction zones with an area reader (Sariwati et al. 2019).

2.15.2. Antifungal

The antifungal screening was executed by the agar disk diffusion procedure with the calculation of the width of restriction areas calculated using P. timoriana extract against various bacterial strains (Candida albicans NBRC 0197, Aspergillus niger NBRC 5376, Aspergillus flavus NBRC 4186, and Aspergillus fumigatus NBRC 4057). Using a sterile syringe, 5 ml each concentration of P. timoriana seed extract (10 mg/ml) was first poured into different Petri-dishes. Ketoconazole (10 mg/ml) was commonly used as a positive control. In addition, sterile Potato Dextrose Agar (PDA) was added to the plates containing the solvent extracts, following that, the plates containing the solvent extracts were gently swirled to ensure proper mixing. Solidify the medium and use a sterile cork drill (5 mm diameter). A matured culture disc was punched out from the advancing margin of a four-day-old pure culture and inoculated at the center of plates, which were then incubated at room temperature (28°C) for 7 days. The experiment was repeated three times. The area of inhibition was measured and recorded daily for 7 days using a meter rule (Akwaji et al. 2016).

2.16. Statistical Analysis

The values were the average of the triplet calculation. During substrate transformation, a student's t-test was used to determine some representational dissimilarity between or within groups. Dissimilarity among procedures was calculated to be statistically representative at a courage level of 5% (P 0.05) by using Excel software.

3. Results
3.1. Phytochemical

The presence of alkaloid, flavonoid, steroid, tannins, terpenoid, saponin and cardiac glycoside was discovered in the phytochemical screening of four extracts of P. timoriana seeds. The presence of alkaloid, flavonoid, steroid, tannins, terpenoid, saponin and cardiac glycoside was discovered in the phytochemical screening of four extracts of P. timoriana seeds. Reducing sugar presence only in the distilled water extract. Anthraquinones was only absent in the ethyl acetate extract shown in Table 1, respectively.

The total phenol content of the methanol extract was the highest (137.42 mg GA/g), an alkaloid (48.90
mg CoE/g, tannin (7.29 mg TAE/g, flavonoid 45.90 mg QE/g, saponin 16.04 mg DE/g and cardiac glycoside 36.53 mg DXE/g. On n-hexane extract, terpenoid had the highest value 91.02 mg linalool Equivalent/g. The greatest result of extraction on methanol solvent with a value of 22.43 mg. This result is shown in Figure 1, respectively.

3.2. Antioxidant Activities

Antioxidant activity can be assessed by observing the reaction with the stable radicals DPPH and ABTS. Figure 2 showed the percentage of DPPH and ABTS scavenging at various concentrations ranging from 10 to 100 µg/ml. The IC₅₀, which is a widely used parameter to measure antioxidant activity and represents the amount of antioxidant required to reduce the initial DPPH concentration by 50%, was calculated using linear regression from a standard curve and expressed as the concentration (µg/ml) of the sample extract used in the test. Methanol extract has the highest antioxidant potential with the lowest IC₅₀ concentrations of DPPH (28.13 µg/ml) and ABTS scavenging (45 µg/ml) shown in Table 2.

3.3. Antidiabetic Activity

Figure 3 shows the α-glucosidase and α-amylase inhibitory potencies of crude methanol, distilled water, ethyl acetate, and n-hexane extract. Crude methanol extract has the highest antidiabetic potential with the lowest IC₅₀ concentrations α-glucosidase 23.04 µg/ml and α-amylase 25.35 µg/ml potencies were observed and shown in Table 2.

3.4. Antimicrobial

Table 3 shows the results of antimicrobial tests with the four extracts against bacteria and fungi. Methanol extract demonstrated the greatest zone inhibition of almost all bacterial and fungal pathogens. To state that the extract was able to inhibit bacteria. Escherichia coli, and Staphylococcus aureus with an inhibition zone diameter of 24 mm with categorized very strongly. Candida albicans had the largest inhibition zone diameter of 16 mm with categorized strong.

