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Phytochemical composition and bioactivity of *Parkia timoriana* leaf extract from Kediri, Indonesia in various solvent polarities

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Abstract. *Sariwati A, Sari F, Suryanti V, Handayani DS, Setyono HA, Yuliati N. 2024. Phytochemical composition and bioactivity of* Parkia timoriana *leaf extract from Kediri, Indonesia in various solvent polarities. Biodiversitas 25: 4900-4908.* The potential therapeutic uses of bioactive chemicals found in natural sources have led to a significant increase in focus on their investigation in recent years. *Parkia timoriana* (DC.) Merr. has secondary metabolites, which have been used as a traditional medicine. This work studies the phytochemical composition and bioactivities evaluation of *P. timoriana* leaf extract of varying solvent polarities, such as methanol, water, ethyl acetate, and hexane. The methanol extract has the highest secondary metabolite contents, excluding terpenoids contents. The Follin-Ciocalteu method showed that the total phenolic content of methanol extract was 302.02 mg GAE/g. The aluminum chloride colorimetric method revealed that the total flavonoid content of the methanol extract was 256.85 mg QE/g. The tannin acid, alkaloids, saponins, and terpenoids contents of methanol extracts were determined by Spectrophotometer UV-Vis, which were found to be 32.07 mg TAE/g, 23.86 mg CoE/g, 18.35 mg DE/g, 5.23 mg Linalool Eq./g, and respectively. The highest terpenoid contents were found in hexane extract, which was 11.34 mg of Linalool Eq./g. Antioxidant activities of the extracts were assessed by measuring the free-radical of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and scavenge of 2,2'-azinobis (3-ethylbenzene-thiazoline-6-sulfonic-acid (ABTS)). The methanol extract was shown to have the strongest antioxidant activity, where the DPPH and ABTS IC_{50} values were 47.78 and 39.54 µg/mL, respectively. The methanol extract exhibited the greatest antimicrobial activities, where the inhibition zone for *Candida albicans* and *Escherichia coli* fungus were 21 and 22 mm, respectively. Antidiabetic effects were assessed in vitro by blocking α-amylase and α-glucoside. The methanol extract shows an inhibition of 50.19 µg/mL for α-glucoside and 42.50 µg/mL for α-amylase. The secondary metabolites of *P. timoriana* leaf are great building blocks for making potent medications.

Keywords: Antibacterial, antidiabetic, antioxidant, *Parkia timoriana* leaf, secondary metabolites

INTRODUCTION

The use of medicinal plants has seen a considerable increase in recent years. This resurgence of interest is due to the need for alternative cures for emerging health concerns and chronic illnesses, as well as the toxicities and health dangers associated with synthetic pharmaceuticals and antibiotics (Akter et al. 2021). Secondary metabolite of plants are responsible for their bioactivities. Pathogenic microbes that are both susceptible and resistant can be inhibited by phenols, flavonoids, alkaloids, tannins, terpenoids, and some other bioactive compounds derived from traditional plants (Ahmadu and Ahmad 2020; Da Silva et al. 2021). These compounds also act as antioxidant agents that inhibit reactive oxygen species and stop oxidative (Rubió et al. 2013; Poulios et al. 2024). The bioactive compounds in medicinal plants offer a promising platform for developing novel antidiabetic medications with diverse modes of action. Plant extracts can increase insulin production and decrease blood glucose levels in vivo (Bouyahya et al. 2021). To optimize their bioactivities, secondary metabolite structure modifications have garnered more attention lately (Suryanti et al. 2018; Wang et al. 2019).

Indonesia has many plant species that have not been thoroughly investigated or utilized for medical purposes (Rani et al. 2023). Genus *Parkia* is commonly cultivated in Southeast Asian countries, including Indonesia. Numerous active compounds in this plant show great potential for ecological and commercial benefits (Hidayati et al. 2019). It is used traditionally to treat several ailments, such as diabetes, diarrhea, wounds, hypertension, cough, chronic piles, conjunctivitis, and measles. Their medicinal values were attributed to the presence of pharmacologically active compounds, such as phenolics, flavonoids, terpenoids, alkaloids, saponins, steroids, tannins, and phytosterol (Saleh et al. 2021; Singha et al. 2021).

