

Antioxidant, Antidiabetic, and Antibacterial Activities of *Terminalia bellerica* Seed Extracts in Various Solvent Polarities

Atmira Sariwati^{1*}, Fita Sari², Venty Suryanti³, Desi Suci Handayani³, Ichiro Kamei⁴, Ninis Yuliaty⁵

¹Department of Tiongkok Traditional Medicine, Faculty of Health Science, Institut Ilmu Kesehatan Bhakti Wiyata Kediri, Kediri 64114, Indonesia

²Department of Pharmaceutical and Food Analysis, Faculty of Pharmacy, Institut Ilmu Kesehatan Bhakti Wiyata Kediri, Kediri 64114, Indonesia

³Department of Chemistry, Faculty of Mathematics and Natural Sciences, Sebelas Maret University, Surakarta 57126, Indonesia

⁴Department of Forest and Environmental Science, Faculty of Agriculture, University of Miyazaki, 1-1, Gakuen-kibanadai-nishi, Miyazaki 889-2192, Japan

⁵Department of Pharmaceutical, Faculty of Pharmacy, Institut Ilmu Kesehatan Bhakti Wiyata Kediri, Kediri 64114, Indonesia

ARTICLE INFO

Article history:

Received January 26, 2024

Received in revised form March 28, 2024

Accepted April 2, 2024

KEYWORDS:

antioxidant activity,
antidiabetic activity,
antibacterial activity,
Terminalia bellerica seeds,
secondary metabolite

ABSTRACT

Terminalia bellerica is well-known for producing edible fruits with pharmacotherapeutic properties. Traditional healers use these species to treat and control diabetes mellitus, its side effects, and other illnesses. Involved in this disease's pathophysiological process, extensive research has been conducted to validate and comprehend these bioactive claims scientifically. This research aims to ascertain the bioactive metabolite contents of different solvent polarities, including ethyl acetate, hexane, distilled water, and methanol. The phenol concentration was determined using the Follin-Ciocalteu procedure to be between 23.45 and 160.41 mg GAE/g. The aluminum chloride colorimetric technique measured flavonoid concentration from 88.52 to 7.12 mg QE/g. The quantitative values of tannic acid, which spanned from 0.78 to 5.32 mg TAE/g, were determined by spectrophotometry UV-VIS. The extracts' capacity to reduce free radical damage ABTS (2, 2' azinobis (3-ethylbenzene-thiazoline-6-sulfonic-acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) was examined. The extract ethyl acetate exhibited the most significant level of antioxidant activity, with IC₅₀ values for DPPH and ABTS of 28.17 and 22.22 g/ml, respectively. *Staphylococcus aureus* (23 mm) and *Aspergillus flavus* were tested for antibacterial and antifungal activity in a methanol extract (21 mm). *In vitro*, antidiabetic activities were assessed using α -glucoside and α -amylase inhibition. The ethyl acetate extract has α -glucoside inhibition IC₅₀ of 23.04 g/ml and α -amylase inhibition IC₅₀ of 25.35 g/ml. *T. bellerica* seed includes secondary metabolites that show promise as lead chemicals in creating potent medications.

1. Introduction

These days, herbal remedies are widely used and, to differing degrees, recognized as significant supplemental and alternative therapies in many nations since they have very little or no adverse side effects. Medicinal plants cure various acute, chronic, and degenerative illnesses, such as neurodegenerative conditions, diabetes, cardiovascular disease, and cancer (Zhang *et al.* 2021).

Degenerative diseases arise due to cell oxidative stress because of the number of free radicals that continue to increase in the body. The increase in free radicals is a chronic inflammatory process, and this provocative response results from innate immunity responding to bacteria, fungi, and viruses (Leyane *et al.* 2021). Antioxidant substances neutralize free radicals and prevent the oxidative processes that cause diabetes mellitus (antidiabetic), which are preventive for chronic inflammation (antimicrobial) (Sariwati *et al.* 2019).

One of the most valuable sources of natural antioxidants is plants as bioactive compound reservoirs (Salem *et al.* 2021). Phytoactive substances

* Corresponding Author

E-mail Address: atmirasari@gmail.com

include tannins, polyphenols, flavonoids, and alkaloids (Prasathkumar *et al.* 2021). Phytomedicines are substances derived from medicinal plants' flowers, seeds, fruits, roots, bark, leaves, and fruits (Abate *et al.* 2022).

Indonesia is a mega-biodiversity country that has diverse living and non-living environments. Due to its equatorial location, Indonesia has a tropical environment that makes it feasible to grow various medicinal plants that might one day be fully developed and put to good use. *Terminalia bellerica* is one of the medicinal herbs that Indonesia frequently uses (Setyawati 2009). Fruit and bark as material health solutions or tonics (body warmers) are generally used by women (Gupta *et al.* 2020). In Bangladesh, the fruit of *T. bellerica* is used to treat menstrual disorders (Mallik *et al.* 2012) and is effective in treating bronchitis, asthma, hepatitis, piles, diarrhea, eye condition, dyspepsia, hair tonic, hoarseness of voice, and is effective in treating bronchitis, asthma, hepatitis, piles, diarrhea, eye condition, dyspepsia, hair tonic, hoarseness of voice, and are also used as a scorpion stings in India (Singh 2011). The green fruit's infusion works well to cure coughs (Deb *et al.* 2016). Leprosy, dropsy, piles, and diarrheal illness are all managed with fruit pulp (Singh *et al.* 2018).

Based on the information above, there have been no scientific reports on seeds from *Terminalia bellerica*, so this study used solvents with different polarities to identify the bioactive components in seeds.

2. Materials and Methods

2.1. Chemicals

Dimethyl sulfoxide (DMSO), methanol, ethyl acetate, *n*-hexane, ferric chloride, sulfuric acid (H₂SO₄), cultures of media (nutrient broth and nutrient agar), hydrochloric acid (HCl), glacial acetic acid, and chloroform, which were procured from Merck, were the substances employed in this investigation. DPPH was acquired through Tokyo Chemical Industries (TCI), Tokyo, Japan. We received gallic acid through Wako Pure Chemical Industries (Osaka, Japan).

2.2. Circumstances for Microbial Cultivated

2.2.1. Bacterial

Salmonella typhi NBRC 14193, *Escherichia coli* NBRC 3301 (NITE Biological Resources Center, NBRC;

Chiba, Japan), *Shyggella flexneri* ATCC 29903 (American Type Culture Collection), *Pseudomonas aeruginosa* (NBRC 3080), *Bacillus subtilis* (NBRC 3009), *Klasiella pneumonia* (NBRC 3319), *Staphylococcus aureus* (NBRC 102135), *Corynebacterium diphtheriae* NCTC 11379T (National Collection of Type Cultures). It was obtained from the Laboratory of Microbial Chemistry Collection Institut Ilmu Kesehatan Bhakti Wiyata Kediri, 100 ml Erlenmeyer flasks with 60 ml of nutritious broth medium were used to cultivate the colony. The culture underwent a 20-hour shaker incubation at 37°C (Sariwati *et al.* 2017).

2.2.2. Fungal

Aspergillus fumigatus NBRC 4057 (NITE Biological Resources Center, NBRC; Chiba, Japan), *Aspergillus flavus* NBRC 4186, and *Aspergillus niger* NBRC 5376. The source of this was the Laboratory of Microbial Chemistry Collection, Institut Ilmu Kesehatan Bhakti Wiyata Kediri. Heat-treated to 37°C, a petri dish measuring 9 cm was used to culture the fungus using PDA (potato dextrose agar, Merck, Darmstadt, Germany). Next, an injection was made into the colony into each of the 100-ml Erlenmeyer flasks after 60 ml of nutrient broth medium had been added. The culture underwent a 20-hour preincubation phase at 37°C and 180 rpm shaker speed (Sariwati and Purnomo 2018).

2.3. Sample Processing

Plant samples were gathered at Kediri, Indonesia's East Java. After being washed in purified water, the seeds of *T. bellerica* were cut into small pieces and let to drain overnight at room temperature. Then, a grinder was used to grind them into a particle mass of 25 mesh.

2.4. Seed Extraction from *T. bellerica*

A 200-milliliter ethyl acetate, *n*-hexane, methanol, and distilled water were then added to a 500 ml flask that held 20 g of dry powder samples. The flask was then securely sealed with aluminum foil. After extraction, an orbital shaker set to spin at 180 rpm for a whole day was used to clean the residues. Whatman No. 1 filter paper is used. Lower temperatures were used to dry the extracts than the solvent's boiling point in a rotatory evaporator to produce drained extracts (Santoso *et al.* 2021). Distilled water, methanol, ethyl acetate, and *n*-hexane have boiling points of 100, 65, 77, and 68°C, respectively. Without the need for solvents, the extracts were put into

extraction vials and kept at 4°C for subsequent use (Sariwati *et al.* 2019).