4. Discussion

Plants, in fact, synthesize chemical compounds for their own needs in order to adapt to their surroundings (Millogo-Kone et al. 2008). To determine the effect of solvent on phytochemical contents, phytochemical screening of the test plant Parkia timoriana seeds with four different solvent
Figure 2. Percentage of scavenging radical DPPH and ABTS by different extract seeds of *Parkia timoriana* (DC.) Merr

![Graph showing scavenging radical DPPH and ABTS by different extract seeds of *Parkia timoriana* (DC.) Merr]

Table 2. IC$_{50}$ of Antioxidant and antidiabetic activity of different extracts of *Parkia timoriana* (DC.) Merr seeds

<table>
<thead>
<tr>
<th>Extract solvent</th>
<th>Antioxidant IC$_{50}$ µg/ml</th>
<th>Antidiabetic IC$_{50}$ µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPPH</td>
<td>ABTS</td>
</tr>
<tr>
<td>Methanol</td>
<td>28.13±0.68&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>45.39±0.45&lt;sup&gt;aA&lt;/sup&gt;</td>
</tr>
<tr>
<td>Distilled water</td>
<td>37.56±0.17&lt;sup&gt;bB&lt;/sup&gt;</td>
<td>54.86±1.50&lt;sup&gt;bB&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>60.22±1.14&lt;sup&gt;cC&lt;/sup&gt;</td>
<td>83.40±2.10&lt;sup&gt;cC&lt;/sup&gt;</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>102.76±0.20&lt;sup&gt;dD&lt;/sup&gt;</td>
<td>124.39±0.25&lt;sup&gt;dD&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Figure 3. Percentage of α-Amylase and α-Glukosyde inhibition and by different extract seeds of *Parkia timoriana* (DC.) Merr

![Graph showing α-Amylase and α-Glukosyde inhibition by different extract seeds of *Parkia timoriana* (DC.) Merr]
extractions was used in this study. The presence of alkaloid, flavonoid, steroid, tannins, terpenoid, saponin and cardiac glycoside was discovered as a result of phytochemical screening. Coumarin and anthocyanins were completely absent from the extract. This result is not different significantly from the previous studies by at methanol extract of *P. timoriana* seeds only possess alkaloids, flavonoids, tannins, saponin, terpenoids (Ralte et al. 2022). Reducing sugar presence only in the ethyl acetate extracts indicated in the Tables 1. Extraction of metabolites using a different solvent extraction method is also important in terms of the number of metabolites extracted (Rafi et al. 2018). The result of Quantitative metabolites secondary was found in Figure 1.

The highest flavonoid content of *P. timoriana* was found in the methanol extract, which was 45.90 mg/g. This is because flavonoid compounds have hydroxyl groups, so they will easily dissolve in polar compounds such as methanol (Dwicahyani et al. 2021). Methanol is a compound that has both polar and nonpolar compounds (Wardoyo et al. 2017). Methanol is universal so it can dissolve all kinds of compounds, including the small intestine (Cinelli and Jones 2021). Alkaloids are plant-friendly chemicals with molecular structure of CH₃OH, is polar because it has a methyl group (-CH₃) (Ramdani et al. 2017). Methanol is universal so it can dissolve both polar and nonpolar compounds (Wardoyo et al. 2021). Alkaloids are plant-friendly chemicals that act as a deterrent to predators and parasites. Alkaloids have been noted to possess antimicrobial, antihtypertensive, antifungal, anti-inflammatory, anti-fibrogenic effects as well as anti-diarrheal effects which may be related to their action on the small intestine (Cinelli and Jones 2021).

