INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
24–0500/2025/33:330615
http://www.fspublishers.org

Full Length Article

Dipeptidyl Peptidase-IV Inhibition and Antioxidant Activity in Leaf, Bark, Fruit and Seed of *Diospyros foxworthyi*

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Received 12 October 2024; Accepted 17 February 2025; Published online 08 March 2025

Editor: Muhammad Farooq

Abstract

The present research was designed to explore the antioxidant activity and DPP-IV inhibition of secondary metabolites from *Diospyros foxworthyi* (foxworthyi ebony) leaf, bark, fruit and seed as well as antidiabetic and antioxidant agents. The sample was extracted in methanol using ultrasound-assisted extraction. It was used to study the extraction yield, Total Phenolic Content (TPC), Total Flavonoid Content (TFC), antioxidant activity and Dipeptidyl Peptidase-IV (DPP-IV) inhibition assay by microplate reader. The % yields of the leaf, bark, fruit and seed were 11%, 15.24%, 22.08% and 50.92%, respectively. Phytochemical screening results showed that the stem bark contained all test compounds (phenolic, alkaloid, tannin, saponin, terpenoid and anthraquinone). These showed TPC of the leaf, bark, fruit and seed were 41.01 mg/g extract, 54.38 mg/g extract, 51.99 mg/g and 64.83 mg/g of the extracts, respectively. TFC was 8.40 mg/g, 3.77 mg/g, 4.70 mg/g and 2.38 mg/g extract, respectively. The IC_{50} values for antioxidant activity using the α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method were 12.10 μg/mL, 5.32 μg/mL, 7.81 μg/mL and 5.86 μg/mL, respectively. The Ferric Reduction Antioxidant Power (FRAP) method was 1.74 $FeSO_4$/g, 4.09 $FeSO_4$/g, 1.91 $FeSO_4$/g and 2.56 g $FeSO_4$/g extract. The % inhibition values of the DPP-IV enzyme for the leaf, bark, fruit, and seed samples were 29.70%, 29.39%, 32.71% and 32.46%, respectively. The test results suggest that the bark and seed of *D. foxworthyi* hold potential for development as a treatment for diabetes mellitus, due to its antioxidant activities and DPP-IV inhibitory.

Keywords: Antioxidant; *Diospyros foxworthyi*; Dipeptidyl peptidase-IV; Total flavonoid content; Total phenolic content

Introduction

Indonesia is known for its biodiversity of wild flora, including numerous traditional medicinal species used by local communities (Buu *et al.* 2023). Indonesia is considered to be part of the richest sources of flora and fauna, as it also possesses a large amount of ethnomedicinal data on plant-based diabetes, hence the potential for further research, especially for the development of new medicine (Arifah *et al.* 2022). In Indonesia, they have used the leaf and bark of Ebenaceae in traditional diabetic healing (Ribeiro *et al.* 2023). The antidiabetic activity from *Diospyros malabarica* bark (Indian persimmon) at 100 μg/mL was identified using alpha-amylase enzyme inhibition method (Zreen *et al.* 2022). The root bark of *Diospyros melanoxylon* (coromandel ebony) has α-amylase inhibitory activity, with an IC_{50} value of 30 μM (Durgam *et al.* 2023). Phenolic compounds of *Diospyros Celebica* leaf (black ebony) showed the highest inhibition to the alpha-glucosidase enzyme, with an IC_{50} value of 14.90 μg/mL (Apriandini *et al.* 2023).

Diospyros foxworthyi (foxworthyi ebony) is a species of Diospyros from Indonesia. The compounds betulin (6.34%), lupeol (2.20%) and lupenone (0.33%) were detected in *D. foxworthyi* (Solehin *et al*. 2022). An inhibitory solid activity of Betulin has been reported on another plant *D. malabarica,* against alpha-amylase enzyme (Nargund *et al*. 2019). The present study suggests that *D. foxworthyi* has the potential to be used as an antidiabetic agent.

There are still rather few publications with *D. foxworthyi*. Nevertheless, supportive data on the compound content in *D. foxworthyi* leaf, which exhibits antidiabetic activity, offers a strong foundation to pursue additional investigations. This study will explore the phytochemical