2.5. Introductory Phytochemical Screening for *T. bellerica*

By exposing seeds to various extracts, *T. bellerica* was phytochemical analyzed for the existence of triterpenoids, saponins, flavonoids, alkaloids, and tannins, using a standardized method (Amalia and Sariwati 2019; Sariwati *et al.* 2022; Suryanti *et al.* 2022).

2.6. Phenolic Concentration

To preserve *T. bellerica* extracts (20 mg), a dispersant consisting of 60% methanol and 5 ml of 3% HCl was added to the relevant alloy (100 L). After three minutes, 100 L of Follin-Ciocalteu phenol reagent was applied to the alloy. Following a 30-minute incubation period, the absorbance at 750 nm was measured with a reference. Calibration curves for concentrations of gallic acid of 2.5, 2.0, 1.5, 1.0, and 0.5 mM were developed using comparable techniques. Gallic acid equivalents (GAE) mg per gram of extract were employed to represent the results (Sariwati *et al.* 2019; Suryanti *et al.* 2022).

2.7. Flavonoid Concentration

The colorimetry of the aluminum chloride technique was used to determine the flavonoid concentration of each extract. The calibration curve and 1 ml of crude extracts were treated by alloying the extracts with denaturing water (4 ml) at denaturing concentrations of 100, 80, 60, 40, and 20 g/ml. A 0.30 cc addition of sodium nitrite (5%) in the flask. Five minutes later, 0.3 ml of 10% aluminum chloride was added to the mixture. After five minutes of waiting, sodium hydroxide 1M (2 ml) was combined, and 10 ml of demineralization water was used to create volume. The absorbance was measured for a blank after alloying the suspension at 510 nm (Sariwati *et al.* 2022; Suryanti 2022).

2.8. Tannic Acid Concentration

One milliliter of the reagent combination was introduced into around 200 microliters of the excerpt from the sample or the standard solution of tannic acid (50–300 grams per milliliter) (8% concentrated HCl in methanol and 4% vanillin in methanol, in a 1:1 ratio). The color obtained was evaluated at 500 nm following a 20-minute incubation period at room

temperature. Umdale *et al.* (2021) state that The outcome was given as mg TAE/g.

2.9. Antioxidant

2.9.1. Activity of Antioxidants (Scavenging of DPPH Radicals)

DPPH (24 mg) and methanol (100 ml) were used to create the assortment solution. The absorbance at 517 nm was measured using a spectrophotometer. *T. bellerica* seed extracts (33 ml) were combined with 1 milliliter of stock DPPH solution at various doses (10–100 g/ml). Store the mixture in the dark for 20 minutes at room temperature after shaking it to stir the reaction. Gallic acid (10–100 g/ml) used as the control was eliminated because sample extracts at various concentrations were absent. The scavenging capability was determined using the DPPH radical scavenger percentage formula.

$$\text{Scavenging of radicals in suppressive (\%)} = \frac{[\text{Control absorbance} - \text{Sample absorbance}]}{\text{Control absorbance (ABTS)}} \times 100$$

It systematically measured the IC₅₀ numbers, which stand for the concentration of the sample appropriate to scavenge half of the ABTS free radical IC₅₀ = (50 - a) / b, by utilizing a variable slope, the values of a and b were standard response patterns (Sariwati *et al.* 2019). The IC₅₀ numbers, representing antioxidant capability, were determined by an equation for linear regression between the sample concentration and the percentage of inhibition.

2.10. Antidiabetic

2.10.1. Suppression of α -amylase

The 3, 5-dinitrosalicylic acid (DNSA) procedure was applied to perform the α -amylase inhibition experiment. Before adding the *T. bellerica* seed extract to a buffer, it was dissolved in at least 10% DMSO (NaCl (0.006 M, Na₂HPO₄ / NaCl₂PO₄ (0.02 M)) at pH 6.9 to produce concentrations varied from 10 to 1,000 g/ml. 200 μ l of extract and 200 μ l solution of α -amylase (2 units/ml) were combined at 20–100 μ g/ml as a single concentration), and the mixture was then allowed to incubate at 30°C for 30 minutes. After that, each tube received 200 μ l of a 1% starch solution in water (w/v), and the tubes were incubated for three minutes. DNSA reagent in 200 μ l (12 g of sodium potassium tartrate tetrahydrate in 8.0 ml of 2 M NaOH and 20 ml of 96 mM 3,5-dinitro salicylic

acid solution) was added to the reaction to end it. A UV-Vis spectrophotometer was required to measure the liquid's wavelength at 540 nm after it had been diluted with 5 ml of distilled water and allowed to come to room temperature. A blank exhibiting 100% enzyme activity was generated by substituting 200 µl of buffer for the plant extract. A blank reaction was created using the plant extract without the enzyme solution at each concentration. Acarbose (20–100 µg/ml), the positive control, was eliminated since it contained no sample extracts. The following equation has to be used to calculate the percentage inhibition of activity of α -amylase inhibitors. By graphing the extract concentration vs the percentage of α -amylase inhibition, the IC_{50} numbers were determined (Wickramaratne *et al.* 2016).

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

Systematically measuring the IC_{50} numbers, which indicate the necessary sample concentration to scuff half of the inhibition caused by α -amylase $IC_{50} = (50 - a) / b$ - Using a variable slope, the numbers of a and b were standard response patterns (Sariwati *et al.* 2019). Through the use of a regression that is a linear formula among the percentage of reticence and the concentration of the sample, the IC_{50} value, which serves as a measure of antidiabetic capacity, is determined.

2.10.2. Suppression of Alfa Glucosidase

After creating a stock solution in phosphate buffer 0.1 M (pH 6.8) at 0.5 U/ml, in the same buffer, the α -glucosidase enzyme was diluted to the proper concentration for the test. Nitrophenyl-D-glucopyranoside (PNPG) was used as a substrate, and the alpha-glucosidase inhibitory activity was quantified spectrophotometrically utilizing the previously described preincubation technique (Kumar *et al.* 2013; Sheikh *et al.* 2016). The 96-well microplate reader was used for this assessment. To put it briefly, 25 µl of the test sample was combined with a solution of enzymes (0.5 U/ml of α -glucosidase in 0.1 M phosphate buffer at pH 6.8), and the combination was incubated at 37°C for ten minutes. Following that, 25 µl of PNPG solution (0.5 mM PNPG in phosphate buffer 0.1M, pH 6.8) was added to the mixture for incubation.) for 30 minutes at 37°C. A multi-well plate reader was applied to adjust the absorbance at 405 nm after adding 100 µl Na_2CO_3

solution (0.2M) to stop the reaction. The unregulated enzyme functioned as the negative control (DMSO control), whereas 20–100 µg/ml of acarbose was the positive control. Three different experiments were conducted to run the test (Sheikh *et al.* 2016; Suryanti *et al.* 2022).

$$\alpha\text{-glucosidase Suppression (\%)} = \frac{[\text{Control absorbance} - \text{Sample absorbance}]}{\text{Control absorbance}} \times 100$$

The IC_{50} numbers were calculated by systematizing the sample concentration needed to scrape half of the α -glucosidase inhibition $IC_{50} = (50 - a) / b$. Using a variable slope, the values of a and b were standardized in the response pattern (Sariwati *et al.* 2019). An equation for linear regression was applied to apprise the IC_{50} numbers or antidiabetic ability, the sample's concentration, and the level of inhibition.

2.11. Antimicrobial Efficacy

2.11.1. Antibacterial

The agar disk diffusion procedure was employed for antibacterial examination *T. ballerica* extract was used to calculate the width of restriction areas against different bacterial strains (including gram-negative bacteria like *Salmonella typhi* NBRC 14193, *Escherichia coli* NBRC 3301, *Bacillus subtilis* NBRC 300, and *Shyggella flexneri* ATCC 29903 and *Klebsiella pneumoniae* NBRC 3319). Gram-positive bacteria include *Pseudomonas aeruginosa* NBRC 3080, *Staphylococcus aureus* NBRC 102135, and *Corynebacterium diphtheriae* NCTC 11379T. The examined bacteria were injected with a 100 µl suspension of nutrient agar (NA), which had been sterilized, into each Petri plate. Whatman filter paper discs of 5 mm in diameter were produced and adsorbed in a 100-microliter extract solution with 10 milligrams per milliliter of DMSO (Sariwati *et al.* 2019). Positive controls were frequently employed, usually ampicillin (10 mg/ml). Incubation of the plates lasted for twenty-four hours at 37°C to promote the most bacterial outgrowth possible. Using an area reader to measure the limitation zones' breadth (in ml), the antibacterial capacity was ascertained (Sariwati *et al.* 2019; Suryanti *et al.* 2022).

