



Exploring the antioxidant and α -glucosidase inhibitor potential properties across three *Ziziphus* species: An insight from HPLC data

[Exploración de las propiedades antioxidantes y de inhibición de la α -glucosidasa en tres especies de *Ziziphus*: Una perspectiva a partir de los datos de HPLC]

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Abstract

Context: Three species from the genus *Ziziphus*, namely *Ziziphus spina-christi*, *Ziziphus mauritiana*, and *Ziziphus jujuba*, are widely recognized for their benefits as traditional medicines. Much literature highlights their antioxidant and antidiabetic properties attributed to their flavonoid components.

Aims: To evaluate the antioxidant capacity and α -glucosidase inhibitor potential of different parts of three *Ziziphus* species by analyzing their antioxidant activity, phenolic and flavonoid contents, identifying key flavonoids using high-performance liquid chromatography (HPLC), and correlating these with their bioactivities.

Methods: This study measured the phenolic and flavonoid contents in the fruit, seeds, and leaves of these plants, evaluated their antioxidant capacity using 2,2-diphenyl picrylhydrazyl (DPPH) and cupric ion reducing antioxidant capacity (CUPRAC) methods, identified compounds via HPLC, and applied Pearson's correlation analysis to the data.

Results: This study found that *Ziziphus* leaves have the highest antioxidant capacity, with *Z. mauritiana* leaves showing the most activity, followed by *Z. spina-christi* and *Z. jujuba* leaves. These results match the total phenolic and flavonoid contents. HPLC analysis identified rutin in all *Ziziphus* leaves, quercetin in *Z. mauritiana* fruit and leaves, kaempferol in *Z. mauritiana* seeds, and apigenin in *Z. mauritiana* fruit and leaves. Pearson's correlation analysis showed a strong correlation between rutin, quercetin, and apigenin and their antioxidant and α -glucosidase inhibitor activities.

Conclusions: The leaves of *Z. mauritiana* possess the highest antioxidant and α -glucosidase inhibitor activities, and these activities are attributed to the presence of specific flavonoid compounds, namely rutin, quercetin, and apigenin.

Keywords: antioxidant; flavonoids; α -glucosidase inhibitor; HPLC; *Ziziphus*.

Resumen

Contexto: Tres especies del género *Ziziphus*, a saber, *Ziziphus spina-christi*, *Ziziphus mauritiana* y *Ziziphus jujuba*, son ampliamente reconocidas por sus beneficios como medicina tradicional. Diversa literatura destaca sus propiedades antioxidantes y antidiabéticas, atribuidas a sus componentes flavonoides.

Objetivos: Evaluar la capacidad antioxidante y el potencial inhibidor de la α -glucosidasa de diferentes partes de tres especies de *Ziziphus* mediante el análisis de su actividad antioxidante, su contenido fenólico y flavonoides, la identificación de flavonoides clave mediante cromatografía líquida de alta resolución (HPLC) y su correlación con sus bioactividades.

Métodos: Este estudio midió el contenido fenólico y flavonoides en el fruto, las semillas y las hojas de estas plantas, evaluó su capacidad antioxidante mediante los métodos de 2,2-difenil picrilhidrazilo (DPPH) y capacidad antioxidante reductora del ion cúprico (CUPRAC), identificó compuestos mediante HPLC y aplicó el análisis de correlación de Pearson a los datos.

Resultados: Este estudio encontró que las hojas de *Ziziphus* poseen la mayor capacidad antioxidante, siendo las hojas de *Z. mauritiana* las que mostraron la mayor actividad, seguidas de las hojas de *Z. spina-christi* y *Z. jujuba*. Estos resultados coinciden con el contenido total de fenólicos y flavonoides. El análisis por HPLC identificó rutina en todas las hojas de *Ziziphus*, quercetina en frutos y hojas de *Z. mauritiana*, kaempferol en semillas de *Z. mauritiana* y apigenina en frutos y hojas de *Z. mauritiana*. El análisis de correlación de Pearson mostró una fuerte correlación entre la rutina, la quercetina y la apigenina y sus actividades antioxidantes e inhibidoras de la α -glucosidasa.

Conclusiones: Las hojas de *Z. mauritiana* poseen las mayores actividades antioxidantes e inhibidoras de la α -glucosidasa, actividades que se atribuyen a la presencia de compuestos flavonoides específicos, concretamente rutina, quercetina y apigenina.

Palabras Clave: antioxidante; flavonoides; HPLC; inhibidor de la α -glucosidasa; *Ziziphus*.

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Abbreviations: AGI: α -glucosidase inhibition; AUC: area under curve; CE: concentration of extract; CUPRAC: cupric ion reducing antioxidant capacity; CS: concentration of standard; DPPH: 2,2 diphenyl picrylhydrazyl; g GAE/100 g DE: grams of gallic acid equivalent per 100 grams of dry extract; g QE/100 g DE: grams of quercetin equivalent per 100 grams of dry extract; HPLC: high performance liquid chromatography; IC₅₀: inhibitory concentration 50; ME: metabolite in extract; pNPG: 4-nitrophenyl- α -D-glucopyranoside; S: standard; SCE: standard content in extract; TFC: total flavonoid content; TPC: total phenolic content; UV/Vis: ultraviolet/visible.

INTRODUCTION

Ziziphus is a plant genus with numerous species worldwide and is utilized by humans across diverse regions for various purposes. The most prominent species of *Ziziphus* are *Ziziphus spina-christi*, *Ziziphus mauritiana*, and *Ziziphus jujuba*. These species hold a significant importance for human consumption and traditional medicine (Arndt et al., 2001; El Maaiden et al., 2020). The *Z. spina-christi* and *Z. mauritiana* are mainly found in the Middle East, Africa, and parts of Europe (Dafni et al., 2005; Pasiecznik, 2019), while *Z. jujuba* has been extensively used in Asia, especially China, for millennia (Jin, 2018; Shahrajabian et al., 2020).