To cite this paper: Clearn AK, B Elya, M Hanafi (2025). Dipeptidyl peptidase-IV inhibition and antioxidant activity in leaf, bark, fruit and seed of *Diospyros foxworthyi*. *Intl J Agric Biol* 33:330615. https://doi.org/10.17957/IJAB/15.2335

compound content of *D. foxworthyi* plant parts to obtain the best antidiabetic activity. The method of testing antidiabetic activity was carried out using the dipeptidyl peptidase (DPP-IV) enzyme inhibition method to get the latest antidiabetic point of view. The principle of antidiabetic activity with DPP-IV enzyme inhibition is by inhibiting the DPP-IV enzyme to reduce incretin hormones (GLP-1 and GIP) so that insulin secretion will continue with the activation of specific receptors on pancreatic beta cells (Amin *et al.* 2019). The antioxidant activity (DPPH and FRAP method) of *D. foxworthyi* plant parts was also explored to support the antidiabetic activity to be studied. Antioxidants can help reduce oxidative stress, improve endothelial function and increase insulin sensitivity (Jeffrey *et al.* 2021). Combining anti-diabetic medications with antioxidants will help improve glycaemic control and reduce oxidative stress in diabetic patients (Krawczyk *et al.* 2023).

Materials and Methods

Experimental details and treatments

Plant material: The leaf, bark, fruit and seed of *Diospyros foxworthyi* were collected from the KST BJ Habibie Research Center for Science and Technology (PUSPIPTEK) BRIN in Serpong, Banten, Indonesia. The authenticity of the plant was confirmed by determination data from the Traditional Medicine Raw Material Standardization Laboratory in Tawangmangu, Central Java, Indonesia (Sample No: 6449-152648-1). Then, samples were cleaned and dried at 35°C, ground into a fine powder, and sieved through 80 meshes. The powdered simplicia was kept in a desiccator at a constant temperature of 16°C until used for extraction.

Chemical: Methanol, sodium carbonate (Merck, Germany), quercetin, gallic acid, Folin-Ciocalteu reagent, DPPH, Gly-Pro-p-Nitroanilide, Trizma base, DPP-IV enzyme (Sigma-Aldrich, USA), aluminum chloride, sodium acetate buffer pH 3.6, HCl 40 mmol/L, 2,4,6-tripyridyl-s-triazine, iron (III) chloride hexahydrate, iron (II) sulfate heptahydrate, water for injection and sitagliptin (Wako Pure Chemical Industries Ltd., Japan).

Extraction

The Ultrasound-Assisted Extraction (UAE) technique, adapted from (Solehin *et al*. 2022) was used to perform the extraction. For each sample, 25 g of powdered leaf and bark were combined with methanol at 1:10 (g/mL) between the sample and the solvent. UAE was used for the extraction, which took place for 30 min at 40°C. For replication, the extraction was carried out three times (Khoshsima *et al*. 2023). The extract was passed through a vacuum rotary evaporator set to 40°C to evaporate it. The extract was dried using an evaporator. The yield was calculated and presented as a percentage.

Phytochemical screening

Phytochemical screening was carried out on the leaf, bark, fruit, and seeds of *D. foxworthyi*. Identification of phytochemical compounds follows the method of Ditjen (1995).

Alkaloids compounds

A total of 50 mg of the sample and 50 mg of *Polyscias scutellaria* (shield aralia) as apositive control were each added to 1 mL of 2 N HCl and 9 mL of distilled water. The solution was heated for 2 minutes, the filtrate formed was filtered. Filtrate in volume 1 mL was reacted with 2 drops of bouchardat reagent. The formation of a brown or black precipitate characterizes positive results.

Phenolic compounds

A total of 50 mg of the sample and 50 mg of gallic acid (positive control) were each added to 3 drops of 1% iron trichloride. The formation of a solid green or black color characterizes positive results.

Tannin compounds

A total of 50 mg of the sample and 50 mg of *Psidium guajava* L. extract (guava), used as a positive control, were each dissolved in 15 mL of hot water. The solution was then filtered. To 1 mL of the filtrate, two drops of 3% $FeCl_3$ reagent were added. The development of a violet-green color indicates a positive result.

Saponin compounds

A total of 50 mg of the sample and 50 mg of *P. scutellaria* extract (positive control) were each dissolved in 10 mL of hot water. The solution was shaken for 5 s. Positive results were characterized by the formation of foam that persisted after adding one drop of HCl 2 N.

Terpenoid compounds

A total of 50 mg of the sample and 50 mg of beta-sitosterol (positive control) were each dissolved in 15 mL of ethyl acetate. Lieberman burchard reagent was added to the solution. Positive results were characterized by the formation of a reddish color at the beginning and became greenish after a few minutes.

Anthraquinone compounds

A total of 50 mg of the sample and 50 mg of *Rheum rhabarbarum* extract (rhubarb) *as* positive control were each dissolved in 5 mL of 2 N sulfuric acid. The solution was heated, and 10 mL of benzene was added. The solution was shaken and allowed to stand until 2 layers were formed.

Positive control was. Positive results were characterized by forming a reddish water layer and clear benzene.