2.11.2. Antifungal

The screening for antifungals procedure was performed using the agar disk diffusion technique, and *T. bellerica* extract was used to measure the

breadth of restriction zones in opposition to different bacterial strains (*Aspergillus flavus* NBRC 4186, *Aspergillus niger* NBRC 5376 and *Aspergillus fumigatus* NBRC 4057). First, *T. bellerica* seed extract (10 mg/ml) with 5 ml of each concentration was drawn using a sterile syringe and, after that, was added to various Petri dishes. Positive controls frequently cited as an example were ketoconazole (10 mg/ml). Following the placement of the solvent extracts onto the plates, Potato Dextrose Agar (PDA), which is sterile, was added, and to guarantee adequate mixing, the plates were gently stirred. After the medium has dried, drill a hole with a clean cork (5 mm in diameter). Four-day-old pure cultures were used to create mature culture discs, which were then injected in the middle of plates and cultured for seven days at ambient temperature (28°C). There were three runs of the experiment. Daily measurements were made of the inhibitory area for seven days using a meter rule. (Akwaji *et al.* 2016; Suryanti *et al.* 2022).

2.12. Analytical Statistics

The figures represented the average of the triplet calculation. Any representational discrepancy among groups during substrate transformation was found by applying a student's t-test. Excel software demonstrated that process dissimilarity was broadly representative statistically at a degree of bravery of 5% (P 0.05) (Suryanti *et al.* 2022).

3. Results

3.1. Phytochemically

Four extracts of *T. bellerica* seeds were subjected to phytochemical screening, and flavonoid, an alkaloid, was found. Cardiac glycoside and steroid tannins were not found in *n*-hexane extracts. Hexane and ethyl acetate extracts contained terpenoids. Saponin

was found to be present in the distilled water and methanol extracts given in Table 1.

According to Figure 1, the maximum yield was achieved by ethyl acetate 19.67 mg, followed by methanol extract (17.36 mg), and the minimum yield on hexane (10.28 mg). The ethyl acetate extract from the seed had the greatest phenolic concentration (160.41±038 mg/g GAE), whereas the methanol extract had the second-highest amount (138.02±0.15 mg/g GAE). The hexane extract had the lowest quantities of phenols (23.45±0.57 mg/g GAE). The maximum total flavonoid concentration (88.52±0.18 mg/g GAE) was detected in the extract of ethyl acetate from the seed. In contrast, the second-highest quantity (76.45±0.24 mg/g GAE) was found in the extract of methanol. Flavonoid concentrations in the hexane extract were the lowest (7.12±0.42 mg/g GAE). The ethyl acetate extract from the seed had the greatest total tannic acid content (5.32±0.73 mg/g TAE), whereas the methanol extract had the second-highest amount (4.54±0.10 mg/g TAE). The hexane extract had the lowest quantity of tannic acid (0.78±0.27 mg/g TAE). Figure 1 displays this outcome appropriately.

3.2. Antioxidant Activities

The interaction of antioxidants can be assessed using the stable radicals ABTS and DPPH. The proportion of acquiring ABTS and DPPH at different concentrations from 10 to 100 µg/ml is displayed in Figure 2. Estimating the antioxidant capability (µg/ml) of the test sample extract included using standard curves for linear regression. This value was then used to express the amount of antioxidants necessary to lower the starting 50% DPPH concentration. One often used metric to measure antioxidant capability is the IC₅₀. According to Table 2, the most potent substance is ethanol acetate extract from an antioxidant perspective, and the lowest

Table 1. Qualitative phytochemical screening of different extracts of *Terminalia bellerica* Roxb seeds

Phytochemical	Extracts solvent of <i>Terminalia bellerica</i> Roxb seeds			
	Methanol	Distilled water	Ethyl acetate	<i>n</i> -hexane
Alkaloid	+	+	+	+
Flavonoids	+	+	+	+
Steroids	+	+	+	-
Tannins	+	+	+	-
Terpenoids	-	-	+	+
Saponins	+	+	-	-
Cardiac glycosides	+	+	+	-

*The designed phytochemical represented as exixtance (+) and absence (-)

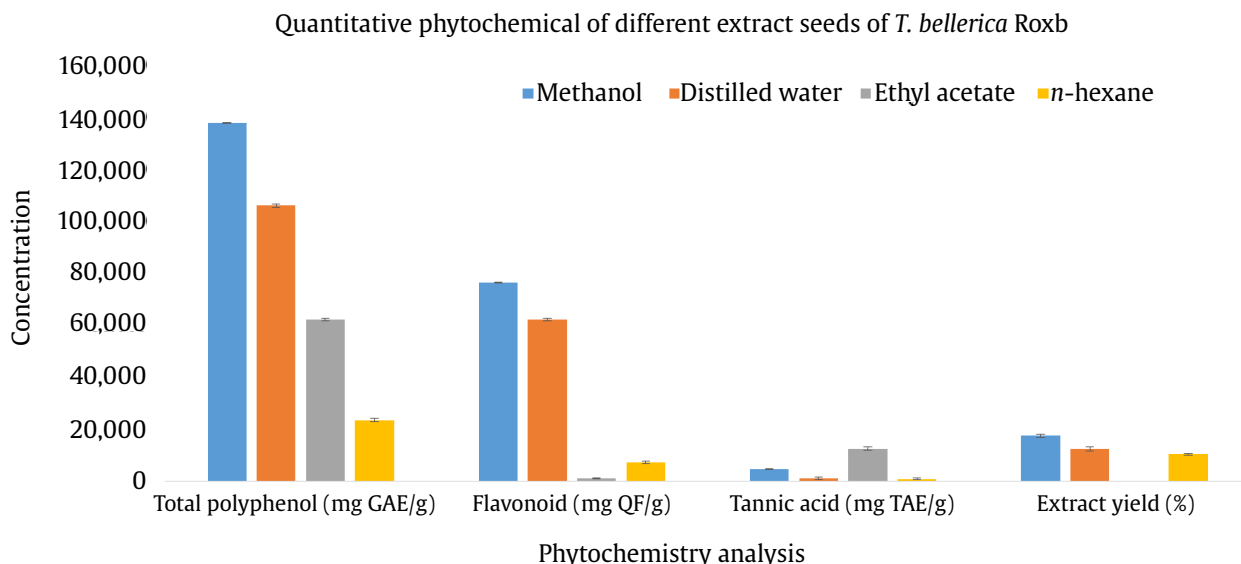


Figure 1. Quantitative phytochemical analysis of different extracts of *T. bellerica* Roxb seeds

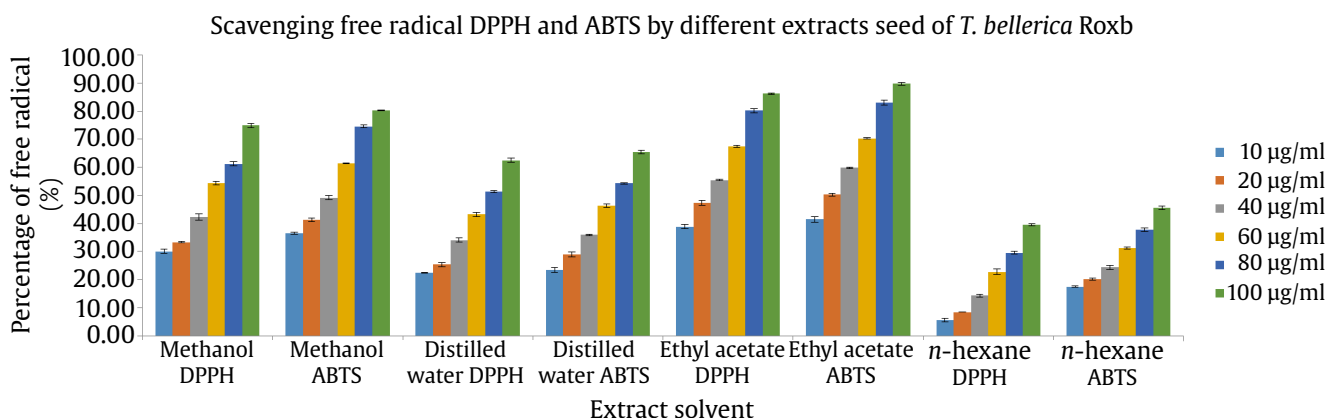


Figure 2. Percentage of scavenging radical DPPH and ABTS by different extract seeds of *T. bellerica* Roxb

Table 2. IC₅₀ of antioxidant and antidiabetic activity of different extracts of *T. bellerica* Roxb seeds

Extract solvent	Antioxidant IC ₅₀ µg/ml		Antidiabetic IC ₅₀ µg/ml	
	DPPH	ABTS	α-amylase	α-glucosidase
Methanol	52.94±0.23 ^{aA}	37.59±0.22 ^{aB}	98.23±0.38 ^{aC}	129.19±0.14 ^{aD}
Distilled water	74.48±0.42 ^{bA}	68.37±0.61 ^{bB}	249.75±0.66 ^{bC}	222.02±0.72 ^{bD}
Ethyl acetate	28.17±0.64 ^{cA}	22.22±0.49 ^{cB}	86.33±0.41 ^{cC}	74.66±0.27 ^{cD}
n-hexane	132.19±0.58 ^{dA}	117.83±0.62 ^{dB}	308.02±0.73 ^{dD}	288.23±0.57 ^{dD}

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)

IC₅₀ numbers for scavenging ABTS (22.22 µg/ml) and DPPH (28.17 µg/ml).

