RESEARCH ARTICLE

Phenolic and Flavonoids Contributions to the Antioxidant, Antidiabetic, and Anticholesterol Activities of *Eriobotrya japonica* Fruit Extract: An *In Vitro* Analysis

Masfria[1,2], Syahputra H[1*], Haro G[1], Nasution L R[1]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia.*
[2]*Nanomedicine Centre of Innovation, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia.*

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ABSTRACT

Eriobotrya japonica has been traditionally used for its medicinal properties. This study investigates its fruit ethanol extract's phenolic and flavonoids profiles and evaluates its antioxidant, antidiabetic, and anticholesterol activities. *Eriobotrya japonica* fruit ethanol extract was tested for total phenolic and flavonoid content using Folin-Ciocalteu and AlCl3 methods. The antioxidant capacity was assessed by DPPH and metal chelation assays. The antidiabetic action was assessed using α-glucosidase inhibition and the Nelson-Somogyi method, whereas the Liebermann-Burchard method was used to measure anticholesterol. The extract exhibited large quantities of phenolics (38.62 mg GAE/g) and flavonoids (4.23 mg QE/g), demonstrating robust antioxidant activity with IC50 of 55 µg/mL by DPPH method and substantial inhibition (71%) by metal ion chelation. In addition, it exhibited strong antidiabetic effects 2.42 µg/mL in the α-glucosidase inhibition test, and at 10 µg/mL using the Nelson-Somogyi method resulted in a 24.02% yield. The extract demonstrated a significant reduction in cholesterol levels during the process of cholesterol reduction, obtaining a maximum reduction of 47.31% at the highest concentration tested. Ethanol extract of *Eriobotrya japonica* can be used in treating oxidative stress and diabetes and introduce its potential to reduce cholesterol levels. The high content of phenolic and flavonoids total contributes to its bioactivities, indicating its potential as a functional food ingredient.

Keywords: Antioxidant, Antidiabetic, Anticholesterol, *Eriobotrya japonica* Lindl, Total Phenolic Flavonoids

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INTRODUCTION

Eriobotrya japonica Lindl is included in the list of economic potential plants in the North Sumatera region that can be used as a functional food.[1] The fruit is rich in several mineral substances and vitamins, especially fiber. The pulp of *Eriobotrya japonica* contains a lot of citric acid, carotene, and vitamins A, B, and C, which are suitable for health. Vitamin content in the pulp of *Eriobotrya japonica* can be used as a source of antioxidants that can inhibit free radicals. *Eriobotrya japonica* extract has a high antioxidant content, so that it can act as an anti-inflammatory, antidiabetic, anticancer, antibacterial, and anti-aging.[2,3] Type 2 diabetes mellitus is a complex metabolic disorder characterized by impaired insulin secretion, insulin resistance, augmented hepatic glucose production, and disrupted lipid metabolism, resulting in elevated blood glucose levels. These elevated glucose concentrations stimulate the production of excessive free radicals, particularly reactive oxygen species (ROS), which exacerbate oxidative stress. This underscores the pivotal role of antioxidants in mitigating or neutralizing free radical chain reactions, thus safeguarding cellular structures from oxidative damage.[4,5] Multiple studies have demonstrated that phenolic compounds and flavonoids possess strong antioxidant properties that can effectively counteract the harmful effects of free radicals. Furthermore, a high concentration of antioxidants can provide protection against hyperglycemia and enhance the absorption of glucose and the effectiveness of insulin.[6,7] In addition, there exists a correlation between phenolic chemicals and flavonoids in their ability to reduce cholesterol levels. Hence, it is imperative to demonstrate the correlation between phenolic compounds and elevated levels of flavonoids in terms of their antioxidant, antidiabetic, and anticholesterol properties.[8,9] *Eriobotrya japonica* fruit has shown significant potential as a candidate for traditional medicine, primarily due to its high content of phenolic and flavonoids compounds, contributing to its antioxidant, antidiabetic, and anticholesterol activities. While these medicinal properties have been well-documented in the plant's leaves and other parts, the fruit, especially those cultivated in specific regions like Sumatra, has received limited attention. Sumatra's unique climate and soil conditions may influence the bioactive compound profile of *Eriobotrya japonica* fruit, potentially offering novel therapeutic benefits not observed in other plant populations. However, there is a notable gap in research on the ethanol extract of the fruit from this region. This study aims to fill this gap by providing a comprehensive analysis of the phenolic and flavonoids levels, as well as the antioxidant, antidiabetic,