Determination of total phenolic content (TPC)

The determination of TPC refers to the study from (Desmiaty and Elya 2021). Twenty microliters of each calibration solution of gallic acid (concentrations 4, 6, 8, 10, 12 and 14 μg/mL) and each sample (200 μg/mL) were added to the 96-well plate. Each gallic acid and sample was added to 80 μL of sodium carbonate (100 g/L) and 100 μL of 25% Folin–Ciocalteu solution. A control solution containing 20 μL extract was mixed with distilled water to a volume of 200 μL. The incubation time was 2 h at 25°C; all experiments were performed in triplicate. Absorbance was read at the 760 nm wavelength. The total phenolic content on all samples was calculated by plotting on the linear regression curve of gallic acid and expressed as mg of gallic acid using the formula below:

$$\text{Total Phenolic Content (mg/g)} = \frac{(\text{concentration } (\mu g/mL) \text{ x total volume of extract (mL)})}{\text{weight of the sample (g) x Dilution factor}}$$

Determination of total flavonoid content (TFC)

A colorimetric assay was used to determine the TFC by the methodology outlined by (Desmiaty and Elya 2021). Twenty microliters of each calibration solution of quercetin (concentrations 30, 40, 50, 60, 70 and 80 μg/mL) and each sample (500 μg/mL leaf extract, 20.000 μg/mL bark, fruit, and seed extract) were added to the 96-well plate. Each quercetin and extract sample was added to 20 μL of 10%, b/v aluminum chloride and 20 μL of 1 *M* sodium acetate then added with aquadest up to a volume of 200 μL. A control solution containing 20 μL samples mixed with aquadest up to a volume of 200 μL. The mixture was incubated for 30 min at room temperature. Samples were prepared in triplicate for each analysis. The absorbance was determined with a microplate reader at 430 nm in wavelength. The total flavonoid content on all samples was calculated by plotting on the linear regression curve of quercetin and expressed as mg of quercetin using the formula below:

$$\text{Total Flavonoid content (mg/g)} = \frac{\text{concentration } (\mu g/mL) \text{ x volume of extract (mL)}}{\text{weight of the sample (g) x Faktor Dilution factor}}$$

DPPH antioxidant activity assay

Antioxidant activity analysis prepared by (Bobo-García *et al.* 2015). 20 μL extract sample (concentration 2 to 16 μL/mL) and quercetin as a positive control (concentration 1, 2, 3, 3.5 and 3.75 μL/mL) were mixed with 180 μL methanol DPPH 150 μM in a 96-well plate then shaken for 60 s for homogenization. The solution was incubated at room temperature in dark conditions for over 40 min. The absorbance was read at 515 nm. The antioxidant activity study was conducted in triplicate. Antioxidant activities were expressed in terms of % scavenging activity and IC_{50} value.

$$\% \text{ Inhibition} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance x } 100\%}$$

IC_{50} value (50% inhibition) = (50-a)/b

FRAP antioxidant activity assay

The assay for FRAP antioxidant activity refers to the study by Pereira *et al*. (2017) with some adaptations. A 30 μL extract sample (leaf concentration: 2.5 μL/mL, bark: 1 μL/mL, fruit: 2.5 μL/mL, and seed: 2 μL/mL) and quercetin as a positive control (0.5 μL/mL) were mixed with 270 μL of FRAP II Reagent (a solution of acetate buffer, 10 mmol/L Tris Pyridyl Triazine, and 20 mmol/L iron (III) chloride hexahydrate in a 10:1:1 ratio). The mixture was incubated for 5 min, and absorbance was measured at 595 nm. The blank contained a mixture of 30 μL of methanol pro analysis and 270 μL FRAP II reagent. Samples were prepared in triplicate for each analysis.

The standard curve solution of iron (II) sulfate heptahydrate (concentrations 7.19; 14.39; 21.58, 28.77, 35.97 and 43.16 μM) was pipetted as much as 30 μL and then FRAP I reagent (acetate buffer pH 3.6, 10 mmol/L Tris Pyridyl Triazine and aquadest in a ratio of 10:1:1) was added as much as 270 μL. The FRAP antioxidants activity on all samples was calculated by plotting on the linear regression curve of iron (II) sulfate heptahydrate and expressed as μmol/g sample using the formula below:

$$\text{FRAP } (\mu\text{mol/g sample}) = \frac{\text{C x V x dilution factor}}{w}$$

Where C is the concentration of $Fe_2SO_4 \cdot 7H_2O$ standard equivalent to the sample (μM), V is the volume of the sample (mL), and w is the weight of the sample (mg).