Parkia timoriana is a synonym name of *Parkia roxburghii* G. Don. It is a species of *Parkia* commonly known as Kedawung in Indonesia. Pods, bark, twigs, fruit,

and leaves of *P. timoriana* tree are consumed either raw or boiled with other ingredients for traditional medical uses, such as diarrhea, dysentery, wounds, fever, ulcers, and skin diseases (Saleh et al. 2021). Various parts of the plant were reported to have antioxidant, antibacterial, antidiabetic, antiproliferative, insecticidal, α-glucosidase, and α-amylase inhibitory properties (Angami et al. 2018). The seeds of *P. timoriana* contain flavonoids, alkaloids, phenolics, saponins, terpenoids, tannins, and cardiac glycosides, which are responsible for their bioactivities (Suryanti et al. 2022). Seed oil extract of *P. timoriana* possesses insecticidal properties and holds promising agents in controlling various insect pests. Lectins isolated from the seed extracts of *P. timoriana* inhibits the proliferation of cancerous macrophage cell lines. Roasted Kedawung seed extract has potent antioxidant properties, as shown by the DPPH technique. *Parkia timoriana* seed extracts have antibacterial, antioxidant, and antidiabetic activities (Sariwati et al. 2024).

The bark extract of *P. timoriana* demonstrated remarkable inhibition of α-amylase and α-glucosidase (Papitha and Selvaraj 2024). Papitha et al. (2024) reported that modified *P. timoriana* bark using nanocomposites ZnO/TiO_2 and CuO/TiO_2 by green synthesis exhibited good biological activities, such as antioxidant and antidiabetic activities. *Parkia timoriana* bark extract exhibits considerably as a natural antibiotic against *Bacillus subtilis, Bacillus pumilus, Pseudomonas aeruginosa*, and *Escherichia coli* (Ralte et al. 2022). *Parkia timoriana* bark extracts have antibacterial, antioxidant, and antidiabetic activities (Sariwati et al. 2024).

The leaf extract of *P. timoriana* has an enormous effect against pathogenic bacteria, such as *E. coli, V. cholerae, S. aureus,* and *B. cereus* (Zuhud et al. 2001). Gold and silver nanoparticles obtained from dried leaves of *P. timoriana* significantly inhibit *S. aureus* compared to *E. coli*. It could be because the Au and Ag NPs accumulated on the cell wall of *S. aureus* (Paul et al. 2016). This work investigates the effects of varying solvent polarities on the chemical diversity contents and bioactivities of *P. timoriana* leaf extracts, such as antibacterial, antifungal, antioxidant, and antidiabetic. Solvent polarity, in particular, plays a critical role in selectively isolating compounds of varying polarities. These findings could significantly contribute to understanding *P. timoriana* leaf as a source of natural bioactive compounds and promote its potential use in pharmaceutical and nutraceutical applications.

MATERIALS AND METHODS

Materials

DPPH was obtained from Tokyo Chemical Industries (TCI), Tokyo, Japan. Gallic acid was purchased from Wako Pure Chemical Industries, Osaka, Japan. Chemicals were purchased from e-Merck, such as linalool, colchine, diosgenin, chloroform, Folin-Ciocalteu, ferric chloride, ethyl acetate, acetic acid, nutrient broth, nutrient agar, hexane, dimethyl sulfoxide, digoxin, glacial acetic acid, enzyme α-amylase and enzyme α-glucosidase, 3,5-dinitrosalicylic acid (DNSA), nitrophenyl-D-glucopyranoside (PNPG).

Microbial cultures

Bacterial

NITE Biological Resources Center (NBRC) Chiba, Japan provides *Pseudomonas aeruginosa* NBRC 3080, *Bacillus subtilis NBRC 3009*, *Propionibacterium acne* NBRC 111530, *Escherichia coli* NBRC 3301, *Porphyromonas gingival* NBRC 115147, *Staphylococcus aureus* NBRC 102135, *Salmonella typhi* NBRC 14193. These bacteria are collection of Chemistry Department, Microbial Chemistry Laboratory, Institut Ilmu Kesehatan Bhakti Wiyata Kediri, Indonesia. The nutrient a media was used to cultivate the colony. In a shaker, the culture was pre-incubated 60 mL of Nutrient Broth (NB) for 20 h at 37°C.

Fungal

The NITE Biological Resources Center (NBRC) Chiba, Japan, provides *Aspergillus niger* NBRC 5376, *Candida albicans* NBRC 0197, *Aspergillus flavus* NBRC 4186, and *Aspergillus fumigatus* NBRC 4057. The fungus was grown in Potato Dextrose Agar (PDA) media at 37°C. The colony was then injected into an erlenmeyer (100 mL) containing 60 mL of nutrient broth and incubated for 20 h at 37°C and 180 rpm (Sariwati and Prunomo 2018).

Samples preparation

Parkia timoriana leaves were collected from Kediri, Indonesia. GPS locationhttps://maps.app.goo.gl/8KwkxrVrMTPyhqCC9. Leaves were washed with water, chopped into small pieces, and left at room temperature overnight. The sample was then ground into a 25-mesh particle size (Sariwati et al. 2024).