**Author for Correspondence: hafid@usu.ac.id*

Table 1. Characteristics of Simplicia

No.	Parameter	Content (%) ± SD
1.	Water content	3.99±1,9
2.	Water soluble juice content	76.37±2.51
3.	Ethanol-soluble juice content	68.21±1.24
4.	Total ash content	3.47±0.19
5.	Acid insoluble ash content	1.73±0.28

and anticholesterol activities of *Eriobotrya japonica* fruit from Sumatra, thereby validating its potential as a candidate for traditional medicine. This research utilized in vitro assays to initially screen the bioactive potential of *Eriobotrya japonica* fruit extract. The in vitro approach was selected to efficiently and effectively evaluate the extract in a controlled environment, offering several advantages, including the ability to isolate specific mechanisms of action, reduce the complexity of biological interactions, and obtain rapid, reproducible results. These attributes make in vitro studies precious in the early stages of research, where the goal is to identify promising bioactivities that warrant further investigation. While in vitro findings provide essential foundational data, it is acknowledged that they must be complemented by in vivo studies to understand the extract's effects within a living organism fully. Thus, this study serves as a critical first step, guiding future in vivo studies to further explore the therapeutic potential of *Eriobotrya japonica* and emphasizing the importance of geographical factors in the phytochemical composition and therapeutic efficacy of medicinal plants.

MATERIALS AND METHODS

Plants and Chemical Compounds

All chemicals used were of pro-analytical quality. *Eriobotrya japonica* fruits were collected from Simalem Farm Brastagi in North Sumatra, Indonesia. Fresh *Eriobotrya japonica* Lindl fruits were peeled, washed, diced into little bits, and subsequently dried and blended.

Ethanol Extract Preparation

The evaluation of simplicia powder includes the analysis of moisture content, water-soluble extractives, ethanol-soluble extractives, total ash, and acid-insoluble ash. To commence the extraction procedure, one part of the dried simplicia powder is mixed with ten parts of solvent in a glass vessel. The mixture is first steeped for 6 hours with periodic stirring, then let to stand for a further 18 hours. The macerate is subsequently isolated. This procedure is conducted a minimum of two times to guarantee comprehensive extraction. All obtained macerates are amalgamated and afterward concentrated utilizing a rotating evaporator.[10]

Qualitative Analysis

The chemical constituents of the ethanol extract from *Eriobotrya japonica* fruit were qualitatively analyzed utilizing Thin Layer Chromatography (TLC), Fourier-Transform Infrared Spectroscopy (FTIR) and phytochemical screening. The FTIR analysis utilized the KBr pellet technique, whereas TLC employed a mobile phase consisting of ethyl acetate and n-hexane in a 4:6 ratio. Seated compounds were identified by analyzing the stain

a b c

Figure 1. Visual Representation of *Eriobotrya japonica* Fruit Processing: a) Fresh Fruit; b) Dried Simplicia; c) Ethanol Extract

spots under UV light at 254 and 366 nm, utilizing quercetin as a reference standard. Phytochemical screening followed standardized methods to identify various phytoconstituents in the extract, including alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenoids/steroids .[11]

Total Phenolic and Flavonoids Content

The total phenolic content was determined via the Folin-Ciocalteu technique, employing gallic acid as the standard (0-250 µg/mL), with absorbance measured at 750.4 nm using UV-Vis spectrophotometry. The Folin-Ciocalteu reagent, sodium carbonate solution, and distilled water were combined and incubated at 37°C for 90 minutes. The phenolic content was quantified as Gallic Acid Equivalents (GAE) in mg/g of the sample[6]. The total flavonoid content was assessed using quercetin as the reference standard at concentrations ranging from 0 to 75 µg/mL. A 0.5 mL extract was combined with methanol, AlCl3, sodium acetate, and distilled water, followed by incubation at 37°C for 30 minutes. Absorbance was recorded at 429.2 nm, and flavonoid concentration was determined using a quercetin standard curve, represented as Quercetin Equivalents (QE) in mg QE per gram of material.[6]

Antioxidant Assays

The ethanol extract of *Eriobotrya japonica* (Thunb.) Lindl fruit was assessed for its antioxidant activity using UV-Vis spectrophotometry, specifically through DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and metal chelation assays. The DPPH assay began by identifying the wavelength at which a 40 µg/mL DPPH solution displayed peak absorbance in the visible spectrum. The antioxidant activity of the fruit extract was evaluated by creating a calibration curve with different concentrations of the extract (25-100 µg/mL). To each concentration, 2 mL of a 200 µg/mL DPPH solution was included. The incubation duration for all mixtures was 30 minutes, and was measured at the specified maximum wavelength. The inhibition percentage (I) was determined using the following formula:

$$I\ (\%) = \frac{Ao - As}{Ao} \times 100$$

The variable "As" represents the sample absorbance, while "A0" refers to the control absorbance. Ascorbic acid (Vitamin C) served as the positive control. The studies were performed three times, and the IC50 values were ascertained by linear regression analysis. The extract's metal chelating activity was assessed by its ability to inhibit the formation of the ferrozine-Fe2+ complex in vitro. In this experiment, a mixture of 2 mM FeSO4, 0.2 mL of the extract, and of 5 mM ferrozine was allowed to react at room