Dipeptidyl peptidase enzyme inhibition activity assay

The activity test for DPP-IV inhibition was performed using a microplate 96-well method, as described previously by Elya *et al*. (2015). The preparation consists of a sample, sample control, blank, and blank control. To prepare the sample, a weighed amount of the sample was dissolved in Tris HCl buffer (pH 7.5) to create a solution with five concentration series within the valid range. Test samples (35 μL each) were mixed with 15 μL DPP-IV enzyme and incubated at 37°C for 10 min. After incubation was over, 50 μL of GPPN substrate 1.2 m*M* was added and incubated for 30 minutes. To stop the reaction, 25 μL of 30% glacial acetic acid was added. To prepare the sample control, use the same procedure as the sample preparation but without the addition of the DPP-IV enzyme. To prepare the blank, use the same procedure as the sample preparation but without a sample solution. The blank control was prepared using the same procedure as the blank solution but without adding the DPP-IV enzyme. Each series of sample concentrations was made

in three replications. The solution was then assayed for absorbance at 405 nm. Calculation of IC_{50} using the regression equation (y = ax + b) the following formula:

$$\% \text{ Inhibition} = \frac{(\text{blank absorbance} - \text{blank control absorbance}) - (\text{sample absorbance} - \text{sample control absorbance})}{\text{blank absorbance} - \text{blank control absorbance}} \times 100$$

Statistical Analysis

Data from TPC, TFC, DPPH, FRAP and DPP-IV antidiabetic activity were analyzed to obtain standard deviation data. The correlation of data from each test variable was analyzed using Pearson correlation with Minitab version 22.

Results

Extraction results and yield

Fresh leaf, bark, fruit and seed simplicia of *D. foxworthyi* in the collection (Fig. 1) were extracted using UAE. The seed sample had the highest dry extract yield of 50.92%. The leaf sample yielded the least amount 11.00% (Table 1).

Phytochemical screening

The results of phytochemical screening are presented in Table 2. The results of phytochemical screening showed that *D. foxworthyi* leaf extract was positive for phenolic compounds, tannins, and terpenoids. *D. foxworthyi* stem bark extract was positive for containing all the indicated compounds, namely alkaloid, phenolic, tannin saponin, terpenoid, and anthraquinone compounds. *D. foxworthyi* fruit extract was positive for phenolic compounds, tannins, terpenoids and anthraquinones. *D. foxworthyi* seed extract was positive for alkaloid, phenolic, tannin, and terpenoid compounds.

Total phenolic and flavonoid content

The total phenolic and flavonoid content from *D. foxworthyi* leaf bark, fruit, and seed extracts was determined in a microplate reader 96 well using a gallic acid standard curve for phenol content and a quercetin standard curve for flavonoid content (Fig. 2). The highest phenolic content in the seed sample was at 64.832 ± 1.085 mg/g extract, while the lowest was in the leaf sample, at 41.010 ± 0.486 mg/g extract. The best TFC results were found in the leaf sample, at 8.400 ± 0.207 mg/g and the lowest in the seed sample, at 2.381 ± 0.037 mg/g (Fig. 3). The results indicate that each part of the plant's total phenolic and flavonoid content is inversely related.

DPPH antioxidant activity

The DPPH antioxidant capacity test revealed that percent inhibition is very high (below 50 μg/mL) for all plant parts of *D. foxworthyi* ones. Altogether, bark extract showed the highest antioxidant activity (IC50: 5.32 μg/mL). Positive control sample DPPH test results: 1.77 μg/mL (Table 3).

Table 1: Yield Data

Plant Parts	Simplicia Weight (g)	Extract Weight (g)	Yields (%)
Leaf	25.00	2.75	11.00
Bark	25.00	3.81	15.24
Fruit	25.00	5.52	22.08
Seed	25.00	12.73	50.92

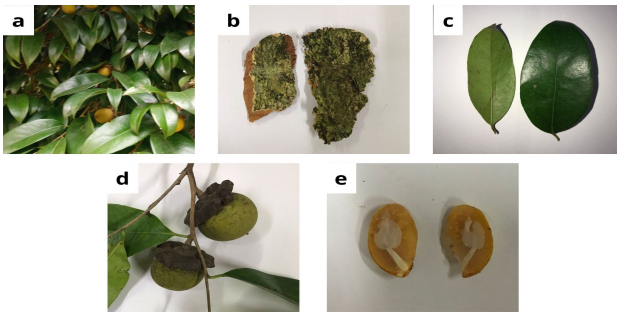


Fig. 1: *Diospyros foxworthyi,* (**a**) *Diospyros foxworthyi* tree; (**b**) bark; (**c**) leaf; (**d**) fruit; (**e**) seed