The greatest tannic acid in methanol extract of *P. timoriana* seeds as much as 48.90 mg/g was found in the methanol extract. Alkaloids are low molecular weight compounds and are generally alkaline due to the presence of a nitrogen atom in their heterocyclic ring (Nobahar et al. 2021), so alkaloids range from polar to semi-polar compounds (Cinelli and Jones 2021). Methanol is a compound that has a molecular structure of CH₂OH, is polar because it has a hydroxyl group (-OH), and is non-polar because it has a methyl group (-CH₃) (Ramdani et al. 2017). Methanol is universal so it can dissolve both polar and nonpolar compounds (Wardoyo et al. 2021). Alkaloids are plant-friendly chemicals that act as a deterrent to predators and parasites. Alkaloids have been noted to possess antimicrobial, antihypertensive, antifungal, anti-inflammatory, anti-fibrogenic effects as well as anti-diarrheal effects which may be related to their action on the small intestine (Cinelli and Jones 2021).

The largest total alkaloid of *Parkia timoriana* seeds as much as 48.90 mg/g was found in the methanol extract. Alkaloids are low molecular weight compounds and are generally alkaline due to the presence of a nitrogen atom in their heterocyclic ring (Nobahar et al. 2021), so alkaloids range from polar to semi-polar compounds (Cinelli and Jones 2021). Methanol is a compound that has a molecular structure of CH₂OH, is polar because it has a hydroxyl group (-OH), and is non-polar because it has a methyl group (-CH₃) (Ramdani et al. 2017). Methanol is universal so it can dissolve both polar and nonpolar compounds (Wardoyo et al. 2021). Alkaloids are plant-friendly chemicals that act as a deterrent to predators and parasites. Alkaloids have been noted to possess antimicrobial, antihypertensive, antifungal, anti-inflammatory, anti-fibrogenic effects as well as anti-diarrheal effects which may be related to their action on the small intestine (Cinelli and Jones 2021).

Table 3. Antimicrobial activity of different extracts of *Parkia timoriana* (DC.) Merr seeds

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Methanol</th>
<th>Distilled water</th>
<th>Ethyl acetate</th>
<th>n-Hexane</th>
<th>Positive control (10 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>12±0.71ᵃ</td>
<td>14±0.00ᵇ</td>
<td>11±1.22ᵃ</td>
<td>18±0.71ᶜ</td>
<td>22±1.22ᵃ</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>13±1.48ᵃ</td>
<td>17±0.71ᵇ</td>
<td>12±0.71ᵃ</td>
<td>24±0.71ᵈ</td>
<td>22±1.00ᵃ</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>11±0.71ᶜ</td>
<td>12±1.00ᶜ</td>
<td>10±0.00ᵇ</td>
<td>20±0.84ᵈ</td>
<td>24±0.00ᵇ</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>14±1.22ᶜ</td>
<td>15±0.48ᵈ</td>
<td>8±0.00ᶜ</td>
<td>18±0.00ᵈ</td>
<td>22±0.84ᶜ</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>11±0.00ᵃ</td>
<td>13±0.00ᶜ</td>
<td>9±1.22ᶜ</td>
<td>16±1.00ᵈ</td>
<td>24±0.00ᵇ</td>
</tr>
<tr>
<td><em>Propioni bacterium acne</em></td>
<td>13±0.84ᵃ</td>
<td>16±0.71ᵇ</td>
<td>10±0.84ᵃ</td>
<td>20±0.71ᵈ</td>
<td>24±1.48ᵇ</td>
</tr>
<tr>
<td><em>Prophyromonas gingival</em></td>
<td>11±0.00ᵃ</td>
<td>11±0.94ᶜ</td>
<td>7±0.00ᵈ</td>
<td>15±0.00ᶜ</td>
<td>20±0.71ᶜ</td>
</tr>
</tbody>
</table>

