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Phytochemical profile, antioxidant, antidiabetic, and antimicrobial activities of *Parkia timoriana* bark extracts

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Abstract. *Sariwati A, Suryanti V, Sari F, Kamei I, Trisnawati EW. 2024. Phytochemical profile, antioxidant, antidiabetic, and antimicrobial activities of* Parkia timoriana *bark extracts. Biodiversitas 25: 2427-2433.* Plants have various bioactive compounds that have pharmacological activity. *Kedawung* (*Parkia timoriana* (DC) Merr) is an indigenous plant in Indonesia that contains bioactive compounds. The leaves, bark, seeds, and roots of *P. timoriana* are commonly used in traditional medicine. The purpose of this study was to determine the chemical composition of *P. timoriana* bark extract and to assess its antioxidant, antidiabetic, and antibacterial activity. The bark of *P. timoriana* was extracted with various solvents that differ in polarity, such as water, methanol, ethyl acetate, and hexane. All extracts were investigated for antioxidant activities by 2,2-diphenyl-1-picrylhydrazyl) (DPPH) free-radical and 2,2'-azinobis (3-ethylbenz-thiazoline-6-sulfonic-acid) (ABTS) scavenging ability. The in-vitro antidiabetic activity was carried out by α-amylase and α-glucosidase inhibition. Antibacterial and antifungal activities were determined by the agar disc diffusion method. *Parkia timoriana* bark extracts contained alkaloids, flavonoids, steroids, tannins, terpenoids, saponins, reducing sugars, and cardiac glycosides. The ethyl acetate extract possessed the highest total phenolics, alkaloids, flavonoids, saponins, tannic acid, and terpenoids. Secondary metabolites in all extracts contributed to free radical scavenging, antidiabetic, antibacterial, and antifungal properties. The *P. timoriana* bark contains natural chemical compounds that might be useful as therapeutic agents for producing bioactive products and pharmaceuticals in the future.

Keywords: Antibacterial, antidiabetic, antifungal, antioxidant, *Parkia timoriana* bark

INTRODUCTION

Indonesia is one of the most biodiverse countries in the world, with over 6000 medicinal plants spread across the archipelago. Plants in Indonesia's tropical forests are diverse, and they contain high bioactive secondary metabolites (Rani et al. 2023). Secondary metabolites produced by plants, such as phenolics, flavonoids, alkaloids, saponins, and terpenoids, are components of the plant defense system but also have the potential to treat various diseases (Roslizawaty et al. 2023). Secondary metabolites are diverse in structure and have health benefits as antioxidants, antifungals, antivirals, antibacterial, antidiabetic, anti-inflammatory, and anticancer agents (Riaz et al. 2023). Exploration of nature's chemo-diversity is an essential and fascinating scientific topic since secondary metabolites play important roles in drug discovery and development. Secondary metabolite structural modification is frequently used to improve their bioactivities (Suryanti et al. 2018; Wang et al. 2019; Mohammed et al. 2023).

Parkia timoriana (DC) Merr belongs to the Leguminosae family and the Mimosoideae subfamily. The distribution range includes Indonesia, Thailand, Malaysia, Myanmar, and India. *Parkia timoriana* is known as *Kedawung* in Indonesia (Hadi et al. 2023). *Parkia* is an ethnobotanically significant multipurpose tree species that is a source of medicine (Mardiyanto et al. 2023), food and wood and has an ecological value. The plants possess α-glucosidase and α-amylase inhibitory properties, insecticidal activities, antioxidant, antibacterial, antiproliferative, and antidiabetic (Angami et al. 2018). The seeds of *P. timoriana* contained abundant protein (albumin and globulin), minerals (magnesium, zinc, potassium, iron, phosphorus, and manganese), essential amino acids (isoleucine, phenylalanine, leucine, and tyrosine), and fatty acids such as oleic and linoleic acid (Angami et al. 2018). The tree reduces soil erosion caused by heavy rains. The pods are consumed as a vegetable, and the ground is used as shampoo (Yusuf and Zuhud 2001; Hidayati et al. 2020). The seeds have a high nutritional value and are often used in traditional remedies as a combination of herbs for bacterial infection and gastrointestinal disorders. The seed extract is also used for contraception (Suryanti et al. 2022). The leaves and bark treat boils and sores (Yusuf and Zuhud 2001; Hidayati et al. 2020).