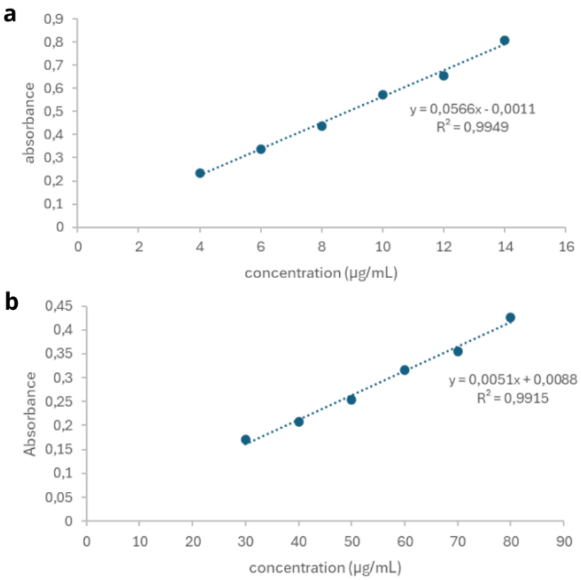
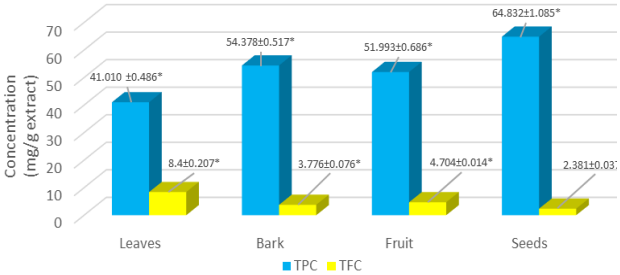


Fig. 2: Standard calibration curve, (**a**) Gallic acid for total phenol content; (**b**) Quercetion for total flavonoid content



*Value is expressed as for the experiment (mean ± SD, n=3)

Fig. 3: Total phenolic content (TPC) and total flavonoid content (TFC) (data from *D. foxworthyi* leaf, bark, fruits and seed.

Table 2: Phytochemical Screening

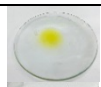
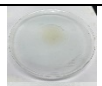
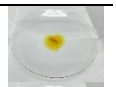
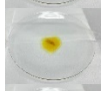
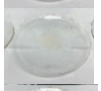
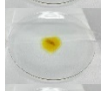
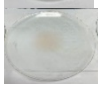
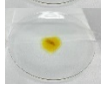
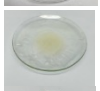
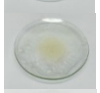
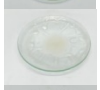
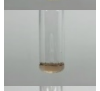
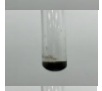
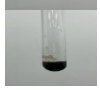
Secondary Metabolites	Plant Parts	Result	Figure		
			Sample	Negative Control	Positive Control
Alkaloids	Leaf	-			
	Bark	+			
	Fruit	-			
	Seed	+			
Phenolic	Leaf	+			
	Bark	+			
	Fruit	+			
	Seed	+			
Tannin	Leaf	+			
	Bark	+			
	Fruit	+			
	Seed	+			
Saponins	Leaf	-			
	Bark	+			
	Fruit	-			
	Seed	-			
Terpenoid	Leaf	+			
	Bark	+			
	Fruit	+			
	Seed	+			

Table 2: Continued

Table 2: Continued

Anthraquinone	Leaf	-	
	Bark	+	
	Fruit	+	
	Seed	-	

Fig. 4: FRAP Standard calibration curve

FRAP antioxidant activity

FRAP methods for the antioxidant activity test were performed by measuring the sample concentration that gave an absorbance equal to the $FeSO_4$ solution using the linear regression equation curve of a Fe(II) standard calibration plot, as previously described in the solution based on the FRAP linear regression equation curve (Fig. 4). The highest antioxidant activity was shown by bark extract, with a value of 14.71 mmol $FeSO_4$/g (Table 4). Similar to the DPPH method, the results indicated that the bark had the highest antioxidant activity.

Dipeptidylpeptidase-IV enzyme inhibitory activity

The DPP-IV inhibitory uses Sitagliptin as the positive control with IC_{50} 0.12 μg/mL (Table 5). The percent inhibition value of each plant part was calculated in a concentration of 140 μg/mL, which is presented in Table 6. The best percent inhibition was shown by *D. foxworthyi* fruit extract with a percent inhibition of 32.71% at a concentration of 140 μg/mL. The percent inhibition of leaf, stem bark, and seed extracts at the same concentration (140 μg/mL) were 29.70%, 29.39% and 32.46%, respectively. The results of antioxidant activity tests (DPPH and FRAP) did not show correlation with inhibitory DPP-IV enzyme, as observed in the above test outcomes.