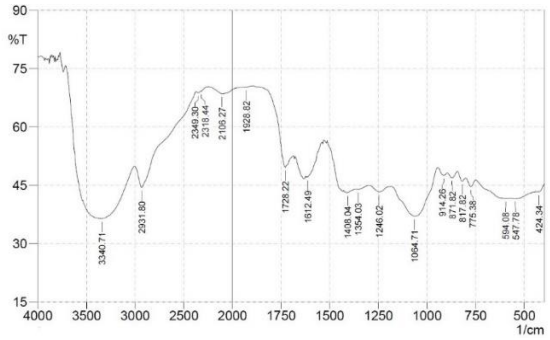
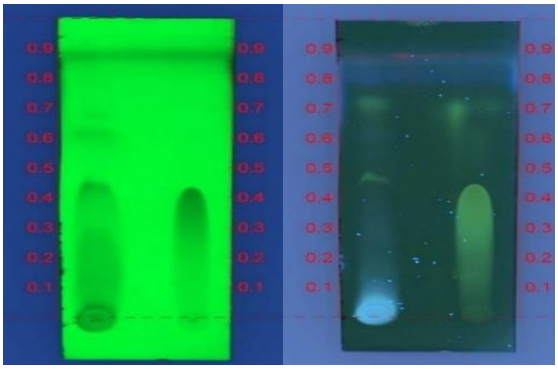


Figure 2. FTIR Spectrum of *Eriobotrya japonica* Fruit Extract



240 nm 360 nm

Figure 3. TLC Profile of *Eriobotrya japonica*

temperature for 10 minutes. The absorbance was subsequently measured at a wavelength of 562 nm. A control solution was produced by replacing FeSO4 and ferrozine with water 12. The percentage suppression of ferrozine-Fe2+ complex (R) production was determined using the subsequent formula:

$$R\ (\%) = \frac{Ao-As}{Ao} \times 100$$

A0 denotes the absorbance of the control reaction (absent of extract), whereas As indicates the absorbance with the extract; Sodium Ethylenediaminetetraacetic Acid (Na-EDTA) served as a positive control. The IC50 value, which represents the concentration of the extract or EDTA necessary to chelate 50% of iron ions, was determined by linear regression of metal chelating activity versus extract concentration.

Antidiabetic Assays

The antidiabetic efficacy of the ethanol extract was assessed using enzymatic and non-enzymatic techniques, with UV-Vis spectrophotometry utilized for analysis. The enzymatic activity was evaluated using the α-glucosidase inhibition test, whereas the non-enzymatic activity was determined utilizing the Nelson-Somogyi method.

The inhibitory action of α-glucosidase was evaluated by quantifying the release of 4-nitrophenol from p-nitrophenyl-α-D-glucopyranoside, the enzyme's substrate. The reaction mixture comprised 5 µL of the extract, 100 mM potassium phosphate buffer (pH 7), α-glucosidase (0.15 units/mL in 10 mM potassium phosphate buffer), and 5 mM p-nitrophenyl-α-D-glucopyranoside. Following a 15-minute incubation at 37°C, the reaction was terminated by the addition of 1000 µL of 200 mM sodium carbonate solution. The absorbance of the combination was quantified at 400 nm. The percentage inhibition (I) was calculated using the subsequent formula:

$$I\ (\%) = \frac{Ao-As}{Ao} \times 100$$

A0 denotes the absorbance of the control reaction (absent of extract), whereas As indicates the absorbance with the extract. The positive control employed dimethyl sulfoxide. The studies were performed thrice, and the IC50 values were ascertained utilizing a linear regression equation.

The evaluation of antidiabetic activity via the Nelson-Somogyi method began with identifying the ideal wavelength, operational duration, and establishing a standard curve for glucose production. A calibration curve was established for *Eriobotrya japonica* fruit ethanol extract at concentrations ranging from 1 to 10 µg/mL. For each concentration, 3 mL of the solution was mixed with 60 µg/mL glucose solution in a test tube. In a 10 mL volumetric flask, 1 mL of the mixture was amalgamated with 1 mL of Nelson reagent. The flask was thereafter wrapped in cotton and heated in hot water for 10 minutes. Following a 5-minute chilling interval, 1 mL of arsenomolybdate reagent was added, and distilled water was then included until the calibration mark was attained. The solution was amalgamated and allowed to remain undisturbed for the specified duration, after which its absorbance was measured at 760.4 nm.[14] The glucose-reducing activity was determined by applying the following formula:

$$\text{Glucose Level Reduction}\ (\%) = \frac{Ao-As}{Ao} \times 100$$

A0 denotes the absorbance of the control reaction (absent of extract), whereas As indicates the absorbance with the extract. Quercetin functioned as the positive control.