The most commonly consumed and used parts of the plant for traditional medicine are leaves and fruit. *Z. spina-christi* and *Z. mauritiana* fruits share similar characteristics, typically featuring a sour taste (Zandiehvakili and Khadivi, 2021; Zarei et al., 2021). In contrast, *Z. jujuba* fruits exhibit a slightly different appearance, resembling dates, and possess a sweeter taste (Khadivi et al., 2021). *Z. jujuba* fruit is the most extensively used and traded commodity among the three species (Shahrajabian et al., 2020). Meanwhile, the *Z. spina-christi* and *Z. mauritiana* leaves are widely consumed and believed to offer various notable health benefits (Abdallah et al., 2016; Al-Ghamdi and Shahat, 2017; Almeer et al., 2019).

The benefits of these three plants have been extensively documented, thereby bolstering their significance for consumption and utilization in traditional medicine. These plants are widely recognized for their antioxidant properties, which are closely linked to various health benefits like antidiabetic, anti-inflammatory, and functional foods (Al-Ghamdi and Shahat, 2017; Ji et al., 2020; Lee et al., 2021). The *Ziziphus* plant's antioxidant capacity and other benefits are attributed to its phenolic and flavonoid compounds. These compounds are known for their ability to scavenge free radicals and contribute to overall health (Sampiev et al., 2020; Xue et al., 2021; Youl et al., 2019; Zandiehvakili and Khadivi, 2021). In this study, all *Ziziphus* species samples were determined to inhibit the α -glucosidase enzyme, leveraging the antidiabetic benefits of the antioxidant ability within the *Ziziphus* genus (Ben et al., 2022; Sakna et al., 2019; 2022; Şöhretoğlu et al., 2022).

These three *Ziziphus* species are of particular interest for comparative studies on their antioxidant and

anti-hyperglycemic efficacy. Such investigations are essential to identify the plant parts exhibiting the highest bioactivity, thereby guiding their potential development as therapeutic agents for diabetes mellitus and other degenerative diseases associated with oxidative stress and free radical-induced damage. Among the three species, *Z. jujuba* has been the most extensively studied regarding its antioxidant activity. Previous studies have examined the antioxidant properties of its leaves (Damiano et al., 2017), comparing antioxidant capacities among different fruit components—pulp, seeds, and whole fruit (Rajaei et al., 2021; Sumālan et al., 2025), and assessed variations in antioxidant activity across different fruit ripening stages (Xie et al., 2017; Yan et al., 2022). Additionally, investigations have explored the impact of post-harvest processing, such as blackening treatments, on antioxidant capacity (Song et al., 2022), as well as the bioactivity of polysaccharides extracted from aqueous fruit residues (Zhu et al., 2021). The antioxidant potential of *Z. mauritiana* and *Z. spina-christi* fruits has also been reported (El Maaiden et al., 2020; Zozio et al., 2014). The bioactivity of these species is frequently associated with their capacity to inhibit key metabolic enzymes involved in diabetes pathophysiology, such as α -glucosidase and xanthine oxidase, which are implicated in postprandial glucose regulation and oxidative stress (Aleryani et al., 2025; Bukhari et al., 2022).

Phenolic and flavonoid compounds from *Ziziphus* species have garnered significant attention, with numerous reports documenting their presence. Notably, catechin, ferulic acid, caffeic acid, chlorogenic acid, syringic acid, apigenin, rutin, quercetin, and kaempferol have been identified in the fruits, leaves, and stems of *Z. spina-christi* (Ghafoor et al., 2012; Saeed et al., 2025; Salem et al., 2025). Similarly, *Z. jujuba* has been found to contain a comparable profile of phenolic and flavonoid compounds. However, additional bioactive compounds such as eriodictyol, *p*-hydroxybenzoic acid, silibinin, taxifolin, and phloretin have also been identified in this species (Lee et al., 2016; Nuralin, 2024; San and Yildirim, 2010).

The three *Ziziphus* species discussed in this study have strong ethnomedicinal significance, as they have been traditionally used across various cultures throughout Asia, the Middle East, parts of Africa, and Europe (Alla et al., 2025; Dafni et al., 2005; Jin, 2018; Pasiecznik, 2019; Shahrajabian et al., 2020). This study conducts a comparative analysis of the flavonoid

composition in these *Ziziphus* species, providing a clearer understanding of their pharmacological potential, particularly in relation to their antioxidant and antidiabetic activities.

This study comprehensively analyzed the total phenolic and flavonoid contents within the leaves, fruits, and seeds of three *Ziziphus* species: *Z. spina-christi*, *Z. mauritiana*, and *Z. jujuba*. Through the high-performance liquid chromatography (HPLC) utilization, flavonoid compounds, including apigenin, luteolin 7-O-glucoside, kaempferol, quercetin, and rutin, were identified and examined for their potential role in providing antioxidant and α -glucosidase inhibitor activities. The primary focus of the investigation was to discern the specific compounds within these plants that contribute significantly to their bioactivity properties.

MATERIAL AND METHODS

Materials

This study employed several solvents, including distilled water, aqua-pro injection, 96% ethanol, pro-analytical, and HPLC-graded methanol. Reagents used for total phenols, total flavonoids, and antioxidant activity analyses comprised gallic acid, quercetin, ascorbic acid, Follin-Ciocalteu, sodium carbonate, aluminum chloride, 2,2-diphenyl-1-picrylhydrazyl (DPPH), cupric chloride, neocuproin, and ammonium acetate. The α -glucosidase (*Saccharomyces cerevisiae*) G5003-100UN Cas number 56180-94-0, 4-nitrophenyl- α -D-glucopyranoside (pNPG) N1377-1G Cas number 3767-28-0 were acquired from Sigma-Aldrich (St. Louis, USA), and acarbose hydrate was obtained from TCI (Tokyo, Japan). The standards, such as apigenin, kaempferol, luteolin 7-O-glucoside, rutin, and quercetin, were utilized in the HPLC analysis. All solvents and reagents were manufactured by Merck. The chromatographic analysis was performed using LiChrospher® 100 RP-18 (Merck). The tools employed in the analysis included high-performance liquid chromatography (HPLC) LC-20AD, CTO-20A oven, CTO-20A pump injector (Shimadzu), and UV/Vis Spectrophotometer (Thermo).