Parkia timoriana is rich in tannins, phenols, and flavonoids (Ruthiran and Selvaraj 2017). Methanol and acetone extract of *P. timoriana* pod contain phenolics and

flavonoids. The acetone extract of pods contained flavonol. Both extracts have a high reducing power and radical scavenging capacity (Angami et al. 2018). *Parkia timoriana* pods could be a potential source of pectin with high antioxidant activity based on 2,2-diphenyl-1-picrylhydrazyl (DPPH); 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS), and Ferric Reducing Antioxidant Power (FRAP) methods (Buathongjan et al. 2020). The ethyl acetate sub-fraction of *P. timoriana* pods is anti-hyperglycemic and hepatoprotective due to hyperin and epigallocatechin gallate. The leaf extract inhibited the growth of pathogenic bacteria such as *Escherichia coli*, *Vibrio cholerae*, *Staphylococcus aureus*, and *Bacillus cereus* (Zuhud et al. 2010; Angami et al. 2018). Gold and silver nanoparticles made from dried leaves of *P. timoriana* inhibited *S. aureus* (Paul et al. 2016). Except for *E. coli*, seed extract demonstrated substantial antibacterial activity against all pathogenic bacteria (Devi et al. 2007). Lectins isolated from the seed extracts of *P. timoriana* suppressed the development of malignant macrophage cell lines. *Parkia timoriana* seed oil extract has insecticidal effects (Angami et al. 2018).

The seeds of *P. timoriana* contain phenolics, flavonoids, alkaloids, saponins, tannins, terpenoids, and cardiac glycosides (Suryanti et al. 2022). The seed extracts have antioxidant properties, and the methanol extract possesses antibacterial and antifungal properties against *E. coli* and *Candida albicans*, as well as antidiabetic. This study aims to determine the chemical contents and evaluate the antioxidant, antidiabetic, and antibacterial activity of *P. timoriana* bark extracts.

MATERIALS AND METHODS

Materials

Chemicals of analytical grade were obtained from E-Merck. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Tokyo Chemical Industries (TCI, Tokyo, Japan). Gallic acid was purchased from Wako Pure Chemical Industries (Osaka, Japan).

Bacterial cultures

Bacillus subtilis NBRC 3009, *E. coli*, *S. aureus*, *Pseudomonas aeruginosa* NBRC 3080, *Salmonella typhy*, *Propionibacterium acne*, and *Prophyromonas gingivalis* (NITE Biological Resources Center, NBRC; Chiba, Japan), were provided by Department of Chemistry, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia. Each bacterial isolate was put into a 100-mL Erlenmeyer flask containing nutrient broth (60 mL). The culture was shaken for 20 h at 37°C before incubation (Sariwati et al. 2017).

Fungal cultures

Candida albicans, *Aspergillus niger*, *A. flavus*, *A. fumigatus* (NITE Biological Resources Center, NBRC; Chiba, Japan) were supplied by the Department of Chemistry, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia. They were cultivated at 37°C on Potato Dextrose Agar (PDA) media. A colony was inoculated into the nutrient broth (60 mL) in 100-mL Erlenmeyer flasks. Pre-incubation was carried out for 20 h at 37°C and 180 rpm (Sariwati et al. 2022).

Extract preparation

The bark of *P. timoriana* was collected in July 2021 in Cagar Alam Besowo, Kediri, Indonesia, which is located at a latitude of 07°51'51"-07°52'40" S and a longitude of 112°18'24"-112°18'30" E. The bark was cleaned with water, chopped into small pieces, and drained overnight. The dried bark was ground to a particle size of 25 mesh. Dried powder of samples (25 g) was macerated in 200 mL solvent at 180 rpm for 24 h. Water, methanol, ethyl acetate, and hexane were utilized as solvents. The filtrate evaporated to obtain concentrated extracts. The solvent-free extracts were transferred to vials and kept at 4°C for future use (Sariwati et al. 2019).