Anticholesterol Assay

The ethanol extract's anticholesterol activity was evaluated using the Liebermann-Burchard method, which measures the sample's effectiveness in decreasing cholesterol levels in a chloroform solution. This technique quantifies the absorbance of cholesterol by utilizing a UV-visible spectrophotometer at its peak wavelength. The ethanol extract was evaluated at concentrations ranging from 50 to 90 µg/mL. Each sample was amalgamated with 2.5 mL of a 400 µg/mL cholesterol reference solution. Each combination was administered anhydrous acetic acid and concentrated sulfuric acid. After dilution with chloroform to the required volume, the mixture was homogenized and left for 15 minutes. The absorbance was measured at 414 nm and then compared with the cholesterol calibration curve prepared. This comparison facilitated the formulation of a linear equation. The extract's cholesterol-lowering capacity was later assessed using the appropriate formula.[15]:

$$I\ (\%) = \frac{Ao-As}{Ao} \times 100$$

The sample absorbance and A0 represent the control absorbance, with simvastatin and fenofibrate serving as positive controls. All tests were conducted in duplicate. The IC50 value is determined by a linear equation.

Statistical Analysis

Table 2. Phytochemical Screening Results of Simplicia and Ethanol

No.	Parameter	Simplicia	Ethanol Extract
1.	Alkaloids	-	-
2.	Flavonoids	+	+
3.	Glycoside	+	+
4.	Saponin	+	+
5.	Tannin	-	-
6.	Triterpenoids /steroids	+	+

The results are presented as the mean ± standard deviation (SD) based on three independent experiments, with each sample analyzed in triplicate (n = 3). Statistical significance was determined using one-way ANOVA, with a p-value of less than 0.05. Post-hoc comparisons were performed using Tukey's HSD test. Levene's test was conducted to assess the homogeneity of variances, and the Shapiro-Wilk test was used to evaluate data normality. All statistical analyses were carried out using SPSS version 22.0.

RESULTS AND DISCUSSION

Characteristics of *Eriobotrya japonica* Fruit Simplicia

Plant identification was conducted at the Herbarium Medanese (MEDA), Faculty of Mathematics and Natural Sciences, University of North Sumatra, confirming the fruit used as *Eriobotrya japonica* (Thumb.) Lindl., a member of the Rosaceae family. The characterization results of the simplicia are presented in Table 1. The water content of simplicia complies with the established minimum limitations, which stipulate that it must not exceed 10%. The assessment of juice content is performed utilizing two solvents: water and ethanol. The concentration of polar chemical constituents in the simplicia is dictated by the aqueous solubility of the juice content. The objective of assessing the ethanol-soluble juice content is to quantify the amounts of both polar and non-polar molecules that are soluble in ethanol. The test findings indicate that the quantity of juice soluble in water exceeds that soluble in ethanol. Consequently, utilizing ethanol for juice extraction is more efficient. The assessment of ash content is performed to identify the presence of internal minerals derived from plant tissues in the sample. The acid-insoluble ash content signifies the amount of silicate, specifically sand, present in the simplicial. The whole ash is dissolved in hydrochloric acid to determine this.[18]

Ethanol Extract of *Eriobotrya japonica* Fruit

The 10 kg of *Eriobotrya japonica* fruit was harvested in the morning when ripe, light yellow in color (Figure 1). Following wet sorting, 8.4 kg of fruit flesh was obtained, which was then dried in an oven, resulting in 0.8 kg of dry simplicia (90.54% yield). Extraction was performed by maceration using 96% ethanol, with 350 g of dry simplicia and a 1:10 ratio of ethanol. After soaking for 24 hours, the extract was concentrated using a rotary evaporator, yielding 253.66 g of extract (72.47% yield).

Qualitative Analysis

FTIR spectroscopy revealed the presence of various functional groups in the ethanol extract of *Eriobotrya japonica* fruit at the following peaks: 3340 cm^{-1} (OH group), 2931 cm^{-1} (C-H stretching vibrations), 1612 cm^{-1} and 1728 cm^{-1} (N-H bending vibrations and C=O bending vibration), and 1354 cm^{-1} and 1246 cm^{-1} (C-H and O-H bending vibrations, respectively). 1064 corresponds to C-O (stretched vibration), while 871 pertains to C-C (stretching vibration).[16] These peaks are shown in the FTIR spectroscopy results in Figure 2. Thin Layer Chromatography (TLC) is a method employed to identify chemical composition by separating the mobile and stationary phases and Quercetin as the reference compound. As shown in the figure, the distance between the ethanol extract of *Eriobotrya japonica* fruit and quercetin corresponds to an Rf value of 0.43.[16] The appearance of stains on TLC can be seen in Figure 3.

Table 3. Total Phenols and Flavonoids

Sample	Total Phenol Content (mg GAE/g Sample)	Total Flavonoids Content (mg QE/g Sample)
Ethanol extract	38,62 ± 0,6	4,23±0,01

Fruit Extract Compared with Quercetin Standard

The phytochemical screening results of simplicia and ethanol extracts of *Eriobotrya japonica* fruit indicate the presence of flavonoids, glycosides, saponins, and triterpenoids/steroids, as detailed in Table 2.