Collection of plant samples

Samples from leaves, fruit, and seeds of three *Ziziphus* species: *Z. spina-christi* were obtained from Depok, West Java, Indonesia (6°23'24"S 106°48'23"E, Herbarium Bandungense, Bandung Institute of Technology 2133/IT1.C11.2/TA.00/2022); *Z. mauritiana* from Pekanbaru, Riau, Indonesia (0°26'35"N 101°22'13"E, Herbarium Medanese, Universitas Sumatera Utara 373/MEDA/2022); and *Z. jujuba* from Miaoli, Taiwan (24°33'25"N 120°50'00"E, Herbarium

Bandungense, Bandung Institute of Technology 74/IT1.C11.2/TA.00/2023). Official botanists identified all plants at Herbarium Bandungense, School of Life Sciences and Technology, Bandung Institute of Technology, and Herbarium Medanese, University of North Sumatra, Indonesia. All samples underwent a rigorous cleaning process to remove impurities, thus separating the fruit flesh and seeds. Subsequently, all samples—leaves, fruit flesh, and seeds—were dried using a freeze dryer (HNZXIB Lab Vertical Freeze Dryer, Henan Zhuoxian Import & Export Trading Co., Ltd., China) at condenser temperature at -58.9°C, preservation temperature at -19.7°C, and vacuum at 4.5 Pa, before grinding them into a powder.

Sample extraction

All sample powders were extracted using a mixture of ethanol and water (7:3), facilitated by a sonicator (WUC-D22H Ultrasonic Bath, Witeg, Republic of Korea). Ten solvent parts were employed for each powder part, followed by sonication at 50°C for 30 minutes. Subsequently, the extract was separated from the residues through filtration. The extract was then concentrated using a rotary evaporator (rotavapor R-215, Buchi, Switzerland) at 50°C until a dry extract was achieved.

The total phenolic contents

Total phenolic contents were determined using gallic acid as a reference standard. The gallic acid solutions were prepared at various concentrations (40-130 μ g/mL). Each concentration was triplicated. Initially, 0.5 mL of gallic acid was mixed with 5 mL of 10% Folin-Ciocalteu reagent. Subsequently, 4 mL of 1 M sodium carbonate was added to the mixture. The solution was incubated for 15 minutes after the sample absorbance was measured at 765 nm wavelength using a UV/vis spectrophotometer. The absorbance values from different concentrations of gallic acid were utilized to construct a calibration curve (Arsul et al., 2024; Insanu et al., 2022; Pourmorad et al., 2006).

The total phenolic contents were determined by initially dissolving each extract in pro-analysis graded methanol. The extract was then mixed with 10% Folin-Ciocalteu reagent and 1 M sodium carbonate, following the same protocol as employed for gallic acid. The extract concentration was adjusted to yield an absorbance within 0.2 to 0.8 and was replicated six times on each sample. The total phenolic contents of the extract were calculated using the equation derived from the gallic acid calibration curve and expressed as grams of gallic acid equivalent per 100 grams of dry extract (g GAE/100 g DE) (Arsul et al., 2024; Insanu et al., 2022; Pourmorad et al., 2006).

The total flavonoid contents

Quercetin, utilized as a reference standard, was prepared in various concentrations (60–130 $\mu\text{g/mL}$). Quercetin was taken at 0.5 mL and mixed with 1.5 mL of methanol, 2.8 mL of distilled water, 0.1 mL of 10% AlCl_3 , and 0.1 mL of sodium acetate 1 M. The mixture was incubated for 30 minutes and measured using an ultraviolet/visible (UV/Vis) spectrophotometer at 415 nm wavelength. The absorbance values for each quercetin concentration were employed to construct a calibration curve (Arsul et al., 2024; Chang et al., 2002; Insanu et al., 2022).

The extract from each sample was dissolved in pro-analysis graded methanol and then mixed with the same reagents for quercetin as described above. The concentration of each sample was adjusted to achieve an absorbance of 0.2 to 0.8. Each sample was analyzed six times. The total flavonoid contents of the extract were calculated using the regression equation derived from the quercetin calibration curve and expressed in grams of quercetin equivalent per 100 grams of dry extract (g QE/100 g DE) (Arsul et al., 2024; Chang et al., 2002; Insanu et al., 2022).

Antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Antioxidant activity was determined using a modified Celep's method (Celep et al., 2015). The ascorbic acid was used as an analysis standard. The ascorbic acid was prepared at 200 $\mu\text{g/mL}$, while the DPPH solution was dissolved in methanol at 50 $\mu\text{g/mL}$. For the ascorbic acid assay, six concentrations (10 μL , 12.5 μL , 15 μL , 20 μL , 25 μL , and 30 μL) were prepared and added to microtubes (Eppendorf). To each microtube, methanol pro analysis was added up to 125 μL , followed by 750 μL of DPPH solution. Each concentration was triplicated. The mixture was then incubated for 30 minutes and subsequently measured using a UV/vis spectrophotometer at 517 nm wavelength. Free radical scavenging by antioxidants was indicated by the shifting purple color of the DPPH solution to yellow. The absorbance values obtained from the ascorbic acid and DPPH mixture were utilized to construct a calibration curve (Arsul et al., 2024; Celep et al., 2015).

The antioxidant activity of each extract was assessed following the same procedure for ascorbic acid. The extract concentration for determining antioxidant ability was adjusted to achieve an absorbance between 0.2 and 0.8. Subsequently, the antioxidant activity was calculated using the linear regression equation derived from the ascorbic acid calibration curve. The antioxidant activity of the extract was ex-

pressed as ascorbic acid equivalent (mg AAE/g) (Arsul et al., 2024; Celep et al., 2015).

Antioxidant activity by cupric reducing antioxidant capacity (CUPRAC)

Antioxidant activity was determined using the modified Özyürek's method (Özyürek et al., 2008). To prepare the CUPRAC stock solution, CuCl_2 and neocuproin were mixed in a 1:1 ratio by volume. For example, the 3061 μL of CuCl_2 solution (1795 $\mu\text{g/mL}$) was mixed with 3061 μL of neocuproin solution (1562 $\mu\text{g/mL}$). Then, the mixture was diluted with ammonium acetate buffer to a final volume of 100 mL, which was used for the CUPRAC reaction. From the ascorbic acid solution, six concentrations were selected (e.g., 15 μL , 17.5 μL , 20 μL , 22.5 μL , 25 μL , and 27.5 μL), with each concentration prepared in triplicate and transferred into separate microtubes (Eppendorf). Next, ammonium acetate buffer was added to each micro tube up to a total volume of 250 μL , followed by the addition of CUPRAC solution (750 μL). The mixture was then incubated for 30 minutes and subsequently measured using a UV-vis spectrophotometer at 450 nm wavelength. The absorbance values from these measurements were utilized to construct a calibration curve. (Arsul et al., 2024; Insanu et al., 2022).

