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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 TAE/g; 50.12-91.02 mg Linalool Ekivalent/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_{50} 28.13µg/ml for DPPH and 45.39 µg/ml for ABTS. Antibacterial 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_{50} α-amylase inhibition (25.35 µg/ml), and IC_{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

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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 -glucoside. (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 (H_2SO_4), 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 respites 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–VISIBLE 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 H_2SO_4 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 DXE/g extract) (Komonlakorn *et al.* 2020).

2.13. Antioxidant

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:

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

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.

2.13.2. Antioxidant Activity 2,2′-Azino-bis (3-ethylbenzothiazoline-6 Sulphonic Acid) (ABTS) Assay

With distilled water, ABTS and potassium persulfate were dissolved to yield concentrations of 7 and 4.9 mM, respectively. Prior to use, these two solutions were mixed in equal volumes and stored in the dark at room temperature for 12-16 hours. The ABTS solution was dissolved in distilled water until the absorbance at 734 nm reached 0.7. Extract (10-100 µg/ml) (10 µl) was added to a 96-well plate with ABTS solution (190 µl) and reacted in the dark at room temperature for 30 minutes. The control Trolox (10-100 µg/ml) was excited as devoid of sample extracts. The absorbance of the mixture was recorded at 734 nm. The following formula was used to calculate ABTS radical scavenging inhibition (Jaafar *et al.* 2017).

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

The IC_{50} values meaningful the concentrate of sample requisite to scratch 50% of the ABTS 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 to indicate antioxidant capacity was calculated by 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 -amylase inhibition assay. *P. timoriana* seed extract was dissolved in a minimum amount of 10% DMSO and then in buffer ((Na_2HPO_4/ $NaCl_2PO_4$ (0.02 M), NaCl (0.006 M) at pH 6.9, to give concentrations ranging from 10 to 1000 µg/ml. A 200 µl α-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 α-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 -amylase inhibition against the extract concentration (Wickramaratne *et al.* 2016).

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

The IC_{50} values meaningful the concentrate of sample requisite to scratch 50% of the α-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 -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 Na_2CO_3 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 IC_{50} values meaningful the concentrate of sample requisite to scratch 50% of the ABTS free radical were measured by systematizing $IC_{50} = \frac{50 - a}{b}$, 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.

2.15. 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, *Prophyromonas 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 of 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 Ekivalent/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 IC_{50}, 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 IC_{50} concentrations of DPPH (28.13 µg/ml) and ABTS scavenging (45 µg/ml) shown in Table 2.

Table 1. Qualitative phytochemical screening of different extracts of *Parkia timoriana* (DC.) Merr seeds

Phytochemical*	Extracts solvent of *Parkia timoriana* (DC.) Merr seeds			
	Methanol	Distilled water	Ethyl acetate	*n*-Hexane
Alkaloid	+	+	+	+
Flavonoids	+	+	+	+
Steroids	+	-	+	+
Tannins	+	+	+	±
Terpenoids	+	+	+	+
Saponins	+	+	+	+
Reducing sugar	-	+	-	-
Cardiac Glycosides	+	+	+	+
Anthraquinones	+	+	-	+
Coumarin	-	-	-	-
Anthocyanins	-	-	-	-

*The designed phytochemical represented as abundant (+), trace (±) and absence (-)

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 IC_{50} 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