3.4. Antidiabetic Activity

Figure 3 displays the inhibitory capacities of crude ethyl acetate, methanol, n-hexane, and distilled water extract opposing α-amylase and α-glucosidase. The finest possible antidiabetic was found in crude

ethyl acetate extract, and it also exhibited the lowest α-glucosidase (74.66 g/ml) and α-amylase (86.33 g/ml) IC₅₀ numbers.

3.5. Antimicrobial

The outcomes of testing using antibiotics using the four extracts in opposition to fungus and bacteria are shown in Table 3. Nearly all fungus pathogens and

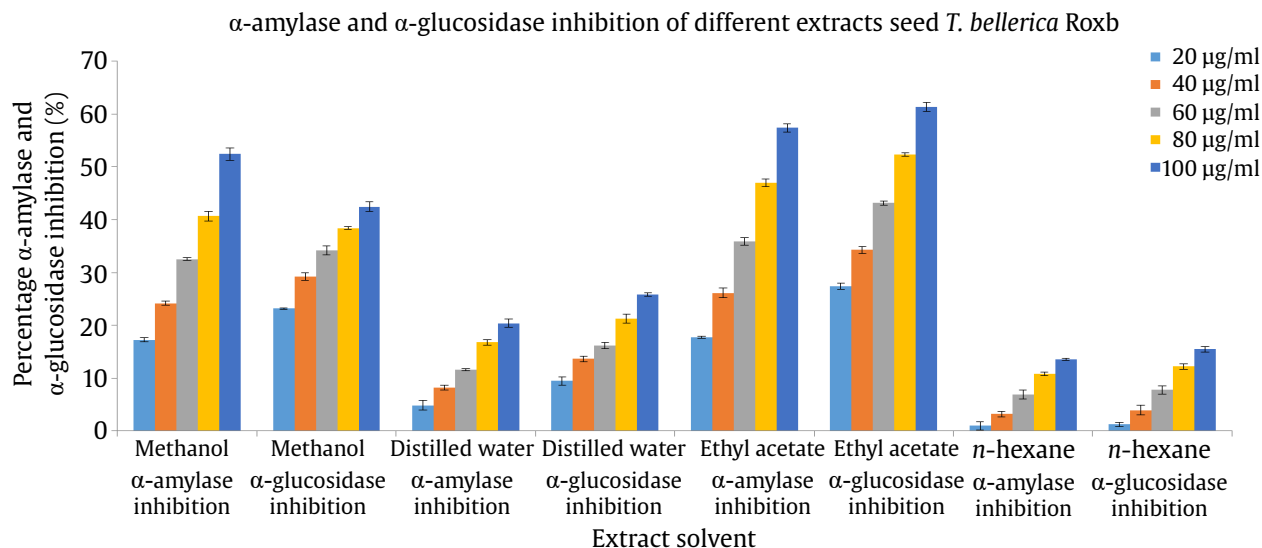


Figure 3. Percentage of α -amylase and α -glucosidase by different extract seeds of *T. bellerica* Roxb

Table 3. Antimicrobial activity of different extracts of *Terminalia bellerica* Roxb seeds

Antimicrobial	Extracts solvent of <i>Terminalia bellerica</i> Roxb seeds (10 mg/mL)				
	Methanol	Distilled water	Ethyl acetate	n-hexane	Positive control (10 mg/ml)
Inhibition zone diameter gram-negative bacterial (mm) with positive control by ampicillin					
<i>Bacillus subtilis</i>	15±0.5 ^{aA}	14±0.2 ^{aB}	11±0.4 ^{aC}	8±0.2 ^{aD}	11±0.4 ^{aC}
<i>Escherichia coli</i>	16±1.0 ^{aA}	13.5±0.4 ^{aB}	14±1.0 ^{bC}	7±0.4 ^{bD}	14±1.0 ^{bC}
<i>Salmonella typhi</i>	14±0.3 ^{bA}	12±0.2 ^{bB}	13±0.5 ^{bC}	9±0.5 ^{cD}	13±0.5 ^{bC}
<i>Shigella flexneri</i>	21±0.5 ^{cA}	10±0.4 ^{cB}	9±0.4 ^{cC}	8±0.5 ^{cD}	9±0.4 ^{cC}
<i>Klasiella pneumoniae</i>	22±0.3 ^{dA}	13±0.3 ^a	9±0.4 ^d	9±0.4 ^d	9±0.4 ^d
Inhibition zone diameter gram-positive bacterial (mm) with positive control by ampicillin					
<i>Staphylococcus aureus</i>	23±0.1 ^{eA}	18±0.1 ^{eB}	12±0.5 ^{bC}	10±1.0 ^{eD}	22±0.4 ^{eA}
<i>Pseudomonas aeruginosa</i>	21±0.2 ^{cA}	17±0.5 ^{eB}	13±0.3 ^{bC}	11±0.3 ^{eD}	24±0.5 ^{fE}
<i>Corynebacterium diphtheriae</i>	22±0.3 ^{dA}	16±0.3 ^{fB}	13±0.4 ^{bC}	11±0.2 ^{eD}	22±0.2 ^{eE}
Inhibition zone diameter fungus (mm) with positive control by ketoconazole					
<i>Aspergillus niger</i>	19±0.2 ^{fA}	15±0.2 ^{gB}	11±1.0 ^{dC}	9±0.4 ^{fD}	23±1.0 ^{eE}
<i>Aspergillus flavus</i>	21±0.5 ^{cA}	17±0.5 ^{eB}	13±0.5 ^{bC}	7±0.5 ^{gD}	24±0.5 ^{fE}
<i>Aspergillus fumigatus</i>	20±1.0 ^{fA}	16±0.3 ^{fB}	15±0.4 ^{eC}	8±0.4 ^{hD}	22±0.2 ^{eE}

Data are mean \pm 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)

bacteria showed the most significant zone inhibition by methanol extract. Claiming that the extract had the power to prevent germs. *Staphylococcus aureus* is firmly categorized and has a 23-mm inhibitory zone diameter. The fungus's most significant inhibition zone diameter (21 mm) and the most substantial classification belonged to *Aspergillus flavus*.

4. Discussion

Plants create chemical molecules to meet their needs and adjust to their environment (Millogo-Kone *et al.* 2008). This study examined

the impact of a phytochemical solvent using screening for phytochemicals of extracted test plant *T. bellerica* seeds, comprising four distinct solvents. Phytochemical screening identified saponins, alkaloids, cardiac glycosides, flavonoids, tannins and steroids, and terpenoids. This finding is not significantly different from other research using methanol extracts of *T. bellerica* seeds that exclusively included terpenoids, tannins, alkaloids, and flavonoids (Sharma *et al.* 2018). Regarding the quantity of metabolites extracted, utilizing a different solvent extraction technique is significant (Rafi *et al.* 2018). The secondary qualitative

metabolites analysis findings are shown in Table 1.

The maximum yield was obtained with ethyl acetate. Different yields are achieved due to changes in the extracted chemicals caused by solvent polarity. Because ethyl acetate is semi-polar, it may remove both water- and oil-soluble components, resulting in a higher yield than when using a different solvent. The purity of the solvent also influences the final yield. Utilizing solvents that include water reduces the solubility of non-polar chemicals in the solvent, which lowers yield (Saputri *et al.* 2023).

Figure 1 displays the findings of measuring the extract of *Terminalia bellirica*'s total phenolic content. Since phenolic group molecules are often semipolar-polar, in semipolar-polar solvents, more phenolic chemicals are removed, such as ethyl acetate extract, which has the greatest total phenolic content (160.41 mg GA/g) among the four. Methanol, distilled water, and *n*-hexane follow arranged of decreasing phenolic concentration. Since it is a semi-polar solvent, several polar and non-polar compounds can be attracted to ethyl acetate (Rahmi and Sari 2021). Total phenolic content assessment was done to determine the relationship between antioxidant activity and phenolic concentration in a sample since compounds with phenolic groups have been demonstrated to have powerful antioxidant activity on several occasions (Akbar and Soekanto 2021). The extract in aqueous of *T. bellerica* seeds had more total phenolic compounds (154.22 mg GAE/g) (Kumar *et al.* 2022).

