Original Article

Extraction and optimization of flavonoids from *Elaeagnus pungens* Thunb and its potential impact on blood plasma cholesterol in a mouse model

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Abstract

Elaeagnus pungens Thunb is a significant source of flavonoids with biological function. This study aims to develop orthogonal tests and alkali alcohol extraction methods for extracting flavonoids from leaves of *E. pungens*. The levels of factors in the orthogonal test were determined by pre-experiment, and the physiological efficacy was verified by the *in vivo* test. The results indicated that the optimal extraction yield was 4.07% when the solid-to-liquid ratio was 1:40, the concentration of ethanol was 60%, the extraction temperature was 80℃, and the extraction time was five h. The alkali-alcohol extraction results demonstrated that the extraction yield of flavonoids could be significantly enhanced by different concentrations of alkali-alcohol solution. When the concentration of the alcohol solution was 0.01 mol/L of sodium hydroxide, the extraction efficiency could be increased by 3 times compared with that of the alcohol extraction method. Furthermore, *in vivo* physiological studies have demonstrated that flavonoid extract from *E. pungens* had a significant role in reducing the total cholesterol in blood plasma. The findings offer a theoretical and experimental foundation for future research and application of flavonoids derived from *E. pungens*.

Keywords: *Elaeagnus pungens*, extraction, flavonoids, lowering cholesterol

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Introduction

Phytochemicals are ingredients that have well-established biological and pharmacological characteristics. Flavonoids are among the most prominent phytochemicals due to their abundance and bioactive characteristics, which include antiviral, antibacterial, antioxidant, antimutagenic, and vasodilator effects. The main causes of flavonoids' antioxidant activity are the free hydroxyl groups and phenolic rings that are present in their chemical structure. By donating hydrogen, these free hydroxyl groups can stop an oxidation process. Hence, flavonoids may be utilized to treat diseases like cardiovascular and neurological disorders that are caused by free radicals (Chua, 2013; Hussain *et al*., 2018; Tarahovsky, 2018). Plants produce secondary metabolites called flavonoids that provide color and UV light protection (Roy *et al*., 2022; Patil *et al*., 2024). Recent phytochemical studies have identified quercetin, rutin, kaempferol derivatives, terpenoids, lignans, and phenolic acids in the *Elaeagnus* genus, which contribute to antioxidative, anti-inflammatory, and lipid-lowering effects (Bammou *et al*., 2025; Zhang *et al*., 2025). Additionally, flavonoids protect vitamins and enzymes from oxidation and help stop fats from oxidizing. Another feature of flavonoids is their ability to chelate metal ions to prevent lipid peroxidation. This has raised interest in the hunt for naturally occurring antioxidants derived from plants (Jomova *et al*., 2025).

The Elaeagnaceae family is a therapeutic plant consisting of 3 genera as well as 70-80 species; a large number of these species originate from eastern and southern regions of Asia. The genus *Elaeagnus* is well-known for its therapeutic features, which include lowering cholesterol, blood sugar, and inflammation while also increasing immunity and acting as an antioxidant. Volatile oils, flavonoids, triterpenoids, alkaloids, steroids, anthraquinones, and fatty acids are among this genus's bioactive constituents; flavonoids have been the subject of the greatest research (Gupta *et al*., 2021; Selvakumar *et al*., 2022). According to recent reports, the methanolic extract of *E. confererta* seeds has anti-ulcerogenic qualities (Gupta *et al*., 2021; Guo *et al*., 2023), indicating possible medicinal uses. In addition to having good food and medicinal value, their leaves, roots, and fruits also provide an environmentally regulating function. Amongst the *Elaeagnus* species, *E. pungens* is a flourishing plant that is also known as "oleaster" or "thorny olive." In traditional Chinese medicine, it is frequently utilized for the relief of respiratory diseases like bronchitis, asthma, and severe coughing (Zhao *et al*., 2006). Previous research showed that *E. pungens* has plenty of amino acids, vitamins, sugar, and trace minerals (Mg, Cu, Zn, Fe, Mn, etc.), and organic acid contributes to its greatest nutritional value, health advantages, and enormous commercial potential (Du and Qu, 2000). The pharmacological effects of leaves, such as their antiasthmatic, antitussive, expectorant, and smooth muscle relaxant properties, have been attributed to their chemical constituents, which include flavonoids, organic acids, lignanoids, and terpenoids. These constituents can also be used to treat chronic bronchitis and asthma (Ge *et al*., 2009, 2013, 2015). Nevertheless, there has not been much research work on *E. pungens* and its level of development. Therefore, this article elaborates on the extraction research and application of flavonoids on blood plasma cholesterol. The extraction and isolation of desired compounds is an extremely essential phase in the processing of plant raw materials. Currently, hot water extraction, ultrasonic-assisted method, microwave-assisted method, organic solvent extraction, enzyme-assisted method, etc., are the primary methods used to extract plant secondary metabolites (Rodríguez De Luna *et al*., 2020). Each of these methods has pros and cons. Therefore, considering the extraction rate and price, the current study implements environmentally pleasant acid precipitation and alkaline extraction techniques. The alkaline alcohol solution is employed to extract flavonoids because they are often acidic, and it is worth noting whether it can increase the rate of extraction. Certain researchers extracted flavonoids from milk thistle, orange peel, and sophora rice using lye extraction (Li *et al*., 2014; Chaves *et al*., 2020).