Phytochemical screening of *P. timoriana* bark extracts

Bark extracts of *P. timoriana* were analyzed for alkaloids, saponins, tannins, flavonoids, and triterpenoids (Abioye et al. 2013).

Total phenolic contents

Bark extracts of *P. timoriana* (20 mg) were dissolved in 5 mL of 3% HCl in 60% methanol. 100 μL of the solution was taken, added to aqueous sodium carbonate (2 mL), and left for 3 mins. After 3 min, the solution was added with Follin-Ciocalteu phenol reagent (100 μL) and incubated for 30 mins. The absorbance of the mixture was measured at 750 nm. Gallic acid solutions at 0.5, 1.0, 1.5, 2.0, and 2.5 mM concentrations were treated using the same procedures to get the calibration standard curve. The Total Phenolic Content (TPC) of the extracts was expressed as Gallic Acid Equivalent (GAE) mg.g^{-1} of extract (Sariwati et al. 2019).

Total flavonoid contents

The aluminum chloride colorimetry procedure was used to determine Total Flavonoid Contents (TFC) (John et al. 2014). Bark extracts of *P. timoriana* (1 mL) were dissolved in demineralization water (4 mL) in 10 mL volumetric flask. The mixture was added 0.30 mL 5% sodium nitrite and left for 5 mins, then added with 10% aluminum chloride (0.3 mL) and left for 5 mins. Then, the mixture was added with 2 mL sodium hydroxide 1M and aquadest up to 10 mL. Finally, the absorbance of the mixture was measured at 510 nm. Quercetin solutions at 20, 40, 60, 80, and 100 μg/mL concentrations were treated in the same procedures to get a calibration standard curve. Total flavonoid contents were expressed as milligram Quercetin Equivalent (QE) per 100 g Dry Weight (DW) (Sariwati et al. 2022).

Antioxidant activity by DPPH assay

DPPH (24 mg) was dissolved in methanol (100 mL), and its absorbance was measured at 517 nm. DPPH stock solution (1 mL) was mixed with *P. timoriana* bark extracts (33 μL) at various concentrations (10-500 μg/mL). The mixtures were left for 20 minutes, and the scavenging capacity was then assessed using Equation 1.

$$\text{Inhibition radical scavenging (\%)} = \frac{(\text{Control absorbance} - \text{Sample absorbance})}{\text{Control absorbance}} \times 100 \quad \ldots(1)$$

The IC_{50} value means the sample concentration needed to inhibit 50% of the DPPH free radical, calculated using Equation (2).

$$IC_{50} = \frac{\frac{1}{y^2} - \text{Weighted non–linear regression}}{\log(\text{Inhibitor concentration})} \quad \ldots(2)$$

Antioxidant activity by ABTS assay

ABTS and potassium persulfate were dissolved in aquadest to obtain 7 and 4.9 mM concentrations, respectively. These two solutions were mixed in equal amounts and kept at room temperature in the dark for 12-16 hours. The ABTS solution was added with aquadest until the absorbance was 0.7 at 734 nm. After adding the extracts (10 μL) to ABTS solutions (190 μL) in 96-well plates, the mixture was left for 30 mins at room temperature in the dark. The absorbance of the mixture was observed at 734 nm. Equation (3) was utilized to calculate the inhibition of ABTS radical scavenging (Jaafar et al. 2017).

$$\text{(\%) ABTS Scavenging} = \frac{[\text{Control absorbance(ABTS)} - \text{Sample absorbance}]}{\text{Control absorbance(ABTS)}} \times 100 \quad \ldots(3)$$