Total Phenolic and Flavonoids Contents

The measurements of total phenols and flavonoids in ethanol extracts are presented in Table 3. The ethanol extract of *Eriobotrya japonica* fruit contained 38.62 mg GAE/g of total phenolic content and 4.23 mg QE/g of total flavonoids content. The significant levels of these compounds suggest a strong presence of bioactive constituent's integral to the plant's antioxidant properties. Phenolic compounds are known to be influential hydrogen donors, which neutralize free radicals by donating electrons. This property makes them crucial in preventing oxidative damage at the cellular level. Although lower than the phenolics, the flavonoids content still contributes significantly to the extract's overall antioxidant potential. Flavonoids have been widely recognized for inhibiting lipid peroxidation, which can lead to cell membrane damage and are implicated in various chronic diseases. Therefore, the combined high levels of phenolics and flavonoids underscore the extract's potential as a potent antioxidant agent capable of counteracting oxidative stress. The quantification of the overall phenolic content was conducted with the Folin-Ciocalteau reagent. The concentration of the gallic acid standard solution was measured to range from 50-250 µg/mL at 750.4 nm. The regression equation for gallic acid is $Y = 0.0036X + 0.0164$. This equation represents a linear relationship, and the correlation coefficient is approximately 0.999. The phenolic content of each sample is quantified in GAE (Gallic Acid Equivalent). GAE represents the quantity of milligram equivalents of gallic acid contained in one gram of the sample. The total flavonoid content was assessed through a colorimetric method utilizing AlCl3 reagent. The concentration of the Quercetin standard solution was

Table 4. Antioxidant Assays

Sample	DPPH Method	Metal chelating Method
	IC_{50} (µg/mL)	%
Ethanol extract	55±0.15	71%±3.14
Positive control	4.88±0.04 (vitamin C)	84%±0.41 (Na-EDTA)

assessed within the range of 25-75 µg/mL at a wavelength of 429.2 nm. The total flavonoid concentration was assessed through a regression equation obtained from a plot correlating the concentration with the absorbance of the standard solution. The calibration curve for quercetin was established through the regression equation Y = 0.01005X + 0.003. There is a linear correlation between absorbance and concentration, with a correlation coefficient of r = 0.9999. To ensure data accuracy, 25 mg of Eriobotrya japonica fruit extract was measured three times during the assessment of total phenolics. The flavonoid content in each sample was quantified as Quercetin Equivalent (QE). The Quercetin Equivalent indicates the amount of quercetin, quantified in milligrams (mg), found in one gram (g) of the sample.[7] The measurement values for the total phenol content were 38.62 ± 0.6 mg GAE/extract. The total phenol content is affected by the choice of solvent, with polar chemicals exhibiting greater solubility in polar solvents such as ethanol. Polar solvents have a greater capacity to dissolve phenols, resulting in higher concentrations of phenols in the extract. The ethanol extract of *Eriobotrya japonica* fruit contains a total of eleven phenol components, which may be classified into five hydroxycinnamic acid group compounds and six flavonol group compounds. Notably, the flavonol group compounds include quercetin and kaempferol. The measurement yielded a total flavonoid content of 4.23 ± 0.01. The ethanol extract of *Eriobotrya japonica* fruit contains two flavonoid components, namely quercetin and kaempferol. These flavonoids belong to the flavonol group and have qualities that are either less polar or semi-polar.[2,3] Total phenol and flavonoids levels in *Eriobotrya japonica* fruit extract have high levels of total phenol and flavonoids. This is because flavonoids compounds are part of phenol compounds. Flavonoids compounds have an -OH group, which includes them in phenol compounds. This means the greater the level of flavonoids compounds in the sample, the greater the level of phenol compounds. Phenolic compounds in the form of flavonoids, namely flavonols and flavones, can act as antioxidants. The higher the total phenol and flavonoids values, the higher the antioxidant ability to donate electrons and suppress the development of free radicals.[2,3]

Antioxidant Assays

The outcomes of the antioxidant assays for the ethanol extract of *Eriobotrya japonica* fruit are presented in Table 4. The extract exhibits DPPH radical scavenging activity, with an IC50 value of 55 µg/mL, indicating a robust ability to neutralize free radicals. This activity is mainly due to the elevated phenolic content in the extract, which donates electrons to decrease DPPH radicals, causing a color transition from purple to yellow. The efficiency of this