The current research aims to enhance the flavonoid extraction efficiency from *E. pungens* leaves using an alkali-alcohol extraction method. Moreover, feeding tests on mice were used to confirm the physiological importance of flavonoids and their potential influence on blood cholesterol, while also being more economical and ecologically friendly by optimizing the extraction conditions. This work demonstrates that alkali-alcohol extraction can significantly increase the total flavonoids extraction yield while also being more economical and ecologically sound, so offering an efficient benchmark for industrial development.

Materials and Methods

Sample collection: The *E. Pungens* Thunb leaves were purchased from Jiangsu Province. After that, the Leaves were ground and run through a 40-mesh filter, further placed in an oven at 78 °C for 24 h, and then stored in a drying cabinet. SPF clean-grade mice and cages were purchased from the Chuzhou city breeding farm in China. The standard sample of rutin was provided by the National Institute of Drug and Biological Products of China. Furthermore, anhydrous ethanol, anhydrous aluminum trichloride, Sodium hydroxide, Sodium carbonate, Sodium citrate, and cholesterol test kits were purchased from Shanghai Meixing Chemical Co., Ltd., Shanghai, P.R. China. All animal procedures were approved by the institution's animal care and use committee (IACUC) of Anhui Agriculture University, China (approval no. AHAU-IACUC-2023-112)

Preparation of the rutin standard curve: Approximately 50 mL of the 5.6 mg rutin standard product was prepared. Then, the stock solution is diluted with a series of standard solutions, such as Quantitative rutin reference solutions 0, 1.0, 2.0, 3.0, 4.0, and 6.0 mL into a 25 mL volumetric flask, diluted with 0.1mol/L aluminum trichloride ethanol solution, mixed well, and let stand for 10 min. The absorbance was then measured at a wavelength of 400 nm using a spectrophotometer. The rutin concentration was used in the horizontal position and the OD400 value in the

vertical position to build a standard curve. Rutin was chosen as the standard because it is commonly found in Elaeagnus species and exhibits spectral characteristics similar to other flavonoid glycosides. Although this method is widely accepted for total flavonoid quantification, more precise methods such as HPLC or LC–MS/MS will be considered in future studies.

Extraction of flavonoid compound: About 1 g of *E. pungens* leaves powder was put into a beaker, and a certain amount of ethanol extract was then sealed with plastic wrap and kept for a while in a water bath at a steady temperature, and further eight layers of gauze were overlapped to filter the extract. Moreover, the obtained crude extract was centrifuged at 4000 r/m for 20 min to obtain the supernatant, which was the flavonoid extract from *E. pungens*. Then, 1 mL of the test solution was added to a 25 mL volumetric bottle, and aluminum trichloride solution with a concentration of 1% as a color-developing agent. The quantity of flavonoid was determined by the rutin standard curve by testing the absorbance OD400 value for 10 min at 400 nm wavelength. All extraction experiments were conducted in triplicate, and results are expressed as mean ± standard deviation.

Single-factor test design: Before designing the orthogonal experiment, the orthogonal test level was determined through a pre-experiment. Firstly, the single-factor pre-experiment was carried out to regulate the test level of each factor, including ethanol concentration, solid-liquid ratio, and extraction time and extraction temperature. Eventually, based on the results of the pre-experiment and careful analysis of the extraction rate and extraction cost, the level indicated in Table 1 was chosen as the test level of the orthogonal experiment.

Table 1 Single-factor experiments design levels were checked.