Parkia timoriana *leaf extracts preparation*

Dried sample powder (20 g) was placed in a 500 mL flask with 200 mL hexane. Similarly, in other vessels, the sample powders were added ethyl acetate, methanol, or water. The flasks were covered with aluminum foil and stirred for 24 h at 180 rpm. The mixtures were filtered, and the solvents were evaporated. The sample extracts were kept at 4°C until needed (Sariwati et al. 2022).

Phytochemical screening of Parkia timoriana *leaf extracts*

Parkia timoriana leaf extracts were treated with specific reagents to determine their phytochemical composition, such as tannins, triterpenoids, flavonoids, saponins, and alkaloids (Sariwati et al. 2024).

Total phenolic contents

Parkia timoriana leaf extracts (20 mg) were kept in 3% HCl (5 mL) in 60% methanol. The mixture (100 µL) was mixed with Na_2CO_3 aqueous (2 mL). After 3 minutes, the mixture was added to the phenol reagent Follin-Ciocalteu (100 µL) and left for 30 minutes. The mixture was then analyzed using a UV-Vis spectrometer at 750 nm. The extract was presented in mL GAE (gallic acid equivalent)

per g of extract. The standard curve was performed at 0.5, 1.0, 1.5, 2.0, and 2.5 mM (Sariwati et al. 2019).

Total alkaloids content

Extract (1 mL), phosphate buffer pH 4.7 (5 mL), and BCG (Bromocresolgreen) solution (5 mL) were mixed. After stirring with chloroform, the mixture was placed into a 10 mL volumetric flask and diluted using the solvent. A series of reference standard solutions for colchicine were made using the same procedure. The absorbance of samples and standard solutions was measured by UV-Vis spectrophotometer at 470 nm. Total alkaloid contents were presented as mg of colchicine per g sample (mg CoE/g) (Umdale et al. 2021).

Total flavonoids content

Total flavonoids content was examined using the colorimetry of the aluminum chloride method. In a 10 mL volumetric flask, samples were added to demineralization water (1 mL) and 0.5% sodium nitrite (0.30 mL). The mixtures were then left for 5 minutes and added with 10% aluminum chloride (0.3 mL). Then, mixtures were left for 5 minutes and added with demineralization water (10 mL) and 1 M NaOH (2 mL). The mixtures were then measured their absorbance by UV-Vis Spectrophotometer at 510 nm. Total flavonoids content was presented as mg per 100 g of dry weight (DW), and the quercetin equivalent (QE) was used to represent the total flavonoid content (Sariwati et al. 2022).

Total saponins content

Sample extract 250 µL (1 mg/mL) was added 72% H_2SO_4 (2.5 mL) and 250 µL vanillin (8 g in 100 mL ethanol). The mixture was heated to 60°C for 10 minutes and chilled in an ice-water bath for 5 minutes. The mixtures were measured for absorbance by UV-Vis spectrophotometer at 544 nm. Diosgenin (5.7-71.4 mg/L) was used for the calibration curve. The total saponins content was presented as mL per g of diosgenin (Chua et al. 2019).

Total tannic acids content

The sample extract was reacted with the Folin-Ciocalteu reagent. After 20 minutes of room temperature incubation, the mixture color change was measured its absorbance by UV-Vis Spectrophotometer at 500 nm. The total tannic acid content was mg TAE/g (Umdale et al. 2021). Tannic acid solution (50-300 g/mL) was used as a standard.

Total terpenoids content

The total terpenoid content is measured by colorimetric methods. The reaction between terpenoids and vanillin or sulfuric acid form a colored complex that can be measured spectrophotometrically at 538 nm. Sample extract (200 µL) was added to chloroform (1.5 mL) and left for 3 minutes. After 10 minutes of incubation, H_2SO_4 (100 µL) was added to each tube. A dark brown precipitate containing terpenoids formed. After carefully decanting the supernatant, 1.5 mL of methanol was used to dissolve the precipitate. The mixture was then measured its absorbance. The concentration was reported in mg of linalool per g sample extract. A standard curve was established with linalool (40-100 g/mL) (Sariwati et al. 2024).