Inhibition zone diameter bacterial (mm) with positive control by Ampicillin

| Candida albicans                  | 11±0.71ᵃ | 12±0.71ᵇ | 8±1.00ᵃ | 16±0.71ᵈ | 20±0.00ᵃ |
| Aspergillus niger                 | 10±0.89ᵃ | 11±1.22ᵃ | 7±0.94ᵃ | 14±0.84ᵇ | 22±1.00ᵃ |
| Aspergillus flavus                | 8±0.94ᵇ  | 9±0.00ᵃ  | 6±0.71ᵃ | 12±0.00ᶜ | 24±0.71ᵇ |
| Aspergillus fumigatus             | 10±0.00ᵃ | 10±0.00ᵃ | 9±0.00ᵃ | 15±0.00ᵈ | 22±0.94ᶜ |

Inhibition zone diameter bacterial (mm) with positive control by Ketoconazole

Data are mean ± standard deviation (n = 3). Data followed by the same capital on each column or by the same minor letter are significantly different (p<0.5).
used to treat intestinal disorders such as diarrhea and dysentery (Adaramola et al. 2012). Tannins present in all medicinal plants are useful sources of antidote to poisons (Ndukwe et al. 2013).

The greatest saponin of Parkia timoriana seed in methanol extract (16.04 mg/g). Saponins are amphiphilic compounds that are made up of polar saccharide chains (hexose, pentose, or uronic acid) that are linked by a non-polar aglycone (Singh 2017). A noticeable amount of saponins in this plant species confirms antibacterial and antibiotic action (Adaramola et al. 2012; Udobi et al. 2008). Saponins are known to have anti-inflammatory effects (Adaramola et al. 2012). Saponins' natural tendency to fight microbes makes them good candidates for the treatment of fungal and yeast infections. These compounds act as natural antibiotics, assisting the body in fighting infections and microbial invasion. (Adaramola et al. 2012; Sodipo et al. 2000).

Terpenoids are mostly found in higher medicinal plants as volatile. They produce a variety of fragrances and are involved in plant protection and pollination of plants. Numerous beneficial biological activities antitumor, anti-inflammatory, antibacterial, antiviral, antimalarial, promoting transdermal absorption, preventing and treating cardiovascular diseases, lowering blood sugar. In addition, some terpenoids also have insecticidal, immunomodulatory, antioxidant, antiaging, and neuroprotective effects. Terpenoids are mostly found in higher medicinal plants as volatile oils (Yang et al. 2020). Terpenoids are lipid-soluble compounds in general (Ludwiczuk et al. 2017). Terpenoids have a low molecular weight and are nonpolar, so they can be extracted using nonpolar solvents such as hexane, supercritical CO, or steam distillation (Kaur et al. 2019). The biggest terpenoid of Parkia timoriana was found in n-hexane extract (91.02 mg/g). The total number of terpenoids in Parkia timoriana seeds is more than of alkaloids and flavonoids, this is supported by previous studies, the crude fat value for Parkia timoriana seeds was 16.14% (Olowokere et al. 2018).

Cardiac glycosides (CGs) are a type of natural product with a steroid-like structure containing unsaturated lactone rings and sugar chains and are considered amphiphilic molecules (Zhu et al. 2017). All extracts have secondary metabolites of cardiac glycosides and the highest cardiac glycoside is found in methanol extract 36.53 mg/g). Cardiac glycosides from several plant species are currently being studied for anti-tumor properties, with the potential to supplement cancer treatment strategies (Adaramola et al. 2012).

This research study on the use of plant materials as powerful treatments for a variety of diseases not only confirmed their efficacy but also revealed the pharmacological roles of individual biological components of these plant materials.