The antioxidant activity of extract samples was assessed using the procedure mentioned above. The concentration of each extract was adjusted to achieve an absorbance of 0.2 to 0.8. Subsequently, the antioxidant activity was calculated using the linear regression equation derived from the calibration curve of ascorbic acid and expressed as milligrams of ascorbic acid equivalent per gram of sample (mg AAE/g) (Arsul et al., 2024).

α -Glucosidase inhibitor activity test

The α -glucosidase inhibition employed a modified approach as detailed by Nor et al. (2023). In the 96-well microplates, 30 μL of the sample, 36 μL of 0.1 M phosphate buffer solution (pH 6.8), and 17 μL of 6 mM p-nitrophenyl- α -D-glucopyranoside substrate were mixed and subjected to a preliminary incubation at 37°C for 5 minutes. After the initial 5-minute period at 37°C, 17 μL of α -glucosidase enzyme at 0.2 units/mL was added, followed by incubation at 37°C for 15 minutes to facilitate the reaction completion. The chemical response was terminated by adding 100 μL of 200 mM Na_2CO_3 to each well. The chemical absorbance was measured using a microplate reader at 405 nm (ThermoFisher). Each measurement was carried out in triplicate to ensure accuracy. For positive control, acarbose was utilized, while the negative control used no inhibitor. For comparative purposes,

Table 1. Total phenol content and total flavonoid content of three *Ziziphus*.

No	Sample		TPC	TFC
			(g GAE/100 g DE)	(g QE/100 g DE)
1	<i>Z. spina-chrsiti</i>	Leaf	4.785 ± 0.04 ^a	1.843 ± 0.06 ^a
		Fruit	0.597 ± 0.02 ^b	0.040 ± 0.00 ^b
		Seed	3.893 ± 0.01 ^c	0.873 ± 0.07 ^c
2	<i>Z. mauritiana</i>	Leaf	6.534 ± 0.05 ^d	2.025 ± 0.08 ^d
		Fruit	2.451 ± 0.06 ^e	0.049 ± 0.00 ^b
		Seed	4.322 ± 0.05 ^f	0.595 ± 0.01 ^e
3	<i>Z. jujuba</i>	Leaf	2.697 ± 0.01 ^g	1.563 ± 0.02 ^f
		Fruit	0.310 ± 0.00 ^h	0.046 ± 0.00 ^b
		Seed	0.937 ± 0.05 ⁱ	0.104 ± 0.00 ^b

Data are expressed as mean ± SEM (n = 3). Treatments not sharing the same letters in the same row are significantly different by ANOVA followed by a Tukey’s test (p<0.05). GAE: gallic acid equivalent; QE: quercetin equivalent.

acarbose was employed as a reference in this study (Febrina et al., 2021; Nor et al., 2023). The percentage of α-glucosidase inhibition was calculated by the following equation [1].

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

[1]

Where A control was the absorbance of the control, and A sample was the absorbance of the *Ziziphus* sample. The inhibitory concentration 50 (IC₅₀) value was calculated from the linear regression between the inhibition percentage and the sample concentration.

Determination of several flavonoid compounds

The marker compounds were determined using Shimadzu’s LC-20AD HPLC instrument (Shimadzu, Japan). Detection was carried out with Shimadzu’s UV/Vis SPD-20A detector at 360 nm. The separation process was performed on LiChrospher® 100 RP-C18 (100 × 4 mm, 5 μm) in stationary phase, connected to a 20 mm pre-column supplied by Merck. The column temperature was meticulously maintained at 30°C, monitored with a CTO-20A oven (Shimadzu, Japan). The mobile phases comprised aqua pro injection (A) and methanol (B). Samples were injected into the 20 μL injector and eluted at a 1 mL/min flow rate, regulated by the CTO-20A pump. A linear gradient system was implemented with the eluent B gradient at 40-60% (minutes 0-5), 60-70% (minutes 6-10), and 60-40% (minutes 11-15) (Pertiwi et al., 2024; Rizaldy et al., 2023). The standard solutions of apigenin, kaempferol, luteolin 7-O-glucoside, quercetin, and rutin were injected at 50 μg/mL. In contrast, extracts from the leaf, fruit flesh, and seed of *Z. spina-christi*, *Z. mauritiana*, and *Z. jujuba* were injected at 10,000 μg/mL. The standard content of the samples was calculated using the following equation [2].

$$\text{SCE} = \frac{\text{AUC}_{\text{ME}}}{\text{AUCS}} \times \frac{\text{CS}}{\text{CE}} \times 100\%$$

[2]

Where SCE: Standard content in extract; AUC: Area under curve; ME: Metabolite in extract; S: standard; CS: Concentration of standard; CE: Concentration of extract.

Statistical analysis

The statistical analysis conducted in this study had two main objectives: First, to assess differences between groups using analysis of variance (ANOVA), and second, to determine the correlation between total phenol content, total flavonoids, antioxidant activity values, and the area under the curve (AUC) of compounds from each sample analyzed by HPLC. Correlation analysis was performed using the Pearson’s a correlation method with the assistance of Minitab® Statistical Software.

RESULTS

Total phenolic and flavonoid contents of three *Ziziphus* species

The total phenolic contents (TPC) of *Ziziphus* samples were determined by using a linear regression equation (y = 0.0028x + 0.0703; R² = 0.9942) with gallic acid as standard. The total flavonoid contents (TFC) were determined using the linear equation (y = 0.0081x - 0.0196; R² = 0.9933) with quercetin as the standard. The TPC and TFC from the leaves, fruit flesh, and seeds of *Z. spina-christi*, *Z. mauritiana*, and *Z. jujuba* are presented in Table 1. The total phenolic contents revealed a significant value difference in all samples (p<0.05). However, the total flavonoids indicate that the fruit flesh of all three species and the seeds of *Z. jujuba* were non-significantly different (p>0.05).