Table 5. Antidiabetic Assays using α-glucosidase Inhibitory Activity Method

Sample	IC_{50} (µg/mL)
Ethanol extract	2.42±0.02
Acarbose	0.82±0.04

reaction suggests that the extract possesses significant free radical scavenging activity, although it is less potent than ascorbic acid (Vitamin C), which acts as a positive control with an IC50 of 4.88 µg/mL. The difference in activity between the extract and Vitamin C may be due to the complex mixture of compounds in the extract, which, while potentially exhibiting synergistic effects, are less concentrated than the pure ascorbic acid. The extract's ability to chelate metal ions was reflected in a 71% inhibition at a 10 mg/mL concentration. Chelating metal ions is a crucial antioxidant mechanism because metals such as iron and copper can catalyze the production of reactive oxygen species (ROS) through Fenton reactions, exacerbating oxidative stress. The observed chelation activity suggests that the extract effectively binds to these metal ions, preventing them from participating in harmful reactions. Compared to Na-EDTA, a standard chelating agent with 84% inhibition, the extract shows considerable chelating efficiency, which may be due to its phenolic content. These phenolics, with their multiple hydroxyl groups, likely interact with the metal ions, forming stable complexes and reducing their availability to catalyze oxidative processes. The operational duration of the DPPH solution in methanol is established by preparing a solution with a concentration of 40 µg/mL and recording its absorbance at the peak absorption wavelength (515.8 nm) at one-minute intervals for 60 minutes. The duration of operation was measured from the 18th to the 21st minute. The time interval from the 18th minute to the 21st minute is selected as the duration for measuring the antioxidant activity of the ethanol extract from *Eriobotrya japonica* fruit. This time period is chosen because it is believed to exhibit the most consistent absorbance value. Assessment of the antioxidant activity of the fruit extract utilizing ethanol as the solvent. The ethanol extract of *Eriobotrya japonica* fruit was produced at a concentration of 1000 µg/mL. A calibration curve was subsequently established using solutions with concentrations varying from 25 to 100 µg/mL. The calibration curve was established utilizing solutions with concentrations varying from 25 to 100 µg/mL. Two milliliters of DPPH solution were added to each flask. Each flask was augmented with 2 mL of DPPH solution at a concentration of µg/mL. Following a 30-minute incubation period, the absorbance of each solution concentration was assessed using a UV-Vis spectrophotometer at the wavelength of maximum intensity (518.5 nm). The duration of the measurement should span from the 18th minute to the 21st minute. From the given data, the regression equation is Y = 0.898X + 0.602, and the IC_{50} value is calculated. The outcomes of the DPPH capture technique are determined by computing the IC_{50}, which represents the substrate concentration capable of diminishing 50% of the DPPH free radicals' activity. IC50 study indicated that the ethanol extract possessed a value of

Table 6. Antidiabetic Assays using Nelson-Somogyi Method

Concentration (µg/mL)	Glucose level reduction value (%)	
Negative control (glucose standard solution 60 µg/mL)	59,94±0.04	
	Ethanol extract	Quercetin
1	8,44±0.05	27,62±0.05
2	10,22±0.09	29,04±0.02
3	12,11±0.08	30,39±0.01
4	13,96±0.23	32,08±0.04
5	15,41±0.15	33,99±0.01
6	16,99±0.08	35,53±0.10
7	18,72±0.15	37,21±0.08
8	20,88±0.12	38,73±0.03
9	22,37±0.13	40,52±0.09
10	24,02±0.07	42,13±0.10

Table 7. Percent Reduction in Cholesterol Levels by Ethanol Extract of *Eriobotrya japonica* Fruit Compared to Simvastatin and Fenofibrate

Concentration (µg/mL)	% Reduction in Cholesterol Levels		
Cholesterol 100 (blank)	99.68 ±0.02		
	Ethanol extract	Simvastatin	Fenofibrate
12.5	35.23 ±0.02	48.89 ±0.07	47.22 ±0.02
15	38.05 ±0.05	52.74 ±0.07	50.11 ±0.01
17.5	40.17 ±0.04	56.26 ±0.04	53.84 ±0.03
20	43.33 ±0.08	58.85 ±0.04	57.26 ±0.09
22.5	47.31 ±0.05	61.53 ±0.01	60.12 ±0.01

55 µg/mL, demonstrating high antioxidant activity. Conversely, vitamin C exhibits potent antioxidant activity, evidenced by an IC50 value of 4.88 µg/mL. This demonstrates that the antioxidant efficacy of vitamin C is stronger than that of the extract of *Eriobotrya japonica* fruit [19,20]. This approach involves chelating metal ions, particularly iron, which is known to cause the production of free radicals in biological systems. The wavelength was measured at 562.5 nm, with an extract concentration of 10 mg/mL. According to the data in Table 4, the ethanol extract concentration of 10 mg/mL demonstrates a high level of chelating activity, with a value of 71%. EDTA, in comparison, exhibits a high chelating capacity of 84%, indicating that the inhibitory effect on ferrozine chelation by the ethanol extract of *Eriobotrya japonica* fruit is relatively comparable to that of EDTA. EDTA exhibits a significant chelating capacity due to the possession of six chelating rings, which can establish coordination connections with Fe^{2+} ions or other metallic elements. Similarly, the extract of *Eriobotrya japonica* fruit includes several phenolic compounds that possess multiple aromatic rings and one or more hydroxyl groups.[20,21] The antioxidant activity of extract was assessed using two distinct methods. These approaches provided a comprehensive understanding of the fruit extract's antioxidant characteristics across several radical scavenging assays. Furthermore, antioxidant activity is not only against DPPH radicals, but also in terms of its ability to counteract oxidative damage generated by metal catalysts in the breakdown reaction. These two approaches were deemed enough to demonstrate the results of the antioxidant activity test methods.[20,21]