Correlation between TPC, TFC, antioxidant and DPP-IV inhibition activity of leaf, Bark, Fruit and Seed

Pearson correlation analysis was measured for TPC, TFC,

Table 3: DPPH Antioxidant Activity

Sample	Concentration	Mean % DPPH Scavenging SD	R2	IC_{50} (μg/mL)	Standard Deviation
Quercetin	1	19.54	0.9916	1.77	1.69
	2	45.16			1.47
	3	74.86			1.08
	3,5	84.68			1.25
	3,75	86.62			0.20
Leaf	6	24.64	0.9784	12.10	0.32
	8	34.67			2.87
	12	53.15			0.69
	14	57.61			0.32
	16	62.53			1.16
Bark	2	27.68	0.9964	5.32	1.25
	3	35.71			0.74
	5	47.57			0.67
	6	55.48			1.87
	7	60.38			1.11
Fruit	5	30.20	0.9901	7.81	0.56
	6	34.81			0.34
	7	42.27			0.60
	8	51.01			0.64
	9	58.52			0.36
Seed	5	43.05	0.9828	5.86	0.13
	6	51.84			0.51
	7	58.79			0.13
	8	63.44			0.65
	9	68.74			0.28

Table 4: FRAP Antioxidant Activity Results

Sample	FRAP (mmol $FeSO_4$/g extract)	Antioxidant Activity (g $FeSO_4$/ g extract)	Standard deviation
Quercetin	34.29	9.53	0.07
Leaf	6.27	1.74	0.01
Bark	14.72	4.09	0.05
Fruit	6.87	1.91	0.00
Seed	9.21	2.56	0.01

*Value is expressed as for the experiment (mean ± SD, n = 3)

Table 5: DPP-IV enzyme inhibitory activity by Sitagliptin

Sample	Concentration (μg/mL)	% Inhibition	R2	IC_{50} (μg/mL)	Standard deviation
Sitagliptin	0.210	69.79	0.9809	0.12	0.49
	0.175	61.43			0.39
	0.105	49.83			1.47
	0.070	39.57			0.61
	0.035	27.75			1.12

*Value is expressed as for the experiment (mean ± SD, n = 3)

Table 6: DPP-IV enzyme inhibitory activity

Plant Parts	Concentration (μg/mL)	% inhibition	Standard deviation
Leaf	140	29.70	0.47
Bark	140	29.39	0.22
Fruit	140	32.71	0.66
Seed	140	32.46	0.54

*Value is expressed as for the experiment (mean ± SD, n = 3)

antioxidant (DPPH and FRAP) and DPP-IV enzyme. A strong correlation was found between TPC with TFC and DPPH with TFC. The correlation between TPC and TFC variables has a *P*-value of 0.031 (< 0.05), so it is concluded that the TPC variable has a significant correlation to the TFC variable. The correlation value obtained is negative (-0.969), so it can be concluded that the direction of the correlation between TPC and TFC variables is in the opposite direction with a strong level of closeness. TFC variables significantly correlate with DPPH variables with a *P*-value of 0.044 (< 0.05). The correlation value of TFC and DPPH is positive 0.956, which is concludes that the direction of the variable correlation was in the same direction with a strong level of closeness (Fig. 5).

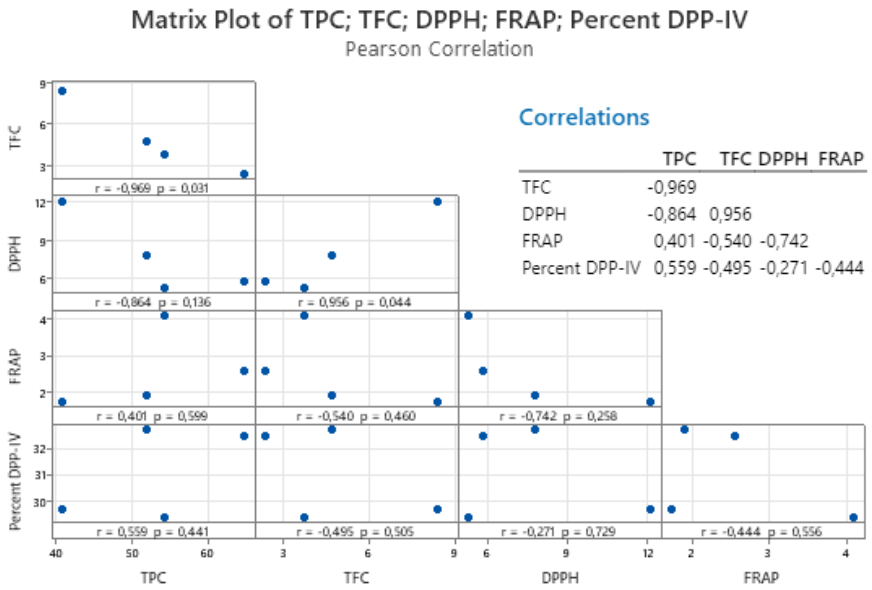


Fig. 5: Pearson Correlation of TPC, TFC, DPPH, FRAP and percent inhibition DPP-IV