When reduced by hydrogen- or electron-donation, DPPH is a stable nitrogen-centered free radical with a characteristic color change from violet to yellow. Free radical scavengers and antioxidants are substances that are capable of performing this reaction (Hinnerburg et al. 2006). The high DPPH radical scavenging activity of various solvent extracts, particularly methanol extracts, is comparable to the standard antioxidants used in most cases, and these extracts have high proton donating capacity and free radical inhibition. It suggests that it may function as an agent. However, the methanol extracts exhibited stronger DPPH radicals quenching ability with lower IC\textsubscript{50} values than Trolox. The computed IC\textsubscript{50} values for the methanol extract (28.13 µg/ml). (Wu et al. 2010) reported IC\textsubscript{50} values as low as those found with these extracts despite hundreds of reports of DPPH radical scavenging activity of plant extracts in different regions of the world. This finding could also imply that these extracts contain potent free radical scavenging phytochemicals that may be able to inhibit free radical upsurge as well as ROS, and thus could be useful therapeutic agents for treating pathological changes caused by radicals (Ibrahim et al. 2013).

The ABTS assay works by producing a blue/green, which is reduced by antioxidants and acts as a free radical scavenger for the ability to donate hydrogen and electrons. Table 2 shows the ABTS scavenger activity of the extract as scavenger concentration at 50% (IC\textsubscript{50}). In this assay, the antioxidant activities of the extracts were compared to a standard reference, Trolox. The methanol extracts of Parkia timoriana reacted quickly with ABTS\textsuperscript{+}, IC\textsubscript{50} values for the methanol extract (45.39 µg/ml).

From the two methods above, it is explained that the methanol extract has potential as an antioxidant potential, this is supported by quantitative data on secondary metabolism, the methanol extract has a high amount of phenol, alkaloids, saponins, tannins, cardiac glycosides compared to other extracts. also has sufficient amount of terpenoids. Phenolics, β-carotene, ascorbic acid, α-tocopherol and flavanoids have been discovered to have antioxidant activities (Suryanti et al. 2016; Suryanti et al. 2021).

DPPH-scapenging reaction is involved in a series of antioxidant mechanisms. four major proposed mechanisms can be used to clarify how antioxidants scavenge free radicals (1) hydrogen atom (H•)
transfer process (HAT). In the process, DPPH\(^{-}\) was thought to convert to a DPPH-H molecule, and the antioxidant was assumed to donate a H\(^{\cdot}\) atom, (2) sequential electron-proton transfer (SEPT). In this mechanism the reaction takes place in two steps, the initial formation of a cation radical by the transfer of an electron from the antioxidant to the free radical, and a proton transfer from the cation radical to the anion, (3) proton loss single electron transfer (SPLET), (Boulebd 2020). (4) proton-coupled electron transfer (PCET), the proton is transferred from the phenolic compound to the radical's lone pair. On the other hand, the electron moves from the 2p lone pair of the antioxidant compounds to the singly occupied molecular orbital (SOMO) of radical (Milenković et al. 2018).

ABTS\(^{2+}\) radical cation however needs an electron (e\(^{-}\)) to neutralize the positive charge, and ABTS\(^{2+}\) scavenging is considered an electron (e\(^{-}\)) transfer reaction. Antioxidant compounds can effectively scavenge ABTS\(^{-}\) radical, which suggests that donating electron (e\(^{-}\)) may be another approach to directly scavenging radicals. The donating electron (e\(^{-}\)) mechanism is further supported by the Cu\(^{2+}\)-directing radicals. The donating electron (e\(^{-}\)) may be another approach to neutralize the positive charge, ABTS\(^{2+}\) scavenging is considered an electron (e\(^{-}\)) transfer reaction (e\(^{-}\)) to neutralize the positive charge, and ABTS\(^{2+}\) scavenging is considered an electron (e\(^{-}\)) transfer reaction. Antioxidant compounds can effectively scavenge ABTS\(^{-}\) radical, which suggests that donating electron (e\(^{-}\)) may be another approach to directly scavenging radicals. The donating electron (e\(^{-}\)) mechanism is further supported by the Cu\(^{2+}\)-directing radicals. The donating electron (e\(^{-}\)) may be another approach to directly scavenging radicals. The donating electron (e\(^{-}\)) mechanism is further supported by the Cu\(^{2+}\)-directing radicals.