Antioxidant activity

Antioxidant activity by DPPH method

The absorbance was measured at 517 nm for DPPH (0.6 mM) (24 mg) solution with methanol (100 mL). A stock DPPH solution (1 mL) was mixed with 33 µL of *P. timoriana* leaf extracts (10-100 µg/mL). The mixture was left for 20 minutes at 28°C in the absence of light. The DPPH radical scavenger ability was evaluated using equation 1. The IC_{50} value was then calculated (Sariwati et al. 2019).

$$\text{Suppression radical scavenging}(\%) = \frac{[\textit{Control absorbance} - \textit{Sample absorbance}]}{\textit{Control absorbance}} x100 \quad \text{.....(1)}$$

Antioxidant activity by ABTS method

The ABTS and potassium persulfate were dissolved in distilled water to obtain 4.9 and 7 mM concentrations, respectively. These solutions were left at room temperature for 12-16 h without light. The ABTS solution was dissolved in distilled water to obtain an absorbance of 0.7 at 734 nm. A 96-well plate was filled with 10 µL of extract (10-100 µg/mL) and 190 µL of ABTS solution. The mixture was left for 30 minutes at 28°C. A control of 10-100 µg/mL trolox was used. The mixtures were measured for their absorbance at 734 nm. The ABTS radical scavenging inhibition was calculated using Equation 2 (Jaâfar et al. 2017).

$$(\%)\ \text{ABTS Scavenging} = \frac{[\textit{Control absorbance}(ABTS) - \textit{Sample absorbance}]}{\textit{Control absorbance}(ABTS)} x100 \quad \text{.....(2)}$$

A linear regression equation was used to get the IC_{50} value and inhibition percentage (Sariwati et al. 2019).

Antidiabetic activity

α-amylase inhibition

The test for α-amylase inhibition was conducted using the 3,5-dinitrosalicylic acid (DNSA) technique (Sariwati et al. 2024). Extracts (200 µL) and α-amylase solution (2 units/mL; 200 µL) were mixed in tubes, and the mixture was incubated for 10 mins at 30°C. Each tube was then filled with 200 µL of the starch solution (1% in water (w/v) as substrate, and the tubes were incubated for 3 mins. DNSA reagent (200 µL) was added to stop the reaction. The mixture was heated for 10 mins at 85-90°C in a water bath and left at room temperature for cooling down. Aquadesh (5 mL) was added for diluting. A reddish-brown or orange complex was observed, and the absorbance was measured at 540 nanometers. The Equation 3 was used to calculate the α-amylase inhibitory activity and represent it as a percentage inhibition. By graphing the percentage of α-amylase inhibition versus the extract concentration, the IC_{50} values were determined (Wickramaratne et al. 2016).

$$\text{α-amylase surpression}(\%) = \frac{[\textit{Control absorbance} - \textit{Sample absorbance}]}{\textit{Control absorbance}} x100 \quad \text{.....(3)}$$

α-glucosidase inhibition

Nitrophenyl-D-glucopyranoside (PNPG) was used as a substrate in the α-glucosidase inhibition test (Sariwati et al. 2024). When PNPG is used as a substrate, α-glucosidase hydrolyzes PNPG into a yellow of *p*-nitrophenol (pNP), where absorbs light at 405 nm in alkaline conditions. The presence of an inhibitor will reduce this enzymatic reaction, decreasing the amount of yellow pNP formed (Kumar and Pandey 2013; Sheikh et al. 2016). The α-glucosidase inhibition was calculated using Equation 4.

$$\alpha\text{-glucosidase inhibition (\%)} = \frac{[Control\ absorbance - Sample\ absorbance]}{Control\ absorbance} x100 \quad .....(4)$$

Antibiotic activity

Antibacterial activity test

Gram-positive bacteria tested are *S. aureus* NBRC 102135, *P. acne* NBRC 111530, and *P. aeruginosa* NBRC 3080. Gram-negative bacteria tested are *P. gingival* NBRC 115147, *E. coli* NBRC 3301, *B. subtilis* NBRC 3009, *P. gingival* NBRC 115147, and *S. typhi* NBRC 14193. The bacteria (100 µL) was suspended in a petri dish containing nutrient agar (NA) media. Ampicillin (10 mg/mL) was used as a positive control. The plates were incubated at 37°C for 12 h, and the inhibition zone was measured in mm (Sariwati et al. 2019).

Antifungal activity test

The fungus used are *A. flavus* (NBRC 4186), *C. albicans* (NBRC 0197), *A. fumigatus* (NBRC 4057), and *A. niger* (NBRC 5376). Sterile potato dextrose agar (PDA) was added into a petri dish containing the 5 mL of *P. timoriana* leaf extract (10 mg/mL), and they were gently spun to ensure proper mixing. Solidify the medium using a sterilized 5-millimeter cork drill. A formed culture dish was found after a four-day-old pure culture was pierced and placed in the center of the plates. The plates were then incubated at 28°C at room temperature for a week. Ketoconazole (10 mg/mL) was used as a control. The inhibition area was measured every day for seven days (Akwaji et al. 2016).