Factor	Level 1	Level 2	Level 3	Level 4
Extraction Time	2 h	3 h	4 h	5 h
Extraction Temperature	50 ℃	60 ℃	70 ℃	80 ℃
Solid-liquid Ratio	1:25	1:30	1:35	1:40
Ethanol Concentration	50%	60%	70%	80%

Orthogonal test: Orthogonal test table L 9(34) was selected to carry out further experiments to examine the flavonoids extraction yield. In this research, solid-liquid ratio (A), ethanol concentration (B), extraction temperature (C), and extraction time (D) were designed as consideration factors. Each factor was designed with three orthogonal test levels based on the pre-experiment results. The specific experimental data are indicated in Tables 2 and 3. As shown in Table 3, 9 groups of orthogonal experiments were selected, corresponding experimental operations were carried out according to the experimental requirements of each group, and the content of extracted flavonoids was quantitatively analyzed.

Alkaline alcohol extraction test: The present study aimed to increase both the overall extraction yield and the flavonoid extraction efficiency. The extraction test was performed utilizing sodium hydroxide alkaline alcohol solution with different concentration gradients (0.01 mol/L, 0.1 mol/L, 0.5 mol/L, 1 mol/L, and 2 mol/L). The obtained *E. pungens* and alkali-alcohol solution of different concentrations were mixed evenly at a 1:25 solid-to-liquid ratio. After soaking in a water bath at 70 ℃ for 5 h, the crude extract was filtered through cotton cloth and centrifuged at 4000 r/min for 20 minutes to obtain the flavonoid extract from *E. pungens*. After the extraction, the pH was adjusted to 5.5, and 1% AlCl3 was added as a color developer to measure the absorbance value at a wavelength of 400 nm.

Experimental animals design: A total of 63 4-week-old healthy SPF-grade male mice, weighing 28-36 g, were selected. After a week of customized feeding, the mice were allocated to three arbitrary groups, namely, a high-cholesterol control group, a high-cholesterol experimental group, and a low-cholesterol experimental group, with three replicates in each group and seven mice in each replicate. The first two groups of mice were fed high-cholesterol and high-fat diets for 4 weeks. After 4 weeks, plasma cholesterol (TC) and triglyceride (TG) were measured, and two groups of mice were established: one in the high-cholesterol group and one in the low-cholesterol group. Both groups were fed feed containing 100 mg/(BW) flavonoid extract. After four weeks of feeding, blood was collected to measure the total cholesterol index in plasma. During the experiment period, the experimental bedding material was changed regularly to ensure that the cage was clean, and the mice could eat and drink freely.

Determination of plasma cholesterol: Blood samples were collected by orbital sampling after 4 weeks of the experimental period. First, the mice were starved for 12 h, then blood was collected into an anti-coagulant blood collection EP tube provided by the laboratory and centrifuged at 12000 r/min for 15 min. After that, the supernatant plasma was taken and stored in a -20℃ refrigerator for later use. The contents of total cholesterol (TC) in blood plasma were determined using a commercial enzymatic colorimetric assay kit (Meixing Chemical Co., Ltd., Shanghai, China) following the manufacturer's instructions.

***Statistical analysis*:** A commercially accessible software program called SPSS version 19.0 was used to perform Statistical analysis. The result was analyzed by a one-way analysis of variance (ANOVA) among the groups. The Significance Difference (LSD) test was applied when significant differences were found. Prior to applying one-way ANOVA, data were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test, and both assumptions were satisfied.

Result

Rutin standard curve: The results of Rutin's standard curve are shown in Figure 1. Rutin, a flavonoid compound, was analyzed in *E. pungens* plant extracts. A rutin standard curve is a graph used to determine the concentration of rutin in a sample by comparing it to a known standard. All extraction experiments, including those in Figure 2, were conducted in triplicate, and results are expressed as mean ± standard deviation.

Single-factor test: A single-factor test was run on each factor of extraction conditions, and the test results are shown in Figure 2, showing the relationship between the extraction of total flavonoids and solid-liquid ratio, extraction temperature, extraction time, and ethanol concentration under certain conditions. Figure 2A shows that as the temperature increases, the content of entire flavonoids initially rises and then declines. Figure 2B shows that as time increased, the content of total flavonoids first surged, attained a higher level at 3 h, and then the level remained without significant fluctuation. Figure 2C indicates that as the solid-to-liquid ratio increases, the extraction rate of flavonoids also increases, but the change is less obvious. Figure 2D revealed that with the change of ethanol concentration, the extracted total flavonoids have a higher level at the ethanol concentration of 60% and 70%, and a lower level at 50% and 80%. Considering the highest target extraction rate and the lowest extraction cost, the conditions in Table 2 were selected as the factor levels for the orthogonal experiment.