Flavonoids act as scavengers of free radicals because they have a stabilized structure that can weaken more reactive free radicals by converting less reactive free radicals into less reactive hydroxyl radicals. Inhibition of these oxidants occurs through the donation of electrons or hydrogens from free hydroxyls, thus playing a moderate role in the propagation of radical damage in biological systems (Hernández-Rodríguez et al. 2019).

Alkaloids were suggested to be mediated via way of means of proton transfer (PT, i.e., H\(^{+}\)-switch) to show antioxidant action. Proton-switch is quite related to H\(^{+}\) ionization from phenolic-OH, in particular in protic solvents. For alkaloids, H\(^{+}\) is a quite touchy species; the nitrogen atom (N) in phenolic alkaloid combines with H\(^{+}\) to shape an amine salt. Therefore, phenolic alkaloid is protonated on the N-atom. The protonated N-atom bears a nice fee and for that reason has robust chickening out electron capacity. The extrude in electron density influences antioxidant stages due to the fact electron-transfer (ET) capacity is an essential component of an antioxidant (Xie et al. 2018). Saponins were electron donors that could bind to free radicals and convert them to more stable products, thereby halting the radical chain reaction (Elekofehinti et al. 2013). Tannins have an antioxidant capacity at least two methods to reflect and differentiate both the single electron transfer (SET) and the hydrogen atom transfer (HAT) phenomena (Ben Ahmed et al. 2017). There are two classes of oxygenated monoterpenes and oxygenated sesquiterpenes, both of which have an oxygen atom in their structure. These compounds' antioxidant activity will be clarified using three antioxidant mechanisms: sequential proton loss electron transfer (SPLET), single-electron transfer followed by proton transfer (SET-PT), and hydrogen atom transfer (HAT) (Ngo et al. 2017).

Salivary and pancreatic -amylase hydrolyze starchy foods into oligosaccharides, which are then hydrolyzed into glucose by \(\alpha\)-glucosidase in the small intestine (Sim et al. 2010). \(\alpha\)-amylase inhibition studies have shown that P. timoriana seed extracts (crude methanol, ethyl acetate, distilled water, and n-hexane) have significant inhibitory potential. The I\textsubscript{50} value of methanol extract (25.35±2.30 μg/ml) is almost identical to acarbose's (28.63±1.21 μg/ml), an anti-diabetic medication that is widely used and marketed. These \(\alpha\)-amylase inhibitors mainly contain 1,4-glycosidic bonds of starch and other oligosaccharides such as maltose, maltotriose, and other simple sugars. (Wickramaratne et al. 2016).
The polar compound is most likely responsible for the inhibitory activity of α-amylase in methanol extract, and more research and isolation of the pure active compound are needed. All polyphenols studied showed higher activity against α-glucosidase than for alpha-amylase. In general, the order of inhibition is flavonoids > biflavonoids > xanthone > tannin for α-glucosidase, and flavonoids > tannins > xanthones for α-amylase (Bomigboye et al. 2020). Methanol extract also contains high flavonoid compounds. The α-glucoside inhibition IC\(_50\) of methanol extract (23.04±1.81 µg/ml) is significantly different from acarbose’s (28.63±1.21 µg/ml). The inhibitory activity of α-glucosidase of flavonoids, phenolic acids, tannins, and plant-derived anthocyanins has been reported (Di Stefano et al. 2018). Flavonoids have been shown to have better inhibitory activity against the α-glucosidase enzyme than phenolic acid. Hydroxybenzoic and hydroxycinnamic were not inhibitory α-glucosidase and α-amylase enzymes at 1 mM. The more pronounced inhibitory activity is most likely due to the presence of additional hydroxyl groups in compounds with a flavone backbone (Gu et al. 2015). The most important forces involved in the complexation of phenolic compounds with -glucosidase are hydrophobic interactions, hydrogen (H) bonds, and Van der Waals interactions (Peng et al. 2016; Yan et al. 2014).