Table 2. Antioxidant capacity of three *Ziziphus* species.

No	Samples		DPPH (mg AAE/g DE)	CUPRAC (mg AAE/g DE)
1	<i>Z. spina-chrsiti</i>	Leaf	62.521 ± 0.01 ^a	518.461 ± 0.01 ^a
		Fruit	7.960 ± 0.01 ^b	86.696 ± 0.03 ^b
		Seed	20.611 ± 0.01 ^c	156.442 ± 0.03 ^c
2	<i>Z. mauritiana</i>	Leaf	94.740 ± 0.01 ^d	560.972 ± 0.01 ^d
		Fruit	22.751 ± 0.01 ^e	430.814 ± 0.03 ^e
		Seed	20.453 ± 0.01 ^c	161.423 ± 0.02 ^c
3	<i>Z. jujuba</i>	Leaf	24.188 ± 0.01 ^f	317.299 ± 0.01 ^f
		Fruit	1.495 ± 0.01 ^g	32.034 ± 0.01 ^g
		Seed	3.693 ± 0.01 ^h	35.659 ± 0.01 ^h

Data are expressed as mean ± SEM (n = 3). Treatments not sharing the same letters in the same row are significantly different by ANOVA followed by a Tukey's test (p<0.05).

Table 3. α-Glucosidase inhibitor activity of three *Ziziphus*.

No	Samples		IC ₅₀ (µg/mL)
1	<i>Z. spina-chrsiti</i>	Leaf	9.135
		Fruit	>800
		Seed	>800
2	<i>Z. mauritiana</i>	Leaf	1.566
		Fruit	8.907
		Seed	>800
3	<i>Z. jujuba</i>	Leaf	55.980
		Fruit	>800
		Seed	>800
4	Acarbose		108.363

Antioxidant capacity

Antioxidant capacity values were determined using two methods: DPPH and CUPRAC. The capacity values of all samples using the DPPH method were expressed in terms of their equivalence with ascorbic acid in scavenging the free radicals. A linear regression equation was obtained using ascorbic acid as a standard ($y = 10.979x + 13.246$; $R^2 = 0.9971$). In the CUPRAC method, the sample's antioxidant capacity was calculated as equivalent to ascorbic acid in terms of increasing the antioxidant capacity. This study derived a linear regression equation ($y = 2.5727x + 44.248$; $R^2 = 0.9915$). The antioxidant activity of all samples is presented in Table 2. Overall, the antioxidant activity capabilities of the two methods showed that all samples exhibited significantly different values, except *Z. spina-christi* and *Z. mauritiana* seeds. Furthermore, no significant difference ($p>0.05$) was

observed in *Z. jujuba* fruit and seeds in increasing antioxidant capacity using the CUPRAC method. Notably, *Z. mauritiana* leaves exhibited the highest antioxidant activity among other samples.

α-Glucosidase inhibitor activity

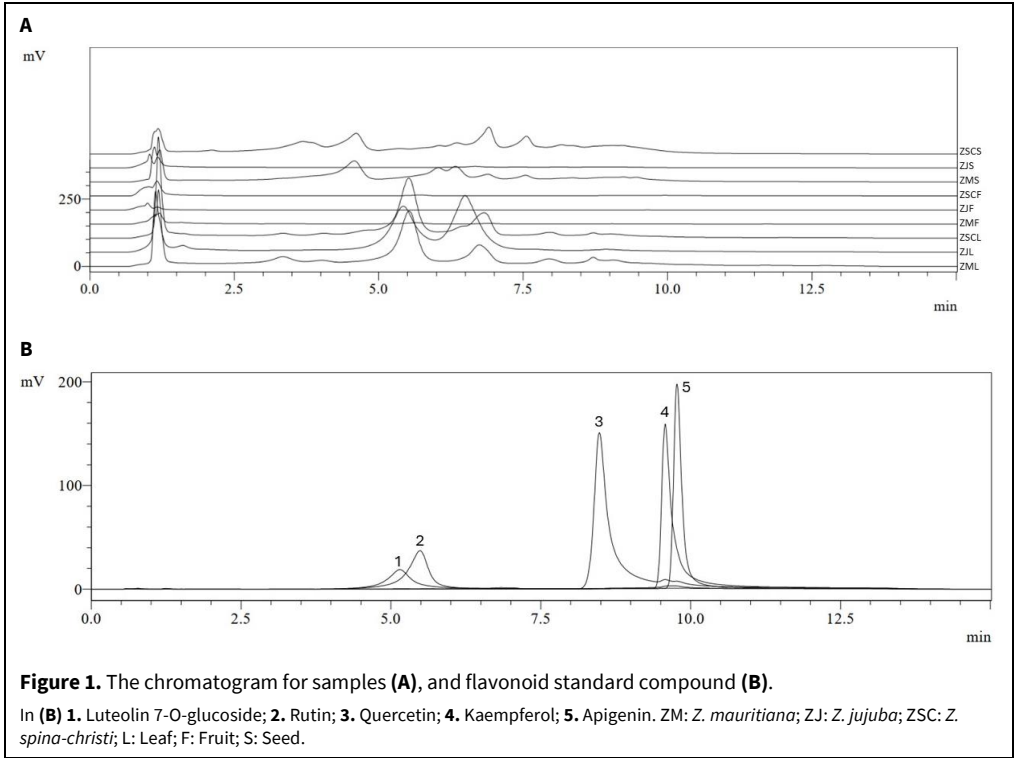
The α-glucosidase inhibitor (AGI) activity of the samples was observed through the IC₅₀ value. *Z. mauritiana* leaves had the lowest IC₅₀ value (1.566 µg/mL), followed by *Z. mauritiana* fruit (8.907 µg/mL), *Z. spina-christi* leaves (9.135 µg/mL), and *Z. jujuba* leaves (55.980 µg/mL). Other samples showed minimal activity, with IC₅₀ values exceeding 800 µg/mL. The results can be seen in Table 3.