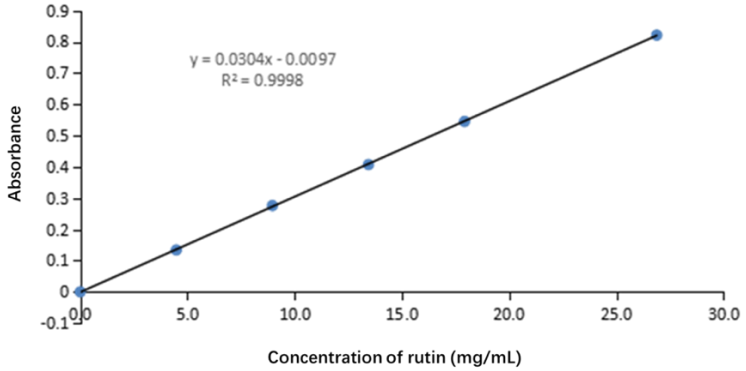

Figure 1 The regression equation of the standard curve (y=0.03040x+0.00097, R2=0.9998). X-axis: Concentration of rutin (mg/mL). Y-axis: Absorbance at 400 nm (OD).

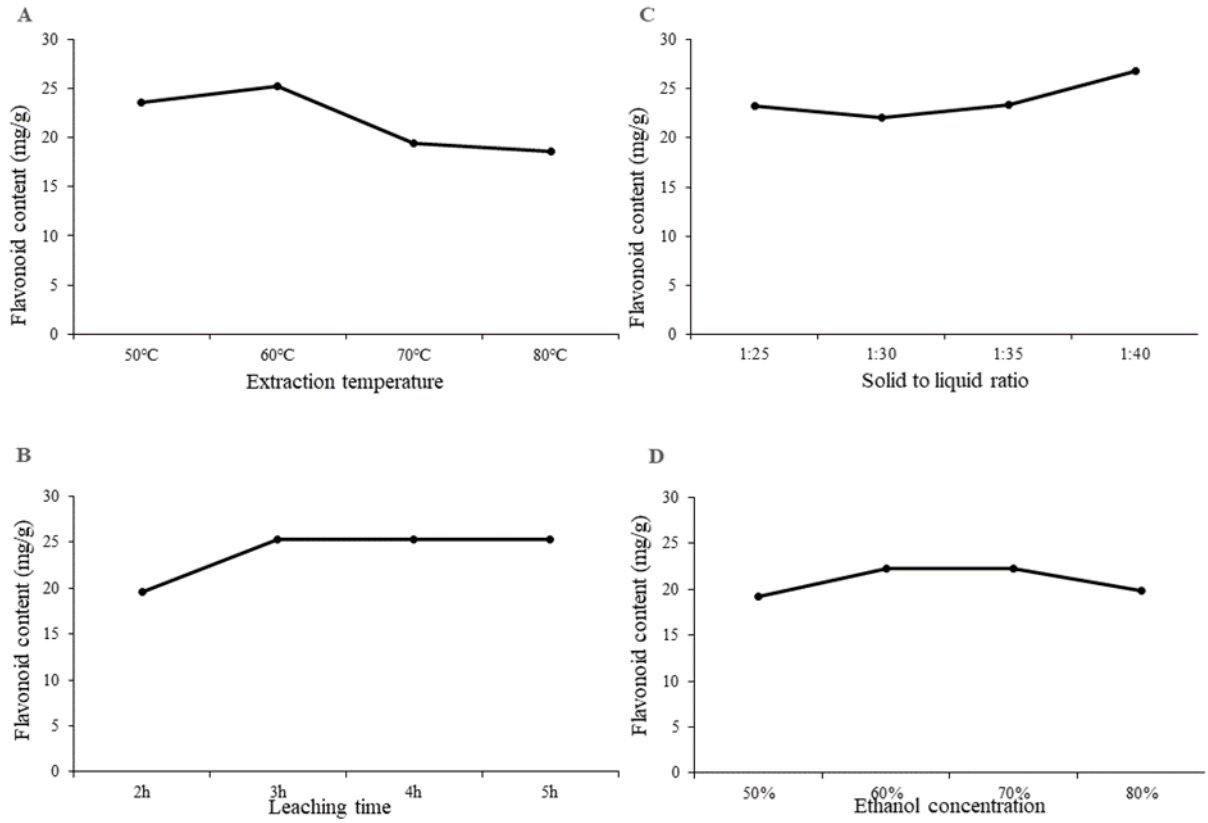

Figure 2 The results of a single-factor test affecting the extraction of flavonoids from *E. pungens* (A: different temperature; B: different time; C: different Solid-to-liquid ratios, D: different ethanol concentrations).