Antidiabetic Assays

The outcomes of the antidiabetic assays for the extract of *Eriobotrya japonica* fruit, utilizing the α-glucosidase inhibitory activity method, are presented in Table 5, while the antidiabetic assays employing the Nelson-Somogyi method are detailed in Table 6. The ethanol extract demonstrated notable α-glucosidase inhibitory action, with an IC50 value of 2.42 µg/mL. This suggests a significant capacity to impede the degradation of carbohydrates into glucose within the digestive system, which may aid in regulating postprandial blood glucose levels in individuals with diabetes. The inhibitory effect is probably attributable to certain flavonoids and phenolic acids in the extract, which have been shown to disrupt the enzyme's active site, thereby diminishing its activity. Although acarbose, a commercial α-glucosidase inhibitor, showed a lower IC_{50} value of 0.82 µg/mL, the extract's performance is noteworthy, especially considering it is a natural product with multiple bioactive components that may offer additional health benefits. The glucose-lowering activity of the extract was further demonstrated through the Nelson-Somogyi method, with a 24.02% reduction in glucose levels at a 10 µg/mL concentration. This reduction indicates the extract's ability to enhance glucose metabolism, possibly by improving insulin sensitivity or directly promoting glucose uptake by cells. The gradual increase in glucose reduction with increasing extract concentration suggests a dose-dependent effect, where higher doses of the extract could potentially result in more significant reductions in blood glucose levels. This finding aligns with traditional uses of *Eriobotrya japonica* in managing diabetes and underscores its potential role as a natural adjunct therapy. The inhibitory activity of the ethanol extract of **Eriobotrya japonica** and acarbose against α-glucosidase was evaluated by IC50 determination. The ethanol extract showed an IC50 of 2.42 µg/mL, while acarbose had an IC50 of 0.82 µg/mL. Both exhibited inhibitory activity, with lower IC50 values indicating higher potential. In particular, acarbose showed lower IC_{50} values than ethanol extract of *Eriobotrya japonica* fruit, indicating that acarbose was more potent in inhibiting α-glucosidase activity. The inhibitory activity of *Eriobotrya japonica* ethanol extract shows its potential as a natural source of α-glucosidase inhibitor, which can be further explored to develop new antidiabetic agents. Additional research is required to clarify the bioactive components that account for the inhibitory activity detected

in the ethanol extract of *Eriobotrya japonica* fruit.[22] The working principle of the Nelson-Somogyi method is reducing the amount of cupro-oxide precipitate that reacts with arsenomolybdate to a blue molybdate complex that a UV-Vis spectrophotometer can measure. Nelson reagent reacts with the remaining glucose to form D-gluconic acid and a brick-red cupro-oxide precipitate. The amount of cupro-oxide formed is equivalent to the amount of reducing sugar present. The cupric ion (Cu^{2+}) from the Nelson reagent will oxidize glucose to gluconic acid and form a cupro-oxide precipitate (Cu_2O) so that the amount of cupro-oxide is equivalent to the amount of glucose present. Potassium sodium titrates contained in the Nelson reagent serve to prevent the precipitation of cupro-oxide.[22] The solution is heated with cotton covered so that the reaction occurs optimally. Heating can increase the kinetic energy of the molecules, thus increasing the speed of the reaction. In addition, heating helps the oxidation process of glucose. The solution that has been heated is then cooled so that the reaction runs stable because if it is too hot, there is a possibility that the compound components will be damaged or vaporized. After that, the arsenomolybdate reagent is added. In this event, the hydro-oxide will reduce the arsenomolybdate to molybdenite blue, which is turquoise, and later measured for absorbance with a UV-Vis spectrophotometer.[22] The results of the data on the decrease in glucose levels show that the percentage decrease continues to increase as the concentration of the test solution added increases because the higher the concentration is given, the more glucose is bound, and the less glucose remains to provide a significant decrease in glucose levels, shows that the ethanol extract of *Eriobotrya japonica* fruit has a lower glucose-lowering activity when compared to quercetin.