The antioxidant capacity value was correlated with the total phenol value, total flavonoid content, and α-glucosidase inhibitor activity. The correlation

Table 4. Pearson’s correlation of TPC, TFC, antioxidant capacity, and α-glucosidase inhibitor activity.

No	Antioxidant capacity parameters	Pearson’s correlation		
		TPC	TFC	AGI
1	DPPH Method	0.878	0.853	-0.707
2	CUPRAC Method	0.765	0.752	-0.934

AGI: α-glucosidase inhibitory; DPPH: 2,2-diphenyl picrylhydrazyl; CUPRAC: cupric ion reducing antioxidant capacity; TFC: total flavonoid content; TPC: total phenolic content.



coefficient for α-glucosidase inhibitor activity is negative (-), following the IC₅₀ parameter, where lower values indicate greater activity. The correlation coefficient values for this data can be seen in Table 4.

Pearson’s correlation value reveals a moderate to very strong correlation (0.700-0.900) between the antioxidant activity in the DPPH and CUPRAC analysis methods and the total phenols and flavonoid contents of the test samples (Akoglu, 2018). Corresponding to the correlation coefficient value, various flavonoid compounds can be identified and quantified using HPLC.

HPLC results for flavonoid determination

All samples were analyzed in HPLC to observe the fingerprint of each extract, compared to the flavonoid standard. This analysis targeted five flavonoids—apigenin, luteolin 7-O-glucoside, kaempferol, quercetin, and rutin—for identification. The chromatogram displays the flavonoid standard; the samples are pre-

sented in Fig. 1. Analysis using HPLC revealed that the luteolin 7-O-glucoside was detected at a retention time of 5.149 minutes, followed by rutin at 5.485 minutes, quercetin at 8.478 minutes, kaempferol at 9.575 minutes, and apigenin at 9.772 minutes. The retention time (RT) value of these standards is a reference for detecting the standard content in each extracted sample.

The chromatogram fingerprints of all samples reveal both similarities and differences in compounds. The presence of identical compounds with matching retention times to the standard metabolites is selected from the array of peaks. Consequently, eight peaks were discerned, with four corresponding to the standard compounds of rutin, quercetin, kaempferol, and apigenin. Table 5 displays the qualitative identification of the chromatogram for each sample.

Following the chromatogram analysis, the standard components within the sample were quantified. The identification and quantification of standard fla-

vonoids present in the sample are depicted in Fig. 2 and detailed in Table 6. Subsequently, the correlation coefficient values reflecting the relationship between

the standard compound content in the sample and its antioxidant activity are provided in Table 7.

Table 5. Compounds detected by HPLC analysis.

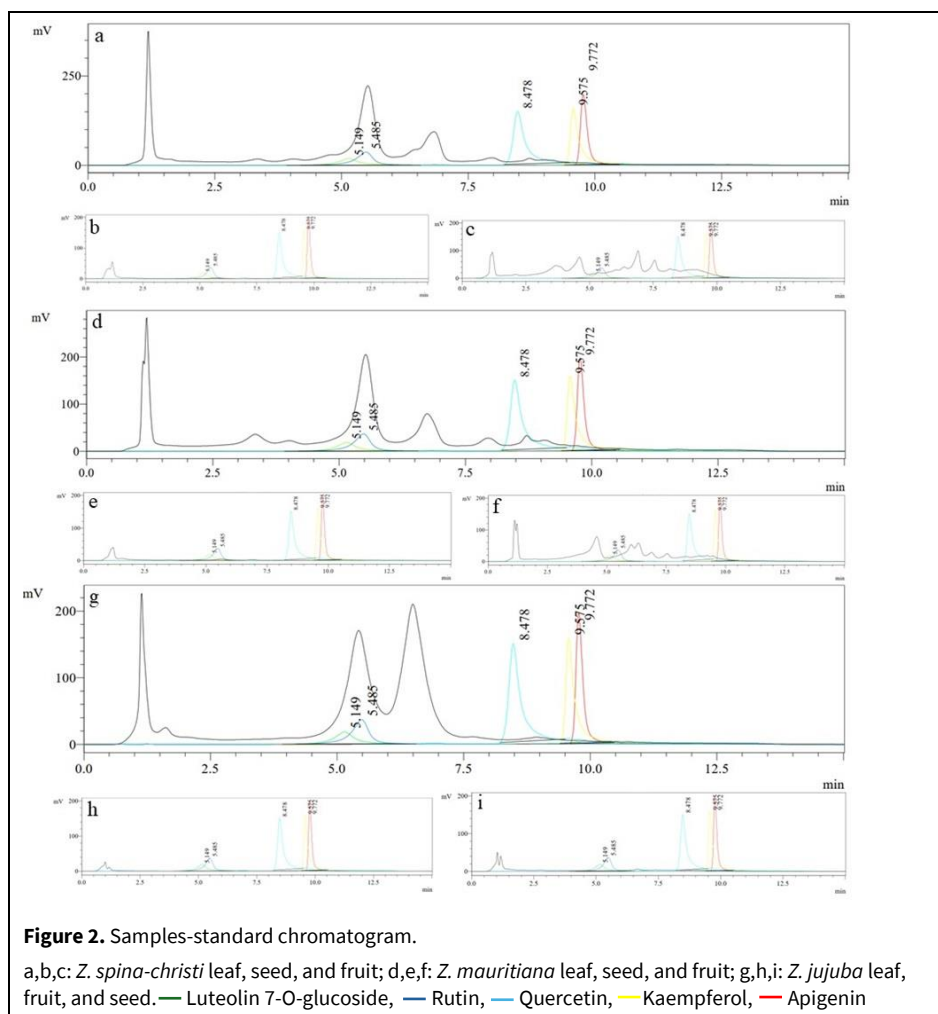
No	Compound	Retention time (minutes)	Sample								
			<i>Z. spina-christi</i>			<i>Z. mauritiana</i>			<i>Z. jujuba</i>		
			Leaf	Fruit	Seed	Leaf	Fruit	Seed	Leaf	Fruit	Seed
1	Compound 1	1.1 – 1.2	+	+	+	+	+	+	+	+	+
2	Compound 2	4.5 – 4.7			+			+			
3	Compound 3 (rutin)	5.4 – 5.6	+			+			+		
4	Compound 4	6.3 – 6.4			+			+			
5	Compound 5	6.7 – 6.9	+	+	+	+	+	+			
6	Compound 6 (quercetin)	8.4 – 9.0	+			+	+		+		
7	Compound 7 (kaempferol)	9.4 – 9.6							+		
8	Compound 8 (apigenin)	9.6 – 9.8							+		+

Table 6. Quantification of flavonoid content in the *Ziziphus* extracts.