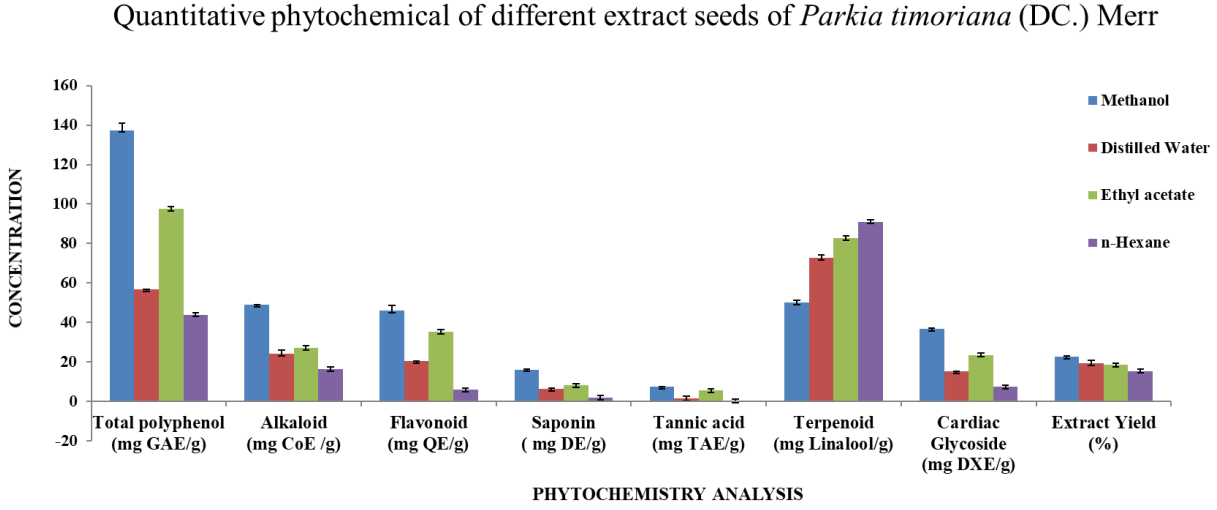

Figure 1. Quantitative phytochemical analysis of different extracts of *Parkia timoriana* (DC.) Merr seeds

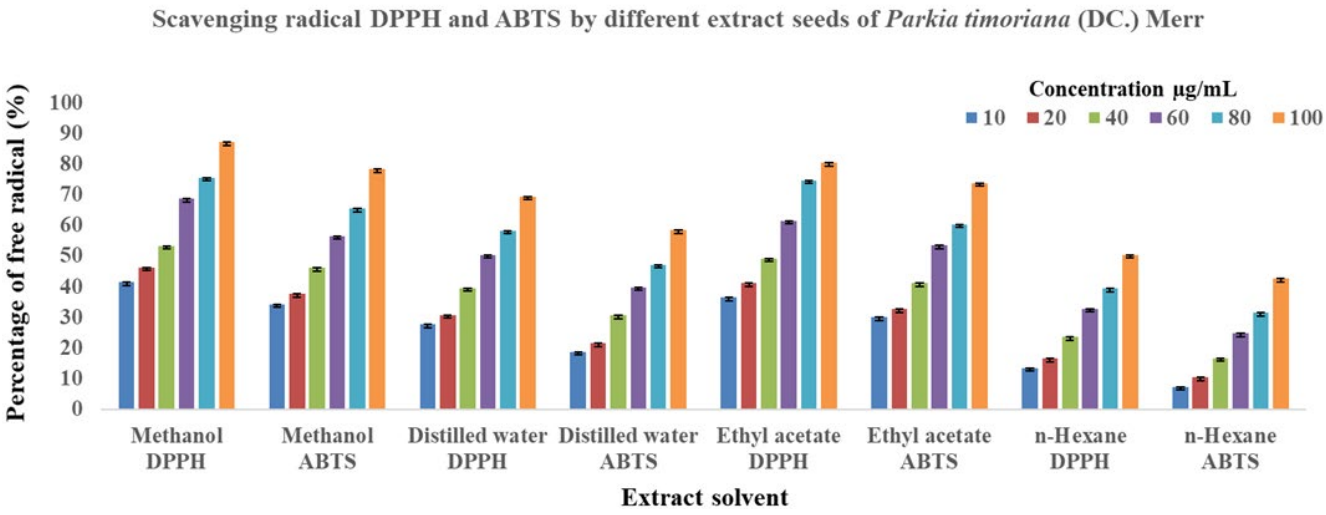

Figure 2. Percentage of scavenging radical DPPH and ABTS by different extract seeds of *Parkia timoriana* (DC.) Merr

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

Extract solvent	Antioxidant IC_{50} µg/ml		Antidiabetic IC_{50} µg/ml	
	DPPH	ABTS	α-Amylase	α-Glukosyde
Methanol	28.13±0.68^{aA}	45.39±0.45^{bA}	25.35±0.66^{aA}	23.04±1.81^{aA}
Distilled water	37.56±0.17^{aB}	54.86±1.50^{bB}	74.51±0.54^{aB}	73.71±2.10^{aB}
Ethyl acetate	60.22±1.14^{aC}	83.40±2.10^{bC}	28.45±0.54^{aC}	26.72±2.10^{aC}
n-Hexane	102.76±0.20^{aD}	124.39±0.25^{aD}	135.33±1.28^{aD}	131.59±1.05^{bD}