Statistical analysis

Experiments were conducted in triplet calculation. An assessment of representational dissimilarity between or among groups was made using a student's t-test during substrate transformation. Excel determined the statistical representativeness of the dissimilarity between processes at a confidence level of 5% (P 0.05) (Suryanti et al. 2022).

RESULTS AND DISCUSSION

Chemical content of *P. timoriana* leaf extracts

Different extract yields are obtained depending on the solvent utilized (Rahmalia et al. 2015). Four different solvent polarities were applied to obtain *P. timoriana* leaf extracts subjected to phytochemical screening. The yield for leaf extracts of methanol, water, ethyl acetate, and hexane were 14.21, 15.46, 1.64, and 1.02 %, respectively. Phytochemical screening revealed the presence of anthocyanins, terpenoids, alkaloids, cardiac glycosides, flavonoids, reducing sugar, tannins, and saponins in all *P. timoriana* leaf extracts. The solvent polarities utilized significantly impact the amount and types of metabolites recovered (Rafi et al. 2018). Steroids were found in both methanol and water extracts. Coumarin is present only in the water extract. Table 1 shows that anthraquinones were able to extract with methanol and water. This result is in line with previous studies that the methanol extract of *P. timoriana* bark and seeds contains terpenoids, alkaloids, tannin, steroids, and flavonoids (Suryanti et al. 2022; Sariwati et al. 2024).

The chemical contents of *P. timoriana* leaf extracts are shown in Table 2. The greatest total phenolics content was found in the methanol extract, which is 302.02 mg GA/g). Methanol is a polar solvent that extracts phenols (Offermanns et al. 2014). The hydroxyl (-OH) groups in phenolic compounds can form hydrogen bonds with methanol, facilitating their dissolution (Cheok et al. 2011; Yanuarti et al. 2017). *Parkia timoriana* leaf methanol extract was found to have the highest flavonoid content at 256.85 mg QE/g. Flavonoids are soluble in methanol due to their polarity. The hydroxyl groups of flavonoids enable the formation of hydrogen bonds with polar solvent (Hikmawanti et al. 2021).

Parkia timoriana leaf methanol extract has the greatest total alkaloid content of 23.86 mg CoE/g. Methanol is an effective alkaloid solvent (Habibian et al. 2020). Alkaloids react with mineral or organic acids to form salts, usually soluble in water and diluted alcohols (Sireesha et al. 2019; Zubairi et al. 2022). Tannic acid content is highest in the methanol extract (32.07% mgTAE/g). Methanol exhibits extraordinary efficacy in the extraction of tannins due to its polarity (Naima et al. 2015; Rhazi et al. 2015). The highest saponin content was found in the methanol extract of *P. timoriana* leaf at 18.35 mg/g. Saponins have high solubility in methanol (Do et al. 2021). Hexane extract of *P. timoriana* leaf had the highest terpenoid content of 11.34 mgDE/g. Terpenoids are soluble in non-polar solvents like n-hexane because of their general lipophilicity (Dewi et al. 2024).

Table 1. Qualitative phytochemical screening of *Parkia timoriana* leaf extracts

Compounds	*P. timoriana* leaf extracts			
	Methanol	Water	Ethyl acetate	Hexane
Alkaloid	+	+	+	+
Flavonoids	+	+	+	+
Steroids	+	+	-	-
Tannins	+	+	+	+
Terpenoids	+	+	+	+
Saponins	+	+	+	+
Reducing sugar	+	+	+	+
Cardiac glycoside	+	+	+	+
Anthraquinones	+	+	-	-
Coumarins	-	+	-	-
Anthocyanins	+	+	+	+

Antioxidant activity

Antioxidant properties were observed by DPPH and ABTS. The IC_{50} values are represented as the sample extract concentration (µg/mL) was needed to reduce the initial DPPH or ABTS concentration by 50%. The highest antioxidant activity was obtained for methanol extract, which had the lowest IC_{50} values for 47.78 µg/mL of ABTS scavenging and 39.54 µg/mL of DPPH (Table 3). In comparison, *P. timoriana* seed in methanol extract has IC_{50} values for 28.13 µg/mL of DPPH of and 45.39 µg/mL of ABTS (Suryanti et al. 2022). Moreover, the IC_{50} values of *P. timoriana* bark in ethyl acetate extracts were found for 66.63 µg/mL of DPPH and 78.72 µg/mL of ABTS (Sariwati et al. 2024). As indicated in the Table 3, the antioxidant activity of extracts quantified through DPPH was higher than the ones obtained by ABTS. This result mainly conforms to Buathongjan et al. (2020), which could be because the ABTS is more sensitive, has lower limitations, and has a heightened response to antioxidants than the DPPH. Further, the reaction kinetics of ABTS with most antioxidants is excessively faster than DPPH.