Anticholesterol Assay

The cholesterol reduction test was conducted utilizing the visible spectrophotometric method with the Lieberman-Burchard reagent. This technique quantifies free cholesterol levels by producing a distinct green hue, which is employed to assess steroid/triterpenoid chemicals, including cholesterol. The addition of anhydrous acetic acid serves to extract cholesterol, maintain anhydrous conditions in the medium, and generate acetyl derivatives, which are subsequently treated with concentrated sulfuric acid to yield a green coloration indicative of steroid and triterpenoid chemicals, including cholesterol. Table 7 displays the findings of the percentage reduction in cholesterol levels achieved by the ethanol extract of *Eriobotrya japonica* fruit, simvastatin, and fenofibrate. The ethanol extract demonstrated a significant reduction in cholesterol levels, achieving a maximum decrease of 47.31% at a concentration of 22.5 µg/mL. This anticholesterol activity is likely due to bioactive compounds that inhibit cholesterol absorption or production. Phenolic chemicals, as detected in the extract, may block the enzyme HMG-CoA reductase, which is essential for cholesterol biosynthesis. The decrease in cholesterol levels was analogous to the effects of simvastatin and fenofibrate, two commonly utilized cholesterol-lowering medications, which attained reductions of 61.53% and 60.12%, respectively. The marginally reduced efficacy of the extract may be ascribed to the existence of many bioactive constituents, which, notwithstanding their synergistic action, are less concentrated than the singular active compounds found in commercial pharmaceuticals. Nevertheless, the extract's ability to reduce cholesterol levels significantly highlights its potential as a natural alternative for managing hypercholesterolemia, particularly for individuals seeking to minimize the side effects associated with synthetic drugs. Elevating the concentration of the sample can effectively lower cholesterol levels. The reduction in cholesterol levels was attributed to a drop in the absorbance value resulting from the combination of the cholesterol solution (100 µg/mL) with the sample and positive control. This demonstrates that both samples and positive controls effectively decrease cholesterol levels, resulting in a low absorbance value and a notable percentage of anticholesterol activity. The ethanol extract of *Eriobotrya japonica* fruit has been found to exhibit anticholesterol activity. At a concentration of 22.5 µg/mL, it is capable of reducing cholesterol levels by 47.31%. Simvastatin and Fenofibrate, at a concentration of 22.5 µg/mL, can reduce cholesterol levels by 61.53% and 60.12% respectively, demonstrating a favorable comparison between the two. The ethanol extract at a concentration of 22.5 µg/mL demonstrated a reduction impact comparable to that of fenofibrate at a concentration of 12.5 µg/mL. This relates to the intricate composition present in the ethanol extract of *Eriobotrya japonica* fruit. It comprises several secondary metabolite compounds, in contrast to the comparison group, which possesses only a single compound. The reduction in cholesterol is significantly different from that observed with simvastatin and fenofibrate, demonstrating a statistically significant difference at a significance level of $p<0.05$. Simvastatin has a greater impact on reducing cholesterol levels compared to Fenofibrate. This is because Fenofibrate is not solely used for lowering cholesterol, but it can also lower triglyceride levels.[23] The ethanol extract of *Eriobotrya japonica* fruit contains phenolic chemicals, flavonoids, and steroids that affect the reduction of total cholesterol levels. The formation of a hemiacetal occurs when the hydroxyl group in cholesterol interacts with the ketone group present in flavonoids. The chemical that remains unattached to the sample is known as free cholesterol, which reacts with anhydrous acetic acid and concentrated sulfuric acid. The concentration of the green chemical product produced is directly proportional to the quantity of free cholesterol available for reaction with the Lieberman Burchard reagent. The concentration of the green hue in the solution directly affects the amount of light that is absorbed. Consequently, when the solution is examined using a visible spectrophotometer, a higher absorbance value will be obtained.[23] Despite the promising results, this study has some limitations that should be acknowledged. The bioactive compounds in *Eriobotrya japonica* were analyzed in vitro, and further in vivo studies are necessary to validate these findings and assess their bioavailability and efficacy in clinical settings. The study primarily concentrated on the ethanol extract of the fruit, while other parts of the plant and various extraction

methods were not examined. Future research should consider investigating these aspects to provide a more comprehensive understanding of the plant's therapeutic potential. Practical applications of these findings include the development of natural supplements for managing oxidative stress, diabetes, and cholesterol levels. These results could also guide the formulation of functional foods or nutraceuticals. Further studies could explore the synergistic effects of combining *Eriobotrya japonica* with other bioactive compounds to enhance its therapeutic efficacy and conduct long-term studies to assess safety and effectiveness in human populations.

CONCLUSION

This comprehensive study confirms that the ethanol extract of *Eriobotrya japonica* fruit significantly contributes to its promising antioxidant, antidiabetic, and anticholesterol activities, thanks to its rich phenolic and flavonoids content. The extract exhibited potent antioxidant properties, with notable efficacy in DPPH radical scavenging and metal chelation assays, demonstrating an IC_{50} of 55 µg/mL and a 71% inhibition rate, respectively. Similarly, the antidiabetic potential was highlighted by an impressive IC_{50} value of 2.42 µg/mL in the α-glucosidase inhibition assay, suggesting its utility in managing blood glucose levels effectively, and Nelson-Somogyi method concentration of 10 µg/mL obtained 24.02%. Moreover, the study revealed a substantial reduction in cholesterol levels, with up to 47.3063% reduction, underscoring the extract's capability as a natural cholesterol level.

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