Antidiabetic activity by α-amylase inhibition assay

The α-amylase inhibition assay was performed using the 3,5-Dinitrosalicylic Acid (DNSA) method. Bark extracts of *P. timoriana* were dissolved in a minimum amount of 10% DMSO. Solutions were then added buffer ($(Na_2HPO_4/NaH_2PO_4$ (0.02 M) and NaCl (0.006 M) at pH 6.9 to get concentrations ranging from 10-1000 µg/mL. The α-amylase solution (200 µL) (2 units/mL) was mixed with extracts (200 µL) and incubated at 30°C for 10 mins. In addition, 200 μL of starch solution (1% in water (w/v)) was added and further incubated for 3 mins. After adding DNSA reagent (200 g), the reaction was stopped by heating at 85°C for 10 mins. The mixture was cooled until it reached room temperature, diluted with aquadest (5 mL), and its absorbance was measured at 540 nm. Equation 4 was utilized to calculate the α-amylase inhibitory activity as a percentage inhibition (Wickramaratne et al. 2016).

$$\text{α–amylase inhibition (\%)} = \frac{[\text{Control absorbance} - \text{Sample absorbance}]}{\text{Control absorbance}} \times 100 \quad (4)$$

Antidiabetic activity by α-glucosidase inhibition assay

A stock solution of α-glucosidase enzyme (0.2 U/mL) was prepared in 0.1M phosphate buffer (pH 6.8) and diluted to the required concentration in the same buffer for the assay. α-Glucosidase inhibitory activities were determined in 96-well microplate using *p*-Nitrophenyl-D-Glucopyranoside (PNPG) as a substrate (Kumar et al. 2013; Sheikh et al. 2016). The extract (25 μL) was mixed with an enzyme solution (0.5 U/mL of α-glucosidase in 0.1 M phosphate buffer, pH 6.8) and incubated at 37°C for 10 mins. The mixture was then incubated for 30 mins at 37°C with 25 μL of PNPG solution (0.1 mM PNPG in 0.1 M phosphate buffer, pH 6.8). After adding 100 μL of 0.2 M Na_2CO3, the absorbance of the solution was measured using A multi-well plate reader at 405 nm. Carbose served as the positive control (Sheikh et al. 2016).

Antibacterial activity

The antibacterial activity of the extracts was examined using the agar disc diffusion method against gram-negative bacteria (*B. subtilis*, *E. coli*, *S. typhi*, and *P. gingivalis*) and Gram-positive bacteria (*S. aureus, P. acne,* and *P. aeruginosa*). Petri dishes were filled with sterilized Nutrient Agar (NA), which was inoculated with 100 μL suspension of the investigated bacteria. Whatman filter paper (5 mm in diameter) was soaked in a bark extract solution in 10 mL DMSO and placed on a surface medium already inoculated with the tested bacteria. The Petri dishes were kept at 37°C for 24 hours. The antibacterial activity was evaluated by measuring the clear zone width (Sariwati et al. 2019).

Antifungal activity

The antifungal activity was conducted by agar disc diffusion procedure against *C. albicans, A. niger, A. flavus and A. fumigatus.* Steam bark extracts (5 mL) were poured into Petri dishes at various concentrations. Potato Dextrose Agar (PDA) was added to the dishes, and the solution was gently swirled to ensure proper mixing. A matured culture disc was inoculated at the center of plates and then incubated at room temperature for 7 days. The experiment was conducted in triplicates. The area of inhibition was measured daily for 7 days (Akwaji et al. 2016).

RESULTS AND DISCUSSION

Phytochemical analysis

The water, methanol, ethyl acetate, and hexane extract of *P. timoriana* bark had a percentage yield of 2.90±0.93, 0.82±0.72, 0.76±2.12 and 0.36±0.29 g, respectively. The aqueous extract had the highest percentage yield. Phytochemical analysis of all *P. timoriana* bark extracts revealed the presence of alkaloids, flavonoids, steroids, tannins, terpenoids, saponins, reducing sugars, and cardiac glycosides (Table 1). There were no different compositions of phytochemicals in all *P. timoriana* bark extracts.