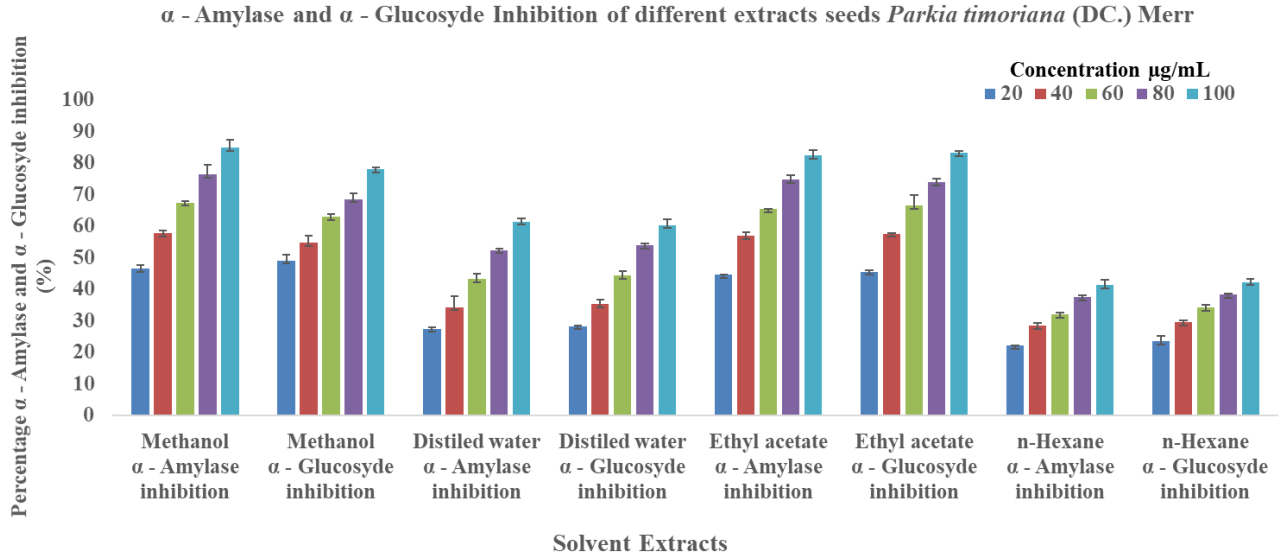

Figure 3. Percentage of α-Amylase and α–Glukosyde inhibition and by different extract seeds of *Parkia timoriana* (DC.) Merr

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

Antimicrobial	Extracts solvent of *Parkia timoriana* seeds (10 mg/ml)				
	Methanol	Distilled water	Ethyl acetate	*n*-Hexane	Positive control (10 mg/ml)
Inhibition zone diameter bacterial (mm) with positive control by Ampicillin					
Bacillus subtillis	12±0.71^{aA}	14±0.00^{bA}	11±1.22^{cA}	18±0.71^{d}	22±1.22^{eA}
Escherichia coli	13±1.48^{aA}	17±0.71^{bB}	12±0.71^{cA}	24±0.71^{d}	22±1.00^{eA}
Staphylococcus aureus	11±0.71^{aB}	12±1.00^{bC}	10±0.00^{cB}	20±0.84^{d}	24±0.00^{eB}
Pseudomonas aeruginosa	14±1.22^{aC}	15±0.48^{bD}	8±0.00^{cC}	18±0.00^{d}	22±0.84^{eA}
Salmonella typhi	11±0.00^{aA}	13±0.00^{bC}	9±1.22^{cC}	16±1.00^{d}	24±0.00^{eB}
Propioni bacterium acne	13±0.84^{aA}	16±0.71^{bB}	10±0.84^{cA}	20±0.71^{d}	24±1.48^{eB}
Prophyromonas gingival	11±0.00^{aA}	11±0.94^{aC}	7±0.00^{bD}	15±0.00^{c}	20±0.71^{dC}
Inhibition zone diameter bacterial (mm) with positive control by Ketoconazole					
Candida albicans	11±0.71^{aA}	12±0.71^{b}	8±1.00^{cA}	16±0.71d^{A}	20±0.00^{eA}
Aspergillus niger	10±0.89^{aA}	11±1.22^{a}	7±0.94^{bA}	14±0.84^{cB}	22±1.00^{dA}
Aspergillus flavus	8±0.94^{aB}	9±0.00^{b}	6±0.71^{cA}	12±0.00^{dC}	24±0.71^{eB}
Aspergillus fumigatus	10±0.00^{aA}	10±0.00^{a}	9±0.00^{bA}	15±0.00^{dD}	22±0.94^{eC}