Discussion

Ultrasound-assisted extraction is one of the newest and most efficient methods for extraction when compared to existing methods. The samples were homogenized to 80 mesh, a process that breaks the hard shell of spores and creates a more extensive surface area in contact with the solvent (Suryanto and Taroreh 2021). UAE extraction for the seed extract of *D. foxworthyi* led to a dry extract yield up to 50%. The high yield was due to the cavitation phenomenon, which led to cell wall breakage of the simplicia, speeding up relative mass transfer between substrate and solvent (Shen *et al.* 2023).

Phytochemical screening showed that stem bark and seed extracts contained alkaloid compounds. The positive control in the alkaloid test was *Rheum rhabarbarum* extract because this plant is known to have high alkaloids used to overcome inflammation, radiation, cancer, and diabetes (Munoz et al. 2018). The positive control in the alkaloid test was *Polyscias scutellaria* extract because this plant is known to have high alkaloids (Kasmara *et al.* 2024). Several types of alkaloids such as boldine, berberine, and sanguinarine, have been shown to have antidiabetic activity by inhibiting α-glucosidase, dipeptidyl peptidase-IV, aldose reductase, α-amylase, tyrosine phosphatase-1B, and increasing insulin secretion (Naseri *et al.* 2022). Numerous tannins such asvescalagin, acutissimin A/B, epiacutissimin A/B, grandinin/roburin E, hexagalloyl glucose, and heptagalloyl, reduce blood sugar levels by blocking the action of α-amylase and α-glucosidase (Hossain *et al.* 2021).

The positive control in the saponin test was *Polyscias scutellaria* extract because this plant is known to have high tannins (Kasmara *et al.* 2024). Saponins from *Momordica charantia* (bitter melon) can improve insulin resistance, lower fasting blood glucose, and return body weight to normal (Sukhikh *et al.* 2023). Terpenoid identification shows that all parts of the plant are positive for containing terpenoids. The plant *D. foxworthyi* had been reported to contain betulin (Solehin *et al.* 2022). Betulin is a pentacyclic triterpenoid found mainly in the bark of persimmon trees (Zhang *et al.* 2024). A 50 mg/kg dose of betulin is used and it shows its anti-diabetic effect through the regeneration of cells along with the protective effects against cytolysis, diabetic nephropathy, and pancreatic damage (Adepoju *et al.* 2024).

Flavonoids and phenolics of the extract part have different levels. Phenolic compounds in flavonoids help to lower blood glucose levels by preventing pancreatic islet of Langerhans cell damage (Parwata *et al.* 2018), Inhibiting the enzyme α-glucosidase from breaking down glucose (Liu *et al.* 2020) and reducing cells resistance to insulin (Zin *et al.* 2022). Phenolic and flavonoid compounds can control blood glucose levels through hydrogen bonding and hydrophobic interactions, which result in structural changes to the α-glucosidase enzyme. These changes block substrate access to the enzyme's active site, ultimately reducing its activity (Le *et al.* 2022; Qin *et al.* 2023).

Among all the parts, bark showed maximum activity in both assays, DPPH and FRAP. These results indicate that the flavonoid and phenolic content contribute to antioxidant potential. The structure of flavonoid molecules often correlates closely with their bioaction, especially for antioxidant action (Jucá *et al.* 2020). This situation resides in the energy necessary to liberate hydrogen from their phenolic groups, concerning the DPPH free radical scavenging mechanism by phenolic and flavonoid

compounds. Flavonoids with lower bond dissociation enthalpy, like quercetin (positive control) are more effective in scavenging DPPH radicals (Veiko *et al.* 2021).

Analysis of DPP-IV enzyme inhibitory activity showed similar activity in all *D. foxworthyi* plant parts. The positive control sitagliptin was able to give an IC_{50} value of 1.20 μg/mL. The results of this positive control align with the literature, which reports that the IC_{50} value of sitagliptin ranges from 0.08 μg/mL (Triadisti *et al*. 2025) to 1.104 μg/mL (Riyanti *et al*. 2019) and up to 9.37 μg/mL (Amin *et al.* 2019). Sitagliptin functions as an inhibitor of the DPP-IV, which stops the breakdown of Glucagon-Like Peptide-1 (GLP-1) and Glucose-Dependent Insulinotropic Polypeptide (GIP), which causes the body to produce more insulin.