Table 1. Phytochemical screening of *P. timoriana* bark extracts

Secondary metabolites	*P. timoriana* bark extracts			
	Water	Methanol	Ethyl acetate	Hexane
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Steroids	+	+	+	+
Tannins	+	+	+	+
Terpenoids	+	+	+	+
Reducing sugar	+	+	+	+
Cardiac glycosides	+	+	+	+

Note: (+): Present; (-): Not present

Phenolics and flavonoids were the two dominant secondary metabolites in all *P. timoriana* bark extracts. Ethyl acetate *P. timoriana* bark extract has the highest contents of total phenolics, alkaloids, flavonoids, saponins, tannic acid, and terpenoids, which were 62.86±0.15, 8.67±0.17, 21.08±0.59, 7.76±1.45, 2.13±0.65 and 4.38±1.27 mg/g, respectively (Table 2). Ethyl acetate is a polar aprotic solvent that can extract these compounds in high contents. Hexane *P. timoriana* bark extract has the lowest contents of total phenolics, alkaloids, flavonoids, saponins, tannic acid, and terpenoids.

Antioxidant activities

DPPH and ABTS radical-scavenging activity were used to assess the antioxidant activity of bark extracts (Table 2). The ability of a compound to donate an electron or a hydrogen atom to DPPH or ABTS to neutralize free radical compounds is utilized in antioxidant assays with DPPH and ABTS (Abu-Lafi et al. 2020; Dubey et al. 2020). An antioxidant's 50% inhibition concentration (IC_{50}) is needed to scavenge 50% of free radicals DPPH or ABTS. High antioxidant activity substances will have a low IC_{50} value.

The IC_{50} values for DPPH and ABTS from *P. timoriana* bark extracts in water, methanol, ethyl acetate, and hexane are presented in Table 3. The highest antioxidant activity was obtained in ethyl acetate extract, with the IC_{50} values in DPPH and ABTS were 66.630.28 and 78.720.64 g/mL, respectively. The IC_{50} values of *P. timoriana* ethyl acetate extract bark was 50-100 ppm, classified as a potent antioxidant. This result corresponds with the secondary metabolite contents of ethyl acetate extract. It can be attributed to compounds in the ethyl acetate extract.

Antioxidant compounds in plants are phenolics, alkaloids, saponins, and tannins (Suryanti et al. 2016, 2021, 2022). Both phenolics and alkaloids are major antioxidants in natural products (Salehi et al. 2019; Omar et al. 2022). Phenolic compounds have radical elimination capabilities due to their ability to donate hydrogen and form stable radical intermediates (Abu-Lafi et al. 2020). The quantity of hydroxyl groups in a phenolic molecule and its chemical structure determines its antioxidant activity. The impact of the structural chemistry of polyphenols on their ability to scavenge free radicals was studied by Rice-Evans et al. (1997). The antioxidative properties of polyphenol flavonoids have several mechanisms, such as chelating metal ions like copper and iron, scavenging free radicals, and blocking enzymes that produce free radicals (Górniak et al. 2019).

The synergistic interactions between phenolic and other antioxidant substances, such as alkaloids, may be linked to ethyl acetate extract's significant antioxidant activity. A wide range and diverse group of alkaloids share basic nitrogen (Thawabteh et al. 2019; Casciaro et al. 2020), which can be a primary, secondary, or tertiary amine. The unique bioactivity of alkaloids is attributed to the presence of nitrogen, capable of accepting a proton, and one or more amine-donating hydrogen atoms, usually accompanied by proton-accepting and -donating functional (Cho et al. 2009).

Antidiabetic activity

Antidiabetic activities of bark extracts were evaluated through α-amylase and α-glucosidase inhibitory assays. Table 4 shows the α α-amylase and α-glucosidase inhibitory potencies of water, methanol, ethyl acetate, and hexane extracts. Ethyl acetate extract had a low IC_{50} value in α-amylase analysis, was 43.14±1.23 µg/mL, and α-glucosidase analysis was 38.08±1.48 μg/mL. These results confirmed that ethyl acetate is an effective α-amylase and α-glucosidase inhibitor with IC_{50} between 25 and 50 μg/mL. On the contrary, hexane extract has the lowest IC_{50} value in α-glucosidase analysis (31.69±1.59 μg/mL) and the highest IC_{50} value in α-amylase analysis (135.57±1.78 μg/mL), indicating it is active as an α-glucosidase inhibitor and inactive as an α-amylase inhibitor.