No	Sample		Flavonoid content (%)			
			Rutin	Quercetin	Kaempferol	Apigenin
1	<i>Z. spina-chrsiti</i>	Leaf	3.118 ± 0.22	0.925 ± 0.13	-	-
		Fruit	-	-	-	-
		Seed	-	-	-	-
2	<i>Z. mauritiana</i>	Leaf	3.267 ± 0.08	1.466 ± 0.50	-	0.079 ± 0.00
		Fruit	-	0.048 ± 0.01	-	0.002 ± 0.00
		Seed	-	-	0.120 ± 0.00	-
3	<i>Z. jujuba</i>	Leaf	2.953 ± 0.10	0.382 ± 0.16	-	-
		Fruit	-	-	-	-
		Seed	-	-	-	-

Table 7. Pearson’s correlation of flavonoid content and bioactivities.

No	Compound	Correlation coefficient		
		DPPH	CUPRAC	AGI
1	Compound 1	0.783	0.756	-0.671
2	Compound 3 (rutin)	0.749	0.740	-0.780
3	Compound 5	0.800	0.635	-0.390
4	Compound 6 (quercetin)	0.857	0.721	-0.573
5	Compound 8 (apigenin)	0.810	0.557	-0.421



DISCUSSION

The correlation coefficient values indicate that compounds 1, 3 (rutin), 5, 6 (quercetin), and 8 (apigenin) contribute significantly as antioxidants. Rutin was consistently present in all *Ziziphus* species samples, while apigenin was detected only in *Z. mauritiana* leaves and fruit flesh. The highest antioxidant activity was observed in *Z. mauritiana* leaves, aligning with these standard compounds. Conversely, the *Z. spina-christi* and *Z. jujuba* leaves only contain rutin per the standard. Apart from the leaves, the pulp of *Z. mauritiana* exhibited notable antioxidant activity, as evidenced in the CUPRAC method, which correlates with the presence of apigenin in this sample.

Several *Ziziphus* plants, including various varieties of leaves, stems, and *Ziziphus jujuba* fruit, were reported to contain rutin compounds, as identified through UHPLC analysis (Elaloui et al., 2016; Sampiev et al., 2020; Xue et al., 2021). Additionally, rutin's presence was documented in the leaves, fruit, and seeds of *Z. spina-christi*, *Z. mauritiana*, and *Z. lotus* (Aldhanhani et al., 2022; Yahia et al., 2020). These species are known to contain flavonoids and exhibit bioactivities such as antioxidant, antimicrobial, anal-

gesic, and anti-inflammatory properties (Aldhanhani et al., 2022; Yahia et al., 2020; Zandiehvakili and Khadivi, 2021). Furthermore, the *Z. joazeiro* leaves from Brazil also exhibited the presence of several flavonoids, including rutin. The existence of flavonoid compounds in *Z. joazeiro* leaves was associated with their antioxidant and antibacterial activities (Andrade et al., 2020; Brito et al., 2015).

Rutin is a flavonoid compound derived from quercetin as a sugar (quercetin 3-O-rutinoside), commonly found in various parts of plants, such as fruit skin, leaves, flowers, and roots (Frutos et al., 2019). Chen et al. (2001) reported that rutin compounds are present in the *Ruta graveolens* plant. Rutin exhibits remarkable antioxidant properties across various test methods, including DPPH-free radical scavenging, hydrogen peroxide, and lipid peroxidase tests (Yang et al., 2008). Its prominent antioxidant and anti-inflammatory capabilities are particularly beneficial, offering protection against various pathological conditions, such as diabetes, cardiovascular disease, and skin cancer, among others (Chua, 2013; Ganeshpurkar and Saluja, 2017; Ghorbani, 2017). Furthermore, rutin has shown potential as a neuroprotective and anti-

Alzheimer's compound, with its antioxidant properties linked to the inhibition of neurodegenerative damage and inflammation in nerve cells (Enogieru et al., 2018; Javed et al., 2012). Rutin is recognized as a phytochemical compound with diverse pharmacological activities, suggesting a nutraceutical potential (Ganeshpurkar and Saluja, 2017; Semwal et al., 2021).

Rutin offers various benefits in enhancing glyemic control by exerting several mechanisms, such as reducing carbohydrate absorption in the small intestine, inhibiting gluconeogenesis, increasing the glucose uptake in tissues, stimulating insulin secretion, and safeguarding Langerhans islets from degeneration (Ghorbani, 2017). Its capability to diminish glucose absorption in the small intestine is attributed to α -amylase and α -glucosidase inhibition, consequently preventing postprandial spikes in blood glucose levels (Adefegha et al., 2015; Dubey et al., 2017). In animal studies, rutin can enhance glucose uptake in rat muscles by stimulating the phosphatidylinositol-3'-kinase (PI3K) pathway, atypical protein kinase C, and mitogen-activated protein kinase (MAPK) pathways (Kappel et al., 2013). Additionally, rutin exhibits favorable effects in chronic hyperglycemia and dyslipidemia conditions linked to tissue damage and diabetic complications by inhibiting the reactive oxygen species (ROS) and advancing the glycation end products (AGE) (Cervantes-Laurean et al., 2006; Dias et al., 2021).