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)

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 presence alkaloids, flavonoids, tannins, saponin, terpenoids (Ralte *et al.* 2022). Reducing sugar presence only in the distilled water extract. Anthraquinones were not present 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.* 2018). The results of this study are not much different from previous studies of 40.2 mg/g by (Dubey *et al.* 2020. Flavonoid presence and high yield justified the potential for radical scavenging is relatively high (antioxidant properties). According to recent research, many flavonoids and related polyphenols significantly contribute to overall antioxidant activity (Adaramola *et al.* 2012; Djeridane *et al.* 2006). Flavonoids are commonly used as plant flavorings. Aside from their function as flavoring agents, they are also expressed in plants in response to microbial infection, implying antimicrobial activity (Kujumgiev *et al.* 1999). Flavonoids are involved as antioxidants in both physiological and pathological conditions (Kumar *et al.* 2013). Tea flavonoids, for example, have been shown to reduce the oxidation of low-density lower the level of cholesterol and triglycerides in the blood (Erdman *et al.* 2007).

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 CH_3OH, is polar because it has a hydroxyl group (-OH), and is non-polar because it has a methyl group (-CH_3) (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 greatest tannic acid in methanol extract (7.29 mg/g). Tannins are complex mixtures of highly polar compounds (Cardullo *et al.* 2018). Tannins are phenolic compounds that have been shown to act through iron sequestration, hydrogen bonding, or specific interactions with vital proteins like enzymes (Adaramola *et al.* 2012) Tannins are powerful antioxidants in addition to their antimicrobial and anticancer properties (Adaramola *et al.* 2012). Plants containing tannins are generally astringents and are

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 antibacterial 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 amphipathic 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_{50} values than Trolox. The computed IC_{50} values for the methanol extract (28.13 µg/ml). (Wu *et al.* 2010) reported IC_{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_{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 $ABTS^{\bullet+}$, IC_{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•-scavenging 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• 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+• radical cation however needs an electron (e) to neutralize the positive charge, and ABTS+• scavenging is considered an electron (e) transfer reaction. Antioxidant compounds can effectively scavenge $ABTS^{+\bullet}$ 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^{+}- reducing power assay. The reducing reaction is actually can take place in two different ways: (i) a single electron transfer (SET) process followed by deprotonation of the formed radical cation or (ii) deprotonation followed by a SET process from the formed anion, it is called sequential electron-proton transfer (SEPT). Even though SEPT and PCET yield the same products as HAT, the influence of the solvent and of the nature of the reacting radical on their feasibility is expected to be different. While a SEPT mechanism is only possible in a polar environment that promotes solvation of the intermediate ionic species, the PCET mechanism may also be viable in a nonpolar medium since the charge separation is smaller than in SEPT (Li *et al.* 2015).

In order to scavenge free radicals, phenolic compounds can donate electrons. The number and position of the hydroxyl group on the aromatic ring binding site, as well as the type of substituents on phenolic compounds, all have an impact on antioxidant activity (Ja'afar *et al.* 2017). By transferring a hydrogen atom from its hydroxyl group, phenolic compounds reduce or inhibit free radicals. A phenolic compound's reaction mechanism with a peroxyl radical (ROO•) involves a concerted transfer of the hydrogen cation from the phenol to the radical, forming a transition state of an H-O bond with one electron. When the reaction medium contains a solvent that is prone to the formation of hydrogen bonds with the phenolic compounds, the antioxidant capacity of the phenolic compounds is greatly reduced (Santos-Sánchez *et al.* 2019).

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 α-glucosidase in the small intestine (Sim *et al.* 2010). α-amylase inhibition studies have shown that *P. timoriana* seed extracts (crude methanol, ethyl acetate, distilled water, and *n*-hexane) have significant inhibitory potential. The IC_{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 α-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 alpha-glucosidase than for alpha-amylase. In general, the order of inhibition is flavonoids >biflavonoids >xanthone >tannin for αglucosidase, and flavonoids >tannins >xanthones for αamylase (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 introduction of a 24-hydroxyl group attenuates the inhibition (Zhang *et al.* 2017). 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. timoriana* 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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