The extract in ethyl acetate of *T. bellerica* has the greatest flavonoid concentration, measuring 88.52 mg/ GAE g. This is because the hydroxyl groups of flavonoid compounds enable them to dissolve quickly in polar molecules like methanol (DwicaHyani *et al.* 2018). According to Dubey *et al.* (2020), the findings of this experiment do not significantly differ from those of previous investigations using 40.2 mg GAE/g.

The high output and abundance of flavonoids supported the comparatively high potential for radical scavenging (antioxidant activities). There is substantial evidence that the overall antioxidant activity is significantly increased by various flavonoids and related polyphenols (Djeridane *et al.* 2006; Adaramola *et al.* 2012). Flavonoids are widely employed as flavorings in plants. In addition to serving as flavoring agents, they also show evidence of antibacterial action when produced by plants'

reaction to an infestation by microbes (Kujumgiev *et al.* 1999). Both physiological and pathological environments benefit from the antioxidant properties of flavonoids (Kumar *et al.* 2013). For example, it has been shown that tea flavonoids reduce blood levels of cholesterol and triglycerides and minimize low-density lipoprotein oxidation (Erdman *et al.* 2007).

The extract with the greatest tannic acid concentration was ethyl acetate (5.32 mg/g). Complex combinations of very polar chemicals make up tannins (Cardullo *et al.* 2018). Tannins belong to the phenolic group. These work through particular interactions with essential proteins, including hydrogen bonding, enzymes, or iron sequestration, according to a study by Adaramola *et al.* (2012).

Apart from their antibacterial and anticancer effects, tannins are also potent antioxidants (Adaramola *et al.* 2012). Tannic plants are typically used as astringents to treat intestinal conditions, including dysentery and diarrhea (Adaramola *et al.* 2012). All medicinal herbs include tannins, effective toxin-counteracting agents (Ndukwe *et al.* 2013).

Additionally, to confirm the effectiveness of these plant-based remedies for various ailments, this research investigation also determined the medicinal purposes of the different biological elements of these herbal treatments. One stable free radical is DPPH, which has a nitrogen core that becomes yellow when it receives an electron or hydrogen donor. Substances that can carry out this process include antioxidants and free radical scavengers (Hinnerburg *et al.* 2006). Many solvent extracts, especially methanol extracts, have strong free radical inhibition and high proton-donating capacities that are equivalent to the typical antioxidants employed in most situations regarding their high DPPH radical scavenging activity. It implies that it might have an agent role. In contrast to Trolox, the ethyl acetate extracts showed a greater capacity to quench DPPH radicals (86.26%) with lower IC₅₀ values. This finding is consistent with Elizabeth *et al.* (2022) research, which found that methanol extract could effectively scavenge DPPH free radicals (84%) and ABTS (81%).

The ethyl acetate extract is calculated IC₅₀ values are 28.17 µg/ml. Although plant extracts effectively scavenge DPPH radicals in many parts of the world, hundreds of publications have indicated IC₅₀ numbers as low as those discovered with these extracts (Wu *et al.* 2010). This discovery may also imply that

the excerpts include potent phytochemicals with scavenging properties for free radicals, which may prevent ROS production and free radicals. For this reason, the extracts may be effective as therapeutic agents to treat pathological alterations by radicals (Ibrahim *et al.* 2013).

To give hydrogen and electrons, the ABTS test produces a green/blue color that is lowered by using antioxidants, it scavenges free radicals. Table 2 displays the extract's ABTS scavenger activity as a 50% (IC_{50}) scavenger concentration. In this experiment, the antioxidant qualities of the extracts were evaluated by comparing them to a standard reference, Trolox. The ethyl acetate extract's IC_{50} value was 22.22 $\mu\text{g}/\text{ml}$, while *T. bellerica* methanol extracts reacted with ABTS+ quickly. The two techniques discussed above have shown that ethyl acetate extract possesses antioxidant capacity. Quantitative data on secondary metabolism further supports this claim. Additionally, the ethyl acetate extract has a higher phenol, flavonoid, and tannic acid content than other extracts.

Numerous antioxidant processes include the DPPH•-scavenging reaction. Antioxidants scavenge free radicals through four key suggested mechanisms: (1) the hydrogen atom ($\text{H}\bullet$) transfer process (HAT). The procedure involved the assumption that the antioxidant would donate an $\text{H}\bullet$ atom and that DPPH• would change into a DPPH-H molecule (2) SEPT (sequential electron-proton transfer) (3) SPLET (Proton loss single electron transfer) is the third stage in this process, which occurs after the first creation of a cation radical by a proton from the cation radical to the anion and the transfer of an electron from the antioxidant to the free radical (Boulebd 2020). (4) Using PCET (proton-coupled electron transfer), the radical's lone pair receives the proton previously in the phenolic molecule. Conversely, the electron shifts from the antioxidant compounds' 2p lone pair to the radical's SOMO (singly occupied molecular orbital) (Milenković *et al.* 2018).

However, ABTS+• scavenging is considered an electron (e) transfer process since the radical cation of ABTS+• requires an electron (e) to neutralize the positive charge. Contributing an electron (e) may be an additional method of directly scavenging radicals, as antioxidant chemicals are capable of scavenging the ABTS+• radical. The Cu^{2+} reducing power test provides further evidence of the donating electron (e) process. One of two processes can be used:

either a sequential electron-proton transfer process that includes the deprotonation of the generated anion or a single electron transfer method that then involves the deprotonation of the radical cation that is created. These are the two real ways that the reduction reaction may occur. Despite producing the same chemicals as HAT, SEPT and PCET are anticipated to have differing feasibility due to the nature of the reacting radical and the solvent. A SEPT process could only happen in a polar environment that promotes the solvation of the intermediate ionic species, even if the PCET mechanism might be possible in a nonpolar medium because of the lesser charge separation than in SEPT (Li *et al.* 2015).

Phenolic compounds can provide electrons to capture free radicals. The kind of substituents on phenolic compounds affects antioxidant efficacy, the amount and location of hydroxyl groups located on the binding site for aromatic rings, and other factors (Ja'afar *et al.* 2017). Phenolic substances either neutralize or suppress free radicals by removing an atom of hydrogen from their hydroxyl group. A phenolic compound reacts with a peroxy radical ($\text{ROO}\bullet$) to produce a transition state where an H-O bond with one electron is formed. The coordinated movement of the radical's hydrogen cation from the phenol initiates this reaction. The reaction medium contains a solvent likely to establish hydrogen bonds with the chemicals, dramatically reducing the antioxidant properties of the components of phenol (Santos-Sánchez *et al.* 2019).

The flavonoids can convert less impulsive unrestrained radicals into less impulsive hydroxyl radicals because of their stabilized structure, which weakens more reactive free radicals. This allows flavonoids to serve as scavengers of free radicals. According to Hernández-Rodríguez *et al.* (2019), these oxidants are inhibited by free hydroxyls providing electrons or hydrogens. This indicates that radical damage is partly propagated in biological systems by free hydroxyls.

Ben Ahmed *et al.* (2017) report that tannins can block two different types of hydrogen atom transfer (HAT) and single electron transfer (SET) processes. Oxygen is a structural component shared by oxygenated sesquiterpenes and monoterpenes, divided into two groups. Three antioxidant processes-sequential proton loss electron transfer (SPLET), single-electron transfer followed by proton transfer (SET-PT), and hydrogen atom transfer

(HAT)—are proposed by Ngo *et al.* (2017) to account for the antioxidant activity of these compounds.

Starchy meals are digested into oligosaccharides through pancreatic α -amylase and salivary, which α -glucosidase subsequently transforms into glucose in the small intestines (Nakamura *et al.* 2014). Studies on α -amylase suppression have demonstrated the strong inhibitory potential of *T. bellerica* seed extracts, including crude distilled water, n-hexane, methanol, and ethyl acetate. The IC_{50} values of ethyl acetate extract ($86.33 \pm 0.41 \mu\text{g}/\text{mL}$) were consistent with previous findings, *T. bellerica* seed aqueous extract demonstrated much higher α -amylase inhibitory action (IC_{50} $74.8 \mu\text{g}/\text{ml}$) (Gupta *et al.* 2020). 1,4-Glycosidic linkages, which are present in maltotriose, maltose, and other simple sugars, as well as starch and other oligosaccharides, are the main constituent of these α -amylase inhibitors (Wickramaratne *et al.* 2016). Further investigation and the isolation of the pure active molecule are required, as the inhibiting α -amylase action extract in methanol most probably originates from the polar component. α -glucosidase was more reactively inhibited by all polyphenols examined than α -amylase. Typically, the inhibition's order is xanthenes < tannins < flavonoids for α -amylase, and tannins < xanthone < bioflavonoids < flavonoids for α -glucosidase (Bomigboye *et al.* 2020). High levels of flavonoid chemicals are also seen in ethyl acetate extract. Acarbose is ($25.17 \pm 0.21 \mu\text{g}/\text{ml}$) and extract in ethyl acetate is ($74.66 \pm 0.27 \mu\text{g}/\text{ml}$) α -glucosidase inhibition.