Quercetin compounds were detected in all leaf samples and *Z. mauritiana* fruit. Quercetin facilitates several plant physiological processes, such as seed germination, pollen growth, antioxidant machinery, and photosynthesis, thereby inducing proper plant growth and development (Singh et al., 2021). Being a potent antioxidant, quercetin has the potential to enhance plant tolerance to various biotic and abiotic threats. Quercetin, a plant pigment found in tea and onions, offers numerous medical benefits, including anti-allergic, anti-inflammatory, anticancer, antidiabetic, and cardiovascular disorder properties. It is a yellow, insoluble crystalline solid substance with a bitter taste. Despite being generally insoluble, quercetin exhibits slight solubility in alcohol, aqueous alkaline solutions, and glacial acetic acid (Becker et al., 2014; Bule et al., 2019; Rauf et al., 2018; Tian et al., 2021).

Quercetin is primarily recognized for its antioxidative characteristics, being the most potent flavonoid antioxidant found in nature, safeguarding the body from the harmful effects of free radicals. In various neurodegenerative diseases, hydrogen peroxide is a major reactive oxygen species. A study conducted in Japan aimed to explore the neuroprotective role of quercetin against apoptosis induced by H_2O_2 in hu-

man neuronal SH-SY5Y cells. The study observed decreased lactate dehydrogenase levels and H_2O_2 -induced cytotoxicity attributable to quercetin. Moreover, quercetin suppressed the expression of the pro-apoptotic Bax gene while enhancing the expression of the anti-apoptotic Bcl-2 gene in neuronal cells. Consequently, quercetin is a preventive measure against neurodegenerative conditions instigated by oxidative stress and apoptosis (Azeem et al., 2023).

Quercetin has undergone successful testing in numerous *in vitro*, *in vivo*, and human studies. In the latest research, quercetin demonstrated synergistic effects when combined with the antidiabetic medication pioglitazone, effectively reversing endothelial dysfunction in diabetic rats. Findings from this and similar studies underscore the significance of quercetin as a promising candidate to combat diabetes mellitus (Achike and Murugan, 2020). Studies conducted on test animals indicate that quercetin has the potential to elevate insulin and glucagon levels, enhance liver function, augment hepatic glycogen reserves, and improve lipid profiles (Abdou et al., 2022). *In vivo* studies further highlight quercetin's potential as a promising agent in combating diabetes and its associated pathophysiological complications. The accumulating evidence suggests that quercetin could be a viable candidate for managing type 2 diabetes and its complications mediated by oxidative stress. Quercetin exhibits its effects by targeting multiple pathways of diabetes and regulating key signaling pathways, thereby facilitating both the symptoms and complications of type 2 diabetes. Nonetheless, additional research is necessary to enhance quercetin's bioavailability and establish an optimal dosing regimen (Dhanya, 2022).

Based on the analysis results, the presence of apigenin in the fruit and leaves of *Z. mauritiana* is intriguing, considering that a previous study conducted by Yahia et al. (2020) could not detect any apigenin compounds in any parts of the *Z. mauritiana* plant. In contrast, the presence of quercetin and luteolin, widely reported in the same *Ziziphus* plants, was undetected in the samples. Such discrepancies are not uncommon, as various factors, including different methodologies, tools, plant varieties, nutrient compositions, climatic conditions, and geographical locations of the samples, can all contribute to variations in analysis results or even differences in metabolite content (John et al., 2016; Kim et al., 2013; Kimura et al., 2014).

Apigenin, a flavonoid aglycone compound also known as 4',5,7-trihydroxyflavone, exists as a yellow solid crystal and is renowned for its myriad of benefits, including antioxidant, anti-inflammatory, antidiabetic, anticancer, neuroprotective, and antimicrobial

properties (Ali et al., 2017; Salehi et al., 2019). Like other flavonoid compounds, apigenin exhibits potent antioxidant activity as a promising candidate for pharmacological intervention in various degenerative diseases associated with reactive oxygen species, aging (Ali et al., 2017), neurodegenerative disorders (Bijani et al., 2022; Nabavi et al., 2018), and antimicrobial defense mechanisms (Morimoto et al., 2015; Wang et al., 2019). Apigenin suppressing the expression of iNOS and COX-2 plays a pivotal role in inflammation mitigation (Kim et al., 2004; Ribeiro et al., 2015). Moreover, as an antidiabetic agent, apigenin inhibits the α -glucosidase enzyme, enhances insulin secretion, and mitigates cellular reactive oxygen species (Jiang et al., 2022; Liu et al., 2024). Several studies have demonstrated the anticancer properties of apigenin, including its ability to impede the proliferation of human head and neck squamous carcinoma cells, induce G2/M cell cycle arrest, and elevate intracellular reactive oxygen species levels (Chan et al., 2012). Additionally, apigenin blocks the progression of progesterin-dependent BT-474 breast cancer cells (Mafuvadze et al., 2012), modulates cell cycle signaling in prostate cancer cells (Shukla et al., 2012), and suppresses proliferation and migration in various colorectal adenocarcinoma cell lines (Xu et al., 2016).

CONCLUSION

This study presents the latest findings on the comparison of flavonoid compound levels in *Z. spina-christi*, *Z. mauritiana*, and *Z. jujuba* and demonstrates their correlation with antioxidant activity and α -glucosidase enzyme inhibition. The comprehensive evaluation of three *Ziziphus* plant species and parts (leaves, fruits, and seeds) involved thoroughly assessing their total phenolic contents, flavonoids, and antioxidant activity using DPPH and CUPRAC methods. In addition, the HPLC analysis uncovered shared characteristics among all samples and successfully identified several key flavonoid compounds. The *Z. spina-christi*, *Z. mauritiana*, and *Z. jujuba* leaves exhibited high antioxidant content and activity. This robust antioxidant performance was attributed to the presence of rutin in these samples, contributing to positive antioxidant and α -glucosidase inhibitor capabilities. Furthermore, the significant presence of quercetin and apigenin in the leaves and fruit of *Z. mauritiana* notably bolstered its overall antioxidant activity, highlighting the importance of flavonoid compositions in determining antioxidant potential across different *Ziziphus* plant varieties.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Hafiz I	Happyana N	Insanu M
Concepts or ideas	x	x	x
Design	x	x	x
Definition of intellectual content	x	x	x
Literature search	x	x	x
Experimental studies	x		
Data acquisition	x		
Data analysis	x	x	x
Statistical analysis	x	x	x
Manuscript preparation	x	x	x
Manuscript editing	x	x	x
Manuscript review	x	x	x

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