Methanol extract has a higher content of flavonoids, alkaloids, phenol, saponin, and tannins than other extracts, contributing to its function as a free radical shield. The presence of terpenoids in the extract supports this result. To attack free radicals, phenolic compounds provide hydrogen atoms through hydroxyl groups of aromatic rings. The mechanism of phenolics scavenge free radicals is called Hydrogen Atom Transfer (HAT). The number of hydroxyl groups of the aromatic ring and the Bond Dissociation Enthalpy (BDE) of the O-H bond significantly impact the free radical scavenging of phenolics (Zhu et al. 2024).

Phenolics, alkaloids, saponins, and tannins are antioxidant compounds found in plants (Suryanti et al. 2016; Suryanti et al. 2021; Suryanti et al. 2022). Major antioxidants found in natural products are phenolics and alkaloids (Salehi et al. 2019; Omar et al. 2022). Hydroxyl groups on the phenyl rings of flavonoids contribute to their antioxidant activity. The hydroxyls in the ortho position are frequently more efficient than those in the meta position at scavenging free radicals (Zhang 1999). Flavonoids inhibit oxidants by removing electrons or hydrogen atoms from the hydroxyl groups. Flavonoids can lower oxidative stress in biological systems by neutralizing free radicals and Reactive Oxygen Species (ROS) (Kumari et al. 2023). The deprotonated alkaloids are antioxidants that can scavenge free radicals through single-electron transfer (SET) (Pérez-González et al. 2020).

Saponins have antioxidant properties that act as electron donors for scavenging free radicals (Gitto et al. 2012; Bhargava 2019). Tannins have free radical scavenging action against ABTS and DPPH radicals through single electron transfer (SET) (Mittal and Kakkar 2021). Terpenoids are against oxidative stress as antioxidants through transferring electrons or hydrogen due to their conjugated double-bond molecular mechanism (Wojtunik-Kulesza et al. 2018; Gutiérrez-Del-Río et al. 2021).

Antidiabetic activity

α-amylase and α-glucosidase are important enzymes that break down carbohydrates (Jones and Rose 2014). Natural compounds, such as α-glucosidase and α-amylase inhibitors, have shown promise to control blood glucose levels in individuals with Type II diabetes (Adisakwattana et al. 2011). Table 4 shows the inhibitory potencies of α-glucosidase and α-amylase for distilled water extract, methanol, ethyl acetate, and hexane extracts. Methanol extract shows the greatest potential for antidiabetic actions, with the lowest IC_{50} value. Table 4 shows α-glucosidase activity of 50.19 µg/mL and α-amylase of 42.50 µg/mL. These values are closed with the IC_{50} values of α-amylase (43.14 µg/mL) and α-glucosidase (38.08 µg/mL) for the ethyl acetate of *P. timoriana* bark (Sariwati et al. 2024). However, these results differ significantly from the IC_{50} values of α-amylase (25.35 µg/mL) and α-glucosidase (23.04 µg/mL) for the methanol *P. timoriana* seed extract (Suryanti et al. 2022).

Table 3. Antioxidant activity of *Parkia timoriana* leaf extracts

Extracts	Antioxidant activity (IC50)	
	DPPH	ABTS
Methanol	39.54±0.34aA	47.78±0.18aB
Water	78.72±0.48bA	88.90±0.53bB
Ethyl acetate	119.88±0.67cA	132.18±0.75c B
Hexane	150.37±0.38dA	159.52±0.48dB

Note: Data are mean ± standard deviation (n = 3). Data followed by the same capital or small letters are statistically significantly different ($p<0.5$)

Table 2. Quantitative phytochemical analysis (mg/g) of *Parkia timoriana* leaf extracts

Phytochemicals	*Parkia timoriana* leaf extracts			
	Methanol	Water	Ethyl Acetate	Hexane
Phenolics (mg GA/g)	302.02±1.32aA	211.71±1.81Ab	156.53±0.04aC	24.75±1.60aD
Flavonoids (mg QE/g)	256.85±2.02bA	207.32±1.42bB	108.33±0.72bC	31.37±0.65bD
Tannin acids (mg TAE/g)	32.07±2.05cA	20.12±0.42cB	25.73±0.35cC	1.97±1.25cD
Alkaloids (mg Coe/g)	23.86±0.38dA	18.78±1.25cB	16.18±0.06dC	22.19±0.75dD
Saponins (mg DE/g)	18.35±0.54eA	14.65±0.86dB	12.49±1.44eC	10.23±0.49eD
Terpenoids (mg Linalool Eq./g)	5.23±0.92fA	8.45±0.67eB	9.56±0.88fC	11.34±1.12fD