Based on past research conducted by (Nampoothiri *et al.* 2011), the methanol extract of *T. bellerica* seeds can inhibit the alpha glucosidase enzyme (IC_{50} $175.35 \text{ g}/\text{ml}$). IC_{50} differs considerably. It has been documented that anthocyanins, tannins, phenolic acid, and flavonoids originating from plants decrease α -glucosidase activity (Di Stefano *et al.* 2018). Research has shown flavonoids exhibit a superior inhibitory effect than phenolic acid against the α -glucosidase enzyme. At one mM, α -glucosidase and α -amylase enzymes were not inhibited by hydroxybenzoic or hydroxycinnamic acid. Molecules with a flavone backbone likely have more hydroxyl groups, so the inhibitory impact is more potent. (Gu *et al.* 2015). The primary modes of interaction between phenolic compounds and α -glucosidase include hydrogen (H) bonds, hydrophobic contacts, and Van der Waals interactions (Yan *et al.* 2014; Peng *et al.* 2016).

Triterpenoids' function depended heavily on the kind and location of their substituents. Transferring a methyl group from C20 to C19 improves the inhibitory action, but adding a 24-hydroxyl group attenuates it. (Zhang *et al.* 2017). The inhibitory action of pentacyclic triterpenes is likewise facilitated by the 17-carboxyl group (Zhang *et al.* 2017). Subsequent investigation demonstrated that α -glucosidase binds to terpenoids at holes 2 and 4 in the enzyme, obstructing their non-competitive activity. (Ding *et al.* 2018). Within the enzyme, terpenoids bind to several residues of amino acids, whereas C28-carboxylic acids form hydrogen bonds with their O atoms. After contact, α -glucosidase underwent a conformational shift, decreasing the enzyme's catalytic activity (Zhang *et al.* 2017; Ding *et al.* 2018). Oxidative damage brought on through the generation of free radicals and the oxidative breakdown of glycosylated proteins during glucose oxidation is anticipated to result in diabetic complications (Wickramaratne *et al.* 2016). Consequently, it is frequently advised to take antioxidants and antidiabetic drugs to prevent these side effects.

Of practically all bacterial and fungal pathogens, ethyl acetate extract showed the highest zone inhibition. With positive control ampicillin, the extract demonstrated a robust and distinct inhibitory effect against *Escherichia coli* germs. The highest fungal zone inhibition, classified as strong, was seen in *Aspergillus fumigatus*; In comparison to the positive control drug ketoconazole, this impact was less pronounced. The significance of this result differs from that of the prior study, which found that methanol extract could suppress *E. coli* growth with a 22 mm inhibition zone diameter (Sharma *et al.* 2018). The prerequisites for antimicrobial power strength are as follows: A 5 mm or less diameter is considered a light inhibition zone, 5 to 10 mm is considered a moderate inhibition zone, 10 to 20 mm is regarded as a zone of strong inhibition, and 20 mm is considered a potent inhibition zone (Maryana *et al.* 2019).

The ethyl acetate extract's elevated flavonoid and tannic acid content will impact its antibacterial properties. By making hydrogen and covalent connections with extracellular proteins in bacteria, flavonoids produce a chemical that impedes the activity of cell transport proteins and enzymes as well as the capacity of bacteria to adhere to

microorganisms via their cell walls (Kumar and Pandey *et al.* 2013). Rubbing down the membrane or cell wall reduces the cell's permeability, which is how tannin chemicals work as an antibiotic (Ajizah 2004). By combining with substrates or enzymes to generate complex compounds, tannins damage cell membranes and have antibacterial properties. Because phenolic substances include carboxyl groups and hydroxyl that may hydrogen bond with cells of bacteria, coagulate proteins, and lyse bacterial cell membranes, they also have antibacterial effects (Erviana and Purwono 2011; Rachmawaty *et al.* 2018). Since lipophilic chemicals like triterpenoids are often present, terpenoids have an antibacterial action that involves breaking down the bacterial cell membrane. When antibacterial active substances interact with the membrane's active side or dissolve lipid components and make them more permeable, cell membrane damage may result. Phospholipids and protein molecules make up the bacterial cell membrane. These bacterial cells' cytoplasm may coagulate due to increased permeability, cell membrane components, or both (Rahmi and Sari 2021). Alkaloids work as antibacterials by turning off the peptidoglycan constituents in bacterial cells. Cells die due to the formation of the cell wall layer, which is not beneficial. The alkaloid components inhibiting the bacterial cell topoisomerase enzymes are DNA intercalators, another alkaloid mechanism (Rahmi and Sari 2021).

In conclusion, the potential antioxidant activity and mechanism of action of *T. bellerica* seed crude ethyl acetate extract were described in this work-the crude ethyl acetate from *T. bellerica* seeds' possible anti-diabetic properties. Based on several standard strains of bacteria and fungi, both Gram-positive and Gram-negative, the experiments demonstrated the efficacy of crude ethyl acetate extract. One potential mechanism for the antibacterial action of *T. bellerica* ethyl acetate extract is DNA damage brought on by ROS.

Acknowledgements

The Directorate of Research and Community Service, Directorate General of Strengthening Research and Development, Ministry of Research, Technology, and Higher Education, Indonesia, provided funding for the research project "Penelitian Kerjasama Antar Perguruan Tinggi," which served as the foundation for this assignment.

References

- Abate, L., Bachheti, R.K., Tadesse, M.G., Bachheti, A., 2022. Ethnobotanical uses, chemical constituents, and application of *Plantago lanceolata* L. *Journal of Chemistry*. 2022, 1-17. <https://doi.org/10.1155/2022/1532031>
- Adaramola, T.F., Ariwaodo, J.O., Adeniji, K.A., 2012. Distribution, phytochemistry, and antioxidant properties of the genus *Parkia* R. Br. (Mimosaceae) in Nigeria. *Int. J. Pharmacogn. Phytochem. Res.* 4, 172-178.
- Ajizah, A., 2004. Sensitivitas *Salmonella typhimurium* terhadap ekstrak daun *Psidium guajava* L. *Bioscientie*. 1, 31-38.
- Akbar, A., Soekamto, N.H., 2021. The antioxidant of *n*-hexane, ethyl acetate, and methanol extracts of *Padina* sp. with DPPH method. *In IOP Conference Series: Earth and Environmental Science*. 800, 012019. <https://doi.org/10.1088/1755-1315/800/1/012019>
- Akwaji, P.I., Okon, E.I., Umana, E.J., Markson, A.A.A., 2016. Phytochemical and antifungal efficacy of leaf and stem bark extracts of *Parkia biglobosa* on fungi associated with seed rot of green bean (*Phaseolus vulgaris* L.) in Akpabuyo, Cross River State, Nigeria. *International Journal of Pharmacology. Phytochemistry and Ethnomedicine*. 3, 27-38. <https://doi.org/10.18052/www.scipress.com/IJPPE.3.27>
- Amalia, A.W., Sariwati, A., 2019. Identifikasi senyawa kimia dan uji aktivitas antioksidan ekstrak etil asetat biji sirsak (*Annona mucicata* Linn.). *JCPS Journal of Current Pharmaceutical Sciences*. 3, 192-197.
- Bamigboye, T.J., Idowu, O.J., Olujide, O.O., 2020. Structure-activity-relationship of the polyphenols inhibition of α -Amylase and α -Glucosidase. *African Journal of Traditional, Complementary and Alternative Medicines*. 17, 55-65. <https://doi.org/10.21010/ajtcam.v17i2.5>
- Ben Ahmed, Z., Yousfi, M., Viaene, J., Dejaegher, B., Demeyer, K., Mangelings, D., Vander Heyden, Y., 2017. Seasonal, gender, and regional variations in total phenolic, flavonoid, and condensed tannins contents and in antioxidant properties from *Pistacia atlantica* ssp. leaves. *Pharmaceutical Biology*. 55, 11. <https://doi.org/10.1080/13880209.2017.1291690>
- Boulebd, H., 2020. Theoretical insights into the antioxidant activity of moracin T. *Free Radical Research*. 54, 221-230.85-1194. <https://doi.org/10.1080/10715762.2020.1747616>
- Cardullo, N., Muccilli, V., Saletti, R., Giovando, S., Tringali, C., 2018. A mass spectrometry and 1H NMR study of hypoglycemic and antioxidant principles from a *Castanea sativa* tannin employed in enology. *Food Chemistry*. 268, 585-593. <https://doi.org/10.1016/j.foodchem.2018.06.117>
- Deb, A., Barua, S., Das, B., 2016. Pharmacological activities of Baheda (*Terminalia bellerica*): a review. *J. Pharmacogn. Phytochem.* 5, 19-197.
- Di Stefano, E., Oliviero, T., Udenigwe, C.C., 2018. Functional significance and structure-activity relationship of food-derived α -glucosidase inhibitors. *Current Opinion in Food Science*. 20, 7-12. <https://doi.org/10.1016/j.cofs.2018.02.008>
- Ding, H., Hu, X., Xu, X., Zhang, G., Gong, D., 2018. Inhibitory mechanism of two allosteric inhibitors, oleanolic acid and ursolic acid on α -glucosidase. *Int. J. Biol. Macromol.* 107, 1844-1855. <https://doi.org/10.1016/j.ijbiomac.2017.10.040>
- Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., Vidal, N., 2006. Antioxidant activity of some algerian medicinal plant extracts containing phenolic compounds. *Food Chemistry*. 97, 654-660. <https://doi.org/10.1016/j.foodchem.2005.04.028>