It has been reported that alkaloids, phenolics, terpenoids, flavonoids, and saponins have antidiabetic activity. They significantly impact hypoglycemic activity, which contributes to the reduction of diabetes (Omar et al. 2022). Tannins promote glucose absorption while suppressing adipogenesis, potentially for Non-Insulin-Dependent Diabetic Mellitus (NIDDM) treatment (Sieniawska 2015).

Bitter melon enhances the glucose tolerance of normal and diabetic mice and in humans, with active components including steroids, momordicosides, acyl glucosyl sterols, fatty acids, amino acids, alkaloids, phenolics, steroidal saponin, vitamins, carbohydrates, and minerals (Tran et al. 2020). *Panax ginseng* root has been shown to have antidiabetic properties both in vitro and in vivo, where the root of *Panax ginseng* contains triterpene glycosides, saponins, Panaxans, amino acids, alkaloids, phenols, proteins, polypeptides, vitamins B1 and B2, vanillic acid, and salicylates (Tran et al. 2020). The antidiabetic activity of garlic has modulated insulin excretion from the cells and enhanced glucose tolerance and glycogen synthesis. Alkaloids, flavonoids, cardiac glycosides, terpenes, steroids, and resin are all found in raw garlic (Tran et al. 2020).

Table 2. Quantitative phytochemical analysis (mg/g) of *P. timoriana* bark extracts

Secondary metabolites (mg/g)	*P. timoriana* bark extracts			
	Methanol	Water	Ethyl acetate	Hexane
Phenolics	51.35±0.64	55.75±0.70	62.86±0.15	21.17±1.36
Alkaloids	3.23±1.02	2.36±0.37	8.67±0.17	1.45±0.48
Flavonoids	13.75±2.23	16.21±1.56	21.08±0.59	8.25±0.39
Saponins	3.82±0.21	4.31±0.53	7.76±1.45	3.54±0.49
Tannic Acids	1.42±0.87	1.59±2.14	2.13±0.65	0.27±0.83
Terpenoids	2.35±1.08	3.56±0.68	4.38±1.27	1.86±0.58

Table 3. The IC_{50} value for antioxidant activity of *P. timoriana* bark extracts by DPPH and ABTS analyses

Extracts	IC_{50}	
	DPPH method	ABTS method
Water	86.23±1.52	96.33±0.69
Methanol	106.85±1.24	127.33±0.74
Ethyl Acetate	66.63±0.28	78.72±0.64
Hexane	142.59±0.61	179.99±1.3

Table 4. The IC_{50} value for the antidiabetic activity of *P. timoriana* bark extracts

Extracts	IC_{50}	
	α-Amilase assay	α-Glucosidase assay
Water	54.68±0.43	47.91±2.64
Methanol	86.33±0.82	81.26±1.41
Ethyl Acetate	43.14±1.23	38.08±1.48
Hexane	135.57±1.78	31.69±1.59

Table 5. Diameter inhibition zone *P. timoriana* bark extracts against bacteria and fungi

Microbes	Antimicrobial activity of *P. timoriana* bark extracts				
	Methanol	Water	Ethyl acetate	Hexane	Gentamycin
Bacterial					
B. subtillis	16±1.24	15±0.35	16±0.50	23±1.00	25±1.32
E. coli	15±0.50	14±0.00	17±0.25	15±0.75	26±1.50
S. aureus	14±0.00	13±0.71	15±0.40	19±0.50	24±0.82
P.aeruginosa	16±0.50	15±0.20	18±0.50	27±0.20	26±0.00
S. typhi	15±1.28	13±1.25	16±0.00	18±0.84	28±0.71
P. acne	14±0.78	13±2.15	16±1.16	20±1.16	24±1.00
P.gingivalis	13±0.25	12±1.48	16±2.25	18±0.86	24±0.94
Fungi					
C. albicans	13±1.40	13±0.89	16±1.86	18±1.25	24±0.20
A. niger	12±0.88	10±0.94	13±0.54	16±0.80	26±1.10
A. flavus	10±1.75	12±0.00	12±0.28	17±0.60	25±0.50
A. fumigatus	8±0.84	8±0.12	10±0.60	16±0.40	26±0.84