The position and nature of the substituents in triterpenoids were critical to their activity. Indeed, the transfer of a methyl group from C20 to C19 increases the inhibitory activity, whereas the transfer of a methyl group from C20 to C19 decreases the inhibitory activity. The 17-carboxyl group also contributes to pentacyclic triterpenes’ inhibitory activity (Zhang et al. 2017). Further research revealed that α-glucosidase inhibits terpenoids in a non-competitive manner, with binding sites identified at holes 2 and 4 of the enzyme (Ding et al. 2018). Terpenoids interact with several amino acid residues in the enzyme, while H-bonds are formed with O atoms in C28-carboxylic acids. The conformational change of α-glucosidase after binding reduced the catalytic activity of the enzyme (Ding et al. 2018; Zhang et al. 2017). Diabetic complications are expected to arise from oxidative stress due to free radical production and oxidative degradation of glycosylated proteins during glucose oxidation (Wickramaratne et al. 2016). Therefore, the use of antioxidants in combination with antidiabetic medications is often recommended to avoid these complications.

Methanol extract demonstrated the greatest zone inhibition of almost all bacterial and fungal pathogens. The extract was able to inhibit bacteria Escherichia coli with the categorized very strong rather than positive control ampicillin. Candida albicans had the largest zone inhibition of fungal with categorized strong, this is smaller than the positive control ketoconazole. According to Maryana et al. 2019, the following are the criteria for antibacterial power strength: 5 mm inhibition zone diameter or less is classified as weak, 5-10 mm inhibition zone is classified as moderate, 10-20 mm inhibition zone is classified as strong, and 20 mm inhibition zone is classified as very strong. This result is different significance from the previous study in methanol extract can inhibit E. coli growth with inhibition zone diameter 17 mm respectively (Ralte et al. 2022). The methanol extract has high levels of flavonoid alkaloids and saponins so it will affect its ability as an antimicrobial. Flavonoids will bind to bacterial extracellular proteins through hydrogen bonds and covalent bonds to form a complex that will interfere with the function of the cell wall bacteria, inactivating microbial adhesion, enzymes, cell transport proteins (Kumar and Pandey 2013). The mechanism of tannin compounds as an antibacterial is by shrinking the cell wall or cell membrane, thereby disrupting the permeability of the cell itself (Ajizah 2004). Tannins are antibacterial by forming complex compounds with enzymes or substrates thereby disrupting cell membranes. The mechanism of saponins as an antibacterial is by causing leakage of proteins and enzymes from within the cell which results in cell death, in other words it is bactericidal, because it has hydrophilic molecules and molecules that can dilute lipids or are lipophilic, thereby lowering cell surface tension (Madduluri et al. 2013). Phenolic compounds are also antibacterial because they have hydroxyl and carboxyl groups that can interact with bacterial cells through hydrogen bonds so that it coagulates proteins and bacterial cell membranes and causes bacteria to become lysed (Erviana and Purwono 2011; Rachmawaty et al. 2018). Terpenoid compounds can inhibit growth by interfering with the process of forming membranes and/or cell walls, membranes or cell walls are not formed or formed imperfectly. Alkaloids have the ability to an antibacterial. The suspected mechanism is by interfering with the peptidoglycan constituent components in bacterial cells so that the cell wall layer is not fully formed and causes the death of the cell (Ajizah 2004).

In conclusion, in this study, we reported the potential antioxidant activity of crude methanol extract of P. timorianna seeds with their mode of action and the potential antidiabetic. The studies showed that the crude n-hexane extract was effective for...
inhibiting of a variety of Gram-negative and Gram-positive bacterial and fungal tested strains. The ROS induced DNA damage is the possible mechanism of antibacterial activity of methanol extract of *P. timoriana* seeds.

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