Note: Data are mean ± standard deviation (n = 3). Data followed by the same capital or small letters are statistically significantly different ($p<0.5$)

Polyphenols effectively inhibit the α-amylase and α-glucosidase. Polyphenols bind to the enzymes through hydrogen bonding and hydrophobic interactions (Dai et al. 2020). The binding occurs in the active side of the enzymes, which could change the structure of the enzymes and reduce their activity (Gao et al. 2013). Flavonoids inhibit both enzymes by acting as competitive inhibitors (Sun et al. 2024). Enzymes and flavonoids interact through van der Waals, π-π forces, and hydrogen bonding, which can change the enzymes' secondary structures and catalytic activity (Sobhy et al. 2019).

The structural properties of flavonoids play a significant role in determining their inhibitory effectiveness. The presence of hydroxyl groups at positions A5 and B3 and a double bond between C2 and C3 are essential for α-amylase inhibition, as they enable flavonoids to align parallel to the catalytic active region of the enzyme. The hydroxyl groups at positions B3 and C3 are essential for inhibiting α-glucosidase because they facilitate the entry of the B-ring into the catalytic active site (Lim et al. 2021). Moreover, a double bond (C2=C3) and a keto group (C4=O) are required for the simultaneous inhibition of both enzymes (Lam et al. 2024).

Tannins inhibit α-glucosidase and α-amylase through hydrogen bonds, hydrophobic and other non-covalent interactions. The enzyme's structure and spatial conformation are altered due to the tannin interactions with specific amino acid residues in the enzyme's active site or allosteric regions (Liu et al. 2023). The non-competitive inhibitory activity of saponins on α-amylase and α-glucosidase is concentration-dependent. They bind to amino acid residues in the enzymes through hydrophobic interactions and intermolecular hydrogen bonding, altering their chemical structure and spatial conformation and reducing their activity (Man et al. 2022).

Alkaloids have effectively blocked α-amylase and α-glucosidase by various binding techniques (Papoutsis et al. 2020). Alkaloids attach to α-amylase and α-glucosidase through a variety of interactions, including electrostatic forces, hydrogen bonds, van der Waals forces, and hydrophobic interactions (Wang et al. 2023). The oxygen atom of C28-carboxylic acids forms hydrogen bonding with amino acid residues in enzymes. Terpenoids may interact with several amino acid residues. Upon contact, α-glucosidase underwent a conformational shift that diminished the enzyme's catalytic activity (Zhang et al. 2017; Ding et al. 2018).

Antibiotic activity

The issue of antibiotic resistance prompted research on a variety of antibacterial treatments. Natural compounds are promising alternatives to traditional antibiotics (Du 2024). Three categories of antibacterial activity are based on the zone of inhibition (ZOI) (i) resistant (ZOI<7 mm); (ii) intermediate (ZOI 8-10 mm); and (iii) sensitive (ZOI>11 mm) (Bharkhavy et al. 2022). Table 5 shows the results of an antibiotic investigation using the four extracts against bacteria and fungus. Methanol extract exhibited the strongest zone inhibition for both bacterial and fungal, confirming the extract's antimicrobial properties. *E. coli* was shown to have an inhibitory zone diameter of 22 mm, making it particularly sensitive. At 21 mm, *C. albicans* exhibited the largest inhibitory zone diameter, categorized as a strong inhibition.

Table 4. Antidiabetic activity of *Parkia timoriana* leaf extracts

Extracts	Antidiabetic activity (IC50)	
	Inhibition of α-amylase	Inhibition of α-glucosidase
Methanol	42.50±0.57aA	50.19±0.83aB
Water	70.91±0.26bA	76.93±0.59bB
Ethyl acetate	114.13±0.49cA	118.34±0.72cB
Hexane	135.15±0.62dA	144.67±0.29dB

Note: Data are mean ± standard deviation (n = 3). Data followed by the same capital or small letters are statistically significantly different (p<0.5)