Antimicrobial activity

Table 5 shows the antimicrobial activity of extracts against bacteria and fungi. Hexane extract demonstrated the highest zone inhibition of all bacterial and fungal tested, except *E. coli*. The highest diameter of inhibition against *E. coli* was obtained in the ethyl acetate extract (17±0.25 mm). Hexane extract demonstrated more than 20 mm inhibition zones against *P. aeruginosa*, *B. subtilis,* and *Propionibacterium acne,* which were 27±0.20, 23±1.00, and 20±1.16 mm, respectively. Hexane extract contains phytochemical compounds, such as alkaloids, flavonoids, steroids, tannins, terpenoids, saponins, reducing sugars, and cardiac glycosides, which synergistically affect antimicrobial properties.

The alkaloid's mechanism of action as an antibacterial agent differs between classes. There are mechanisms of antibacterial actions, such as inhibition of nucleic acid synthesis (Rao and Venkatachalam 2000), disturbance in the bacterial hemostasis, disturbing bacterial membrane integrity, the perturbation of the Z-ring, and cell division inhibition. The natural quinolone alkaloids act as respiratory inhibitors by reducing oxygen consumption in the treated bacteria (Thawabteh et al. 2019).

Flavonoids are hydroxylated phenolic compounds produced by plants in response to microbial infection. In vitro, they have antibacterial activities against a variety of pathogens. Polyphenolic flavonoids bind to bacterial cell walls and participate in biological processes (Hassan et al. 2020).

Steroids have antimicrobials, anticancer, and antidiabetic properties (Cox-Georgian et al. 2019; Manurung et al. 2019; Hassan et al. 2020). Steroids are substances used to increase bone marrow function and stimulate growth. It increases lean body mass and helps to prevent bone loss in older men (Amalraj and Gopi 2017). Steroids modify lipid membrane properties, so they interdigitate into membranes and change membrane fluidity. Steroids influence lipid segregation by changing the packing of the saturated lipid and thus promote or disrupt domain formation (Crowley et al. 2021). Tannins are commonly hazardous water-soluble antibacterial agents due to their potential to form metal complexes (Cano et al. 2021). Tannins can prevent the growth of various microorganisms, including fungi, yeasts, bacteria, and viruses. Tannins have a variety of properties, including analgesic, cardioprotective, anticancer, antidiabetic, anti-inflammatory, anti-carcinogenic, and anti-mutagenic. Tannins can be an effective kidney relief treatment (Sieniawska 2015).

Terpenes are antibacterial agents, and their action is the weakening of microbe tissue and cell walls. Saponins serve various roles, including the ability to precipitate and coagulate red blood cells and bind cholesterol. Saponins have traditionally been used as detergents and molluscicides due to their capacity to generate foam in aqueous solutions and their hemolytic effect. Saponins, in addition to their industrial applications as foaming and surface-active agents, have medicinal uses against various diseases (Olas et al. 2020).

Parkia timoriana bark extracts possess alkaloids, flavonoids, steroids, tannins, terpenoids, saponins, reducing sugars, and cardiac glycosides. The highest content of secondary metabolites was obtained in ethyl acetate extract. All *P. timoriana* extracts showed antioxidant, antidiabetic, antibacterial, and antifungal activities. Ethyl acetate extract has the highest antioxidant activity. Hexane extract demonstrated the highest zone inhibition against all bacterial and fungal tested, except for *E. coli*. The highest inhibition of *E. coli* was obtained from ethyl acetate extract. *Parkia timoriana* bark could potentially be a new source of medicinal ingredients.

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