- Dubey, R.K., Upadhyay, G., Singh, V., Pandey, S., 2020. Antioxidant potential and free radical scavenging activity of *Parkia timoriana*, G. Don, a lesser known leguminous tree from North East India. *South African Journal of Botany*. 131, 454-461.844-1855. <https://doi.org/10.1016/j.sajb.2020.03.013>
- Dwicahyani, T., Sumardianto, S., Rianingsih, L., 2018. Uji bioaktivitas ekstrak teripang keling *Holothuria atra* sebagai antibakteri *Staphylococcus aureus* dan *Escherichia coli*. *Jurnal Pengolahan dan Bioteknologi Hasil Perikanan*. 7, 15-24.
- Elizabeth, L., Bupesh, G., Susshmitha, R., 2017. *In vitro*, antioxidant efficacy of *Terminalia bellirica* seed extract against free radicals. *International Journal of Pharmaceutical Sciences and Research*. 8, 4659-4665.
- Erdman Jr, J.W., Balentine, D., Arab, L., Beecher, G., Dwyer, J.T., Folts, J., Burrowes, J., 2007. Flavonoids and heart health: proceedings of the ILSI North America flavonoids workshop, May 31-June 1, 2005, Washington, DC. *The Journal of Nutrition*. 137, 718-737. <https://doi.org/10.1093/jn/137.3.718S>
- Erviana, R., Purwono, S., 2011. Active compounds isolated from red betel (*Piper crocatum* Ruiz & Pav) leaves active against *Streptococcus mutans* through its inhibition effect on *Glucosyltransferase* activity. *Journal of Medical Science*. 43, 71-78
- Gu, C., Zhang, H., Putri, C.Y., Ng, K., 2015. Evaluation of α -amylase and α -glucosidase inhibitory activity of flavonoids. *Int J Food Nutr Sci*. 2, 174-179.
- Gupta, A., Kumar, R., Bhattacharyya, P., Bishayee, A., Pandey, A.K., 2020. *Terminalia bellirica* (Gaertn.) roxb. (Bahera) in health and disease: a systematic and comprehensive review. *Phytomedicine*. 77, 153278. <https://doi.org/10.1016/j.phymed.2020.153278>
- Hernández-Rodríguez, P., Baquero, L.P., Larrota, H.R., 2019. Flavonoids: potential therapeutic agents by their antioxidant capacity, in: Campos, M.R.S. (Eds.), *Bioactive Compounds*. Woodhead Publishing: Darya Ganj, Delhi, pp. 265-288. <https://doi.org/10.1016/B978-0-12-814774-0.00014-1>
- Hinnerburg, I., Damien, H.J., Hiltunen, R., 2006. Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chem*. 97, 122-129. <https://doi.org/10.1016/j.foodchem.2005.03.028>
- Ibrahim, M.A., Koorbanally, N.A., Islam, M.S., 2013. *In vitro* anti-oxidative activities of the various parts of *Parkia biglobosa* and GC-MS analysis of extracts with high activity. *African Journal of Traditional, Complementary and Alternative Medicines*. 10, 283-291. <https://doi.org/10.4314/ajtcam.v10i5.10>
- Jaâfar, M.K., Jamil, S., Basar, N., 2017. Antioxidant activity of leaf extracts of *Globimetula braunii* (Engler) van Tiegh parasitizing on *Piliostigma thonningii* and *Parkia biglobosa*. *Jurnal Teknologi*. 79, 43-47. <https://doi.org/10.11113/jt.v79.10574>
- Kujumgiev, A., Tsvetkova, I., Serkedjieva, Y., Bankova, V., Christov, R., Popov, S., 1999. Antibacterial, antifungal, and antiviral activity of propolis of different geographic origins. *Journal of Ethnopharmacology*. 64, 235-240. [https://doi.org/10.1016/S0378-8741\(98\)00131-7](https://doi.org/10.1016/S0378-8741(98)00131-7)
- Kumar, S., Pandey, A.K., 2013. Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal*. 2013, 1-16. <https://doi.org/10.1155/2013/162750>
- Kumar, D., Gupta N., Ghosh, R., Gaonkar, R.H., Pal, B.C., 2013. α -Glucosidase and α -amylase inhibitory constituent of *Carex baccans*: bio-assay guided isolation and quantification by validated RP-HPLC-DAD. *Journal of Functional Foods*. 5, 211-218. <https://doi.org/10.1016/j.jff.2012.10.007>
- Kumar, V., Kumari, P., Arora, V., Dixit, H., Singh, R., Sharma, N., 2022. Comparative analysis of phytochemicals, *in vitro* antimicrobial and *in vitro* antidiabetic activity of different parts of *Terminalia bellerica* fruits. *Arabian Journal of Medicinal and Aromatic Plants*. 8, 115-130.
- Li, X., Hu, Q., Jiang, S., Li, F., Lin, J., Han, L., Chen, D., 2015. Flos chrysanthemi indici protects against hydroxyl-induced damages to DNA and MSCs via antioxidant mechanism. *Journal of Saudi Chemical Society*. 19, 454-460. <https://doi.org/10.1016/j.jscs.2014.06.004>
- Leyane, T.S., Jere, S.W., Houreld, N.N., 2021. Cellular signalling and photobiomodulation in chronic wound repair. *International Journal of Molecular Sciences*, 22, 11223. <https://doi.org/10.3390/ijms222011223>
- Mallik, J., Das, P., Karon, B., Das, S., 2012. A review on phytochemistry and pharmacological activity of *Terminalia bellirica*. *Indian J. Drug Formul. Res*. 3, 1-7.
- Maryana, D., Malaka, R., Maruddin, F., 2019. Antibacterial activity of pasteurized milk supplemented with binahong leaf extract (*Anredera cordifolia* (Ten) Steenis) and sukrose toward *Escherichia coli* and *Staphylococcus aureus*. *In IOP Conference Series: Earth and Environmental Science*. 247, 012065. <https://doi.org/10.1088/1755-1315/247/1/012065>
- Milenković, D., Đorović, J., Petrović, V., Avdović, E., Marković, Z., 2018. Hydrogen atom transfer versus proton-coupled electron transfer mechanism of gallic acid with different peroxy radicals. *Reaction Kinetics, Mechanisms and Catalysis*. 123, 215-230. <https://doi.org/10.1007/s11144-017-1286-8>
- Millogo-Kone, H., Guissou, I.P., Nacoulma, O., Traore, A.S., 2008. Comparative study of leaf and stem bark extracts of *Parkia biglobosa* against enterobacteria. *African Journal of Traditional, Complementary and Alternative Medicines*. 5, 238-243. <https://doi.org/10.4314/ajtcam.v5i3.31279>
- Nakamura, S., Takami, M., Tanabe, K., Oku, T., 2014. Characteristic hydrolyzing of megalosaccharide by human salivary α -amylase and small intestinal enzymes, and its bioavailability in healthy subjects. *International Journal of Food Sciences and Nutrition*. 65, 754-760. <https://doi.org/10.3109/09637486.2014.908168>
- Nampoothiri, S.V., Prathapan, A., Cherian, O.L, Raghu, K.G., Venugopalan, V.V., Sundaresan, A., 2011. *In vitro* antioxidant and inhibitory potential of *Terminalia bellerica* and *Emblica officinalis* fruits against LDL oxidation and key enzymes linked to type 2 diabetes. *Food Chemistry and Toxicology*. 49, 125-131. <https://doi.org/10.1016/j.fct.2010.10.006>
- Ndukwe, O.K., Awomukwu, D., Ukpabi, C.F., 2013. Comparative evaluation of phytochemical and mineral constituents of the leaves of some medicinal plants in Abia State Nigeria. *International Journal of Academic Research in Progressive Education and Development*. 2, 244-252. <https://doi.org/10.6007/IJARPE/v2-i3/148>
- Ngo, T.C., Dao, D.Q., Nguyen, M.T., Nam, P.C., 2017. A DFT analysis on the radical scavenging activity of oxygenated terpenoids present in the extract of the buds of *Cleistocalyx operculatus*. *RSC Advances*. 7, 39686-39698. <https://doi.org/10.1039/C7RA04798C>
- Peng, X., Zhang, G., Liao, Y., Gong, D., 2016. Inhibitory kinetics and mechanism of kaempferol on α -glucosidase. *Food Chem*. 190, 207-215. <https://doi.org/10.1016/j.foodchem.2015.05.088>
- Prasathkumar, M., Anisha, S., Dhriya, C., Becky, R., Sadhasivam, S., 2021. Therapeutic and pharmacological efficacy of selective Indian medicinal plants-a review. *Phytomedicine Plus*. 1, 100029. <https://doi.org/10.1016/j.phyplu.2021.100029>