The percentage inhibition activity of the DPP-IV enzyme from the leaf, bark, fruit, and seeds of *D. foxworthyi* did not show any significant difference in the range of 29.39% (leaf) to 32.71% (bark) at a 144 μg/mL concentration. The inhibition activity from *D. foxworthyi* plant parts was more active than the *Anogeissus latifolia* plants (gum ghatti) (IC_{25} 590 μg/mL) and *Aegle marmelos* (maja fruit) (IC_{25} 446 μg/mL), which have long been used as antidiabetic activities by the community in Southeast Asia (Ansari *et al*. 2021). The results of the correlation data between DPP-IV inhibition activity and DPPH and FRAP antioxidant activities were -0.271 and -0.444, respectively. These results indicate no linear correlation between antioxidant activity and DPP-IV inhibition activity in *D. foxworthyi* extract. However, the antioxidant activity of DPPH in *D. foxworthyi* fruit flesh showed high activity with an IC_{50} of 7.80 μg/mL. *D. foxworthyi* fruit extract showed the best DPP-IV inhibition; this was in line with the highest yield and phenolic content in the seed extract. Phenolic compounds inhibit the DPP-IV enzyme through hydrogen bonding to the active site of the DPP IV enzyme (Huang *et al*. 2019). Compounds with more hydroxyl groups tend to have better inhibitory effects (Jia *et al*. 2021).

Antioxidant and antidiabetic activities are closely related. Oxidative stress is a fundamental factor in the pathogenesis, progression, and complication of diabetes (Caturano *et al.* 2023). Diabetes arises partly from oxidative stress that causes organ damage through free radicals (Ghasemi-Dehnoo *et al.* 2020; Bhatti *et al.* 2022). Antioxidants, mainly phenolic compounds, help reduce organ damage by scavenging free radicals and inhibiting reactive oxygen species production (Zeb 2020; Akbari *et al.* 2022). Additionally, phenolic acids are essential for managing diabetes because they influence the activities of insulin and glucose receptors, boost pancreatic β-cell production of the GLUT2 glucose transporter, and facilitate GLUT4 translocation (Kumar and Goel 2019).

Correlation data between TPC, TFC, antioxidants, and inhibition of the DPP-IV enzyme of each sample were conducted using Minitab version 2.2. Correlation analysis was conducted to evaluate the strength and direction of the relationship between quantitative variables (Schober *et al.* 2018). This study uses the Pearson correlation type. Pearson correlation is used to determine the strength of the linear relationship between two continuous variables (Rovetta 2020). According to this analysis, the correlation will be stronger when the values are closer to 1 and less when the values are closer to 0. Positive values indicate a positive correlation and negative values indicate a negative correlation (Schober *et al.* 2018). The correlation value obtained is negative (-0.969), so it can be concluded that the direction of the correlation between TPC and TFC variables is in the opposite direction with a strong level of closeness. The correlation value of TFC and DPPH is positive (0.956), which means that the greater the flavonoid content, the higher the antioxidant activity using the DPPH method.

Conclusion

All parts of the *D. foxworthyi* plant (leaf, bark, fruit and seed) have shown antioxidant and antidiabetic effects as a potent mechanism of DPP-IV enzyme inhibition. *D. foxworthyi* fruit extract showed the best DPP-IV inhibition; this was in line with the highest yield and phenolic content in the seed extract. *D. foxworthyi* fruit extract showed the best DPP-IV inhibition; this is in line with the results of very high phenolic content, namely 51.993 mg/g equivalent of gallic acid. Although the antioxidant activity and DPP-IV inhibition in *D. foxworthyi* plant parts are not significantly correlated, *D. foxworthyi* fruit extract, which has the best DPP-IV inhibition activity of 32.71% at a concentration of 144 μg/mL, also has very active DPPH antioxidant activity, namely with IC_{50} 7.80 μg/mL. The DPP-IV enzyme inhibitory activity in *D. foxworthyi* does not always depend on antioxidant activity, but both can play a synergistic role in diabetes management. More research into pharmacologic variants is warranted for treatment advances of higher magnitude in this species.

Acknowledgments

The first author acknowledges Universitas Indonesia for the support and facilities provided, which enabled us to conduct this research. We also thank all those who offered guidance, insights and valuable contributions to this study.

Author Contributions

Each author has made an equal contribution to this work and has given their approval for its publication.

Conflict of Interest

There is no conflict of interest reported by all authors.

Data Availability

The corresponding author may grant access to the data in this study on a reasonable request.

Ethics Approval

Outside the purview of this manuscript

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