Table 5. Antimicrobial activity of *Parkia timoriana* leaf extracts

Microbial	Extracts				
	Methanol	Water	Ethyl acetate	Hexane	Positive control
Inhibition zone diameter gram-negative bacterial (mm)					
Bacillus subtilis	14±0.3aA	13±0.2aB	11±0.2aC	13±0.5aB	24±0.4aD
Escherichia coli	22±0.5bA	12±0.4bB	12±0.3bB	17±0.4bC	25±0.5bD
Salmonella typhi	15±1.0cA	10±0.2cB	10±1.0aB	20±0.4cC	23±0.2cD
Propionibacterium acnes	21±0.3dA	11±0.5dB	11±0.4aB	19±0.2dC	25±1.0aD
Inhibition zone diameter gram-negative bacterial (mm)					
Staphylococcus aureus	18±0.2eA	14±0.4aB	12±0.5bC	17±0.3eD	22±0.5dE
Pseudomonas aeruginosa	14±0.3aA	11±0.5dB	11±0.4bB	16±0.4fC	24±0.2aD
Salmonella typhi	17±0.1fA	10±0.3cB	10±0.4aB	14±0.5aC	25±0.3bD
Porphyromonas gingivalis	19±1.0eA	13±0.2aB	11±0.2bC	18±0.4gA	24±1.0aD
Inhibition zone diameter fungus (mm)					
Candida albicans	21±0.5dA	11±0.4dB	10±0.3aC	18±0.4gD	24±0.5aE
Aspergillus niger	18±0.3eA	12±1.0bB	9±0.5cC	16±1.0eD	22±0.3dE
Aspergillus flavus	19±1.0eA	10±0.5cB	9±1.0aB	14±0.3hC	24±0.2aD
Aspergillus fumigatus	17±0.2fA	9±0.2eB	7±0.4dC	13±0.5aD	23±0.4cE

Note: Data are mean ± standard deviation (n = 3). Data followed by the same capital or small letters are statistically significantly different (p<0.5)

Previous research has examined the potential antibacterial activities of *P. timoriana* bark (Sariwati et al. 2024) and seed (Suryanti et al. 2022) in n-hexane extract; however, the outcomes differ considerably. Zone inhibition values for *C. albicans* and *E. coli* from *P. timoriana* seeds are 16 an 24 mm, respectively. Zone inhibition values of *P. timoriana* seeds are 18 mm for *C. albicans* and 27 mm against *P. aeruginosa* (Sariwati et al. 2022).

The methanol extract contained flavonoids, alkaloids, and saponins and showed significant antibacterial activity against various microorganisms (Omekudo et al. 2022). Flavonoids bind to extracellular proteins of bacteria through hydrogen bonds and form a complex that prevents the bacteria's cell wall from bonding to microorganisms, inhibits enzyme activity, and functions as a transport protein for cells (Kumar and Pandey 2013).

Peptidoglycan hydrolase activity and the effectiveness of other cell wall-targeting antibiotics are closely linked. When peptidoglycan synthesis is inhibited, these enzymes can play a role in cell death; generally, they repair the cell wall during growth (Salamaga et al. 2021). Saponins generate antibacterial properties by interacting with and breaking down bacterial cell membranes through their amphiphilic properties. According to Dong et al. (2020), this disruption increases membrane permeability, which permits internal elements like proteins and enzymes to leak out and ultimately results in cell death.

Phenolic compounds exhibit antimicrobial activity through several mechanisms. They interact with bacterial cell structures, especially the cell membrane and proteins. The hydroxyl (-OH) group of phenolics forms hydrogen bonds with phospholipids or proteins of bacterial cell membranes, disrupting the membrane's structural integrity. By interacting with proteins, phenolics cause protein denaturation and coagulation, leading to the loss of protein function and inhibiting essential cellular processes. The disruption of the cell membrane and protein coagulation results in cell lysis, allowing the contents of the bacterial cell to leak out and further contribute to its death (Erviana and Purwono 2011; Rachmawaty et al. 2018). The main ways that tannins have antibacterial effects on bacteria are by destroying the integrity of bacterial membranes, inhibiting the development of cell walls, changing the permeability of cell membranes, and obstructing the pathways that create fatty acids. According to Farha et al. (2020), kojic acid, a related molecule, has shown that a free $-CH_2OH$ group at the C-2 position can dramatically affect the antibacterial activity against Gram-negative bacteria (Wu et al. 2018). Tannins utilize several techniques to prevent the growth of bacteria, making them promising antibacterial agents. Their several modes of action, which include iron chelation, membrane disruption, and protein synthesis suppression, are thought to be responsible for their effectiveness against a range of bacterial strains, including antibiotic-resistant ones (Farha et al. 2020). In conclusion, quantitative data on secondary metabolism support the antibacterial, antioxidant, and antidiabetic effects of parkia leaf methanol extract. The methanolic extract has the highest total phenol, flavonoids, alkaloids, tannins, and saponins among the other extracts.

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