- Rachmawaty, F.J. Akhmad, M.M., Pranacipta, S.H., Nabila Z., Muhammad, A., 2018. Optimasi ekstrak etanol daun sirih merah (*Piper crocatum*) sebagai antibakteri terhadap bakteri *Staphylococcus aureus*. *Mutiara Medika. Jurnal Kedokteran dan Kesehatan*. 18, 13-19. <https://doi.org/10.18196/mm.180109>
- Rafi, M., Febriany S., Wulandari P., Suparto I.H., Ridwan T., Rahayu S., Siswoyo D.M., 2018. Total phenolics, flavonoids, and anthocyanin contents of six *Vireya* *Rhododendron* from Indonesia and evaluation of their antioxidant activities. *J. Appl. Pharm. Sci.* 8, 49-54. <https://doi.org/10.7324/JAPS.2018.8908>
- Rahmi, M., Sari, T.M., 2021. Antibacterial activity of ethanol extract, *n*-hexan, ethyl acetate and butanol fraction of *Momordica charantia* L. seed against *Staphylococcus epidermidis*. In *Journal of Physics: Conference Series*. 1918, 052013. <https://doi.org/10.1088/1742-6596/1918/5/052013>
- Salem, M.Z., Mervat, E.H., Ali, H.M., Abdel-Megeed, A., El-Settawy, A.A., Böhm, M., Salem, A.Z., 2021. Plant-derived bioactives: novel utilization as antimicrobial, antioxidant, and phyto-reducing agents for the biosynthesis of metallic nanoparticles. *Microbial Pathogenesis*, 158, 105107. <https://doi.org/10.1016/j.micpath.2021.105107>
- Santoso, B., Imaduddin, F., Sukanto, H., Triyono, J., Lambang, R.L., Widodo, P.J., Siswantoro, D.H., 2021. Procurement and operation technical for meniran (*Phyllanthus niruri*) extraction equipment. *Mekanika: Majalah Ilmiah Mekanika*. 20, 34-43. <https://doi.org/10.20961/mekanika.v20i1.45487>
- Santos-Sánchez, N.F., Salas-Coronado, R., Villanueva-Cañongo, C., Hernández-Carlos, B. 2019. *Antioxidant Compounds and Their Antioxidant Mechanism*. IntechOpen, London.
- Saputri, D.R., Listyadevi, Y.L., Adiwibowo, M.T., Alhanif, M., Damayanti, D., Fahni, Y., ... Kurniawansyah, A., 2023. The effect of solvent type on the extraction of soybean crude oil and its laboratory-scale cost analysis. *Teknika: Jurnal Sains dan Teknologi*. 19, 129-135.
- Sariwati, A., Purnomo, A.S., Kamei, I., 2017. Abilities of cocultures of brown-rot fungus *Fomitopsis pinicola* and *Bacillus subtilis* on biodegradation of DDT. *Current Microbiology*. 74, 1068-1075. <https://doi.org/10.1007/s00284-017-1286-y>
- Sariwati, A., Purnomo, A.S., 2018. The effect of *Pseudomonas aeruginosa* addition on 1, 1, 1-trichloro-2, 2-bis (4-chlorophenyl) ethane (DDT) biodegradation by brown-rot fungus *Fomitopsis pinicola*. *Indonesian Journal of Chemistry*. 18, 75-81. <https://doi.org/10.22146/ijc.25158>
- Sariwati, A., Fitri, I., Purnomo, A.S., Fatmawati, S., 2019. Phytochemical, antibacterial, and antioxidant activities of *Anthurium Hookerii* leaves extracts. *HAYATI Journal of Biosciences*. 26, 101-109. <https://doi.org/10.4308/hjb.26.3.101>
- Sariwati, A., Fatmawati, S., Rizqi, H.D., Purnomo, A.S., 2022. Antioxidant properties of the by-product Indonesian favourable fruits. *ASM Science Journal*. 16, 107-118.
- Setyawati, T., 2009. Kajian etnobotani di beberapa Kawasan Hutan Cagar Alam, Jawa Timur. *Jurnal Tumbuhan Obat Indonesia*. 2, 114-123.
- Sharma, R., Raizada, S., Gautam, A., Bhatia, A.K., 2018. Phytochemical and antibacterial analysis of *Terminalia chebula* and *Terminalia bellirica*, in: Parmar, V., Malhotra, P., Mathur, D. (Eds.), *Green Chemistry in Environmental Sustainability and Chemical Education*. Springer: Singapore, pp. 131-137. https://doi.org/10.1007/978-981-10-8390-7_13
- Sheikh, Y., Maibam, B.C., Talukdar, N.C., Deka, D.C., Borah, J.C., 2016. *In vitro* and *in vivo* antidiabetic and hepatoprotective effects of edible pods of *Parkia timoriana* and quantification of the active constituent by HPLC-PDA. *Journal of Ethnopharmacology*. 191, 21-28. <https://doi.org/10.1016/j.jep.2016.06.015>
- Singh, A.S., 2011. *Herbalism Phytochemistry and Ethnopharmacology*, first ed. CRC Press, Boca Raton. 357-361. <https://doi.org/10.1201/b10878>
- Singh, M.P., Gupta, A., Sisodia, S.S., 2018. Ethno and modern pharmacological profile of Baheda (*Terminalia bellerica*): a review. *The Pharmaceutical and Chemical Journal*. 5, 153-162.
- Suryanti, V., Sariwati, A., Sari, F., Handayani, D.S., Risqi, H.D. 2022. Metabolite bioactive contents of *Parkia timoriana* (DC) Merr seed extracts in different solvent polarities. *Hayati Journal of Biosciences*. 29, 681-694. <https://doi.org/10.4308/hjb.29.5.681-694>
- Umdale, S., Mahadik, R., Otari, P., Gore, N., Mundada, P., Ahire, M., 2021. Phytochemical composition and antioxidant potential of *Frerea indica* Dalz.: a critically endangered, endemic, and monotypic genus of the Western Ghats of India. *Biocatalysis and Agricultural Biotechnology*. 35, 102080. <https://doi.org/10.1016/j.bcab.2021.102080>
- Wickramaratne, M.N., Punchihewa, J.C., Wickramaratne, D.B.M., 2016. *In-vitro* alpha-amylase inhibitory activity of the leaf extracts of *Adenanthera pavonina*. *BMC Complementary and Alternative Medicine*. 16, 1-5. <https://doi.org/10.1186/s12906-016-1452-y>
- Wu, N., Zu, Y., Fu, Y., Kong, Y., Zhao, J., Li, X., Li, J., Wink, M., Efferth, T., 2010. Antioxidant activities and xanthine oxidase inhibitory effects of extracts and main polyphenolic compounds obtained from *Geranium sibiricum* L. *J Agric Food Chem*. 58, 4737-4743. <https://doi.org/10.1021/jf904593n>
- Yan, J., Zhang, G., Pan, J., Wang, Y. 2014. α -Glucosidase inhibition by luteolin: Kinetics, interaction and molecular docking. *Int J Biol Macromol*. 64, 213-223. <https://doi.org/10.1016/j.ijbiomac.2013.12.007>
- Zhang, B.W., Xing, Y., Wen, C., Yu, X.X., Sun, W.L., Xiu, Z.L., Dong, Y.S., 2017. Pentacyclic triterpenes as α -glucosidase and α -amylase inhibitors: structure-activity relationships and the synergism with acarbose. *Bioorganic and Medicinal Chemistry Letters*. 27, 5065-5070. <https://doi.org/10.1016/j.bmcl.2017.09.027>
- Zhang, J., Hu, K., Di, L., Wang, P., Liu, Z., Zhang, J., Qiao, H., 2021. Traditional herbal medicine and nanomedicine: Converging disciplines to improve therapeutic efficacy and human health. *Advanced Drug Delivery Reviews*, 178, 113964. <https://doi.org/10.1016/j.addr.2021.113964>