Table 2 Factors affecting the extraction efficiency of flavonoids from the *E. pungens* and orthogonal test level.

Factor	Level 1	Level 2	Level 3
A: Solid to liquid ratio	1:25	1:30	1:35
B: Ethanol concentration	50%	60%	70%
C: Extraction temperature	60 ℃	70 ℃	80 ℃
D: Extraction time	3 h	4 h	5 h

Orthogonal test extraction parameter: The nine groups of processing data obtained according to the specific treatment scheme of the orthogonal experiment in Table 3 are indicated in Table 4. The table showed that the seventh treatment of the nine groups of schemes listed in the orthogonal table has the optimal yield. The results reflect that the level of each factor has different effects on the extraction yield. After conducting a range analysis, it was concluded that the optimal extraction conditions combination for alcohol extraction was A3, B2, C3, and D3. The optimal extraction conditions of flavonoids from *E. pungens* leaves were as follows: solid-liquid ratio 1:40, ethanol concentration 60%, extraction temperature 80℃, and extraction time 5 h. According to the range analysis data in Table 4, Ethanol concentration has the least impact on the extraction rate of flavonoids from *Elaeagnus* leaves. The K-value analysis was consistent with the results of the single factor, so the single factor test was verified again. Among them, the solid-to-liquid ratio factor and extraction temperature have a greater impact on the extraction yield, and as the solid-to-liquid ratio and temperature increase, the extraction yields also increase significantly, and their changing association showed an obvious positive correlation. The influence of extraction time on the results also increased with the extension of time, and the extraction rate increased correspondingly, but this change gradually decreased with the continuous extension of extraction time. These results demonstrated that the main factors in the extraction process were the solid-to-liquid ratio and extraction temperature, and the concentration of ethanol had the least effect. This may be due to the water solubility of the flavonoids in *E. pungens* leaves, while higher extraction temperature could promote the dissolution of flavonoids and molecular deconstruction, separation, and dissolution. The results of the analysis of variance using SPSS 19.0 are shown in Table 5. The F-value of the test model is 5.391, and the possibility of error is only 0.001, which is significant. Ethanol concentration had the least effect on the extraction rate of total flavonoids, followed by extraction time (P>0.05). The ratio of solid to liquid and the extraction temperature had significant effects on the extraction results (P<0.01).

Table 3 The specific classification scheme of orthogonal experiment.

Level	Factor			
	A	B	C	D
1	A1	B1	C1	D1
2	A1	B2	C2	D2
3	A1	B3	C3	D3
4	A2	B1	C2	D3
5	A2	B2	C3	D1
6	A2	B3	C1	D2
7	A3	B1	C3	D2
8	A3	B2	C1	D3
9	A3	B3	C2	D1

Table 4 The combination of orthogonal test design and test results.

Treat	Solid-liquid ration	Ethanol concentration	Extraction temperature	Extraction time	Test results
	A	B	C	D	Content (mg/g)
1	A1	B1	C1	D1	26.57
2	A1	B2	C2	D2	29.54
3	A1	B3	C3	D3	35.84
4	A2	B1	C2	D3	36.42
5	A2	B2	C3	D1	38.90
6	A2	B3	C1	D2	31.07
7	A3	B1	C3	D2	40.72
8	A3	B2	C1	D3	37.47
9	A3	B3	C2	D1	32.97
K1	91.95	103.71	95.11	98.44	
K2	106.39	105.91	98.93	101.33	
K3	111.16	99.88	115.46	109.73	
K1	30.65	34.57	31.70	32.81	
K2	35.47	35.30	32.98	33.78	
K3	37.05	33.29	38.48	36.58	
R	6.4	2.01	6.78	3.77	

Table 5 The combination of orthogonal test design and test results.

Source of Variations	Sum of squares	df	Mean square	F	Sig
Calibration model	521.726a	8	65.216	5.391	0.001
Intercept	31928.020	1	31928.020	2639.159	0.000
A	200.449	2	100.225	8.285	0.003
B	18.598	2	9.299	0.769	0.478
C	233.850	2	116.925	9.665	0.001
D	68.829	2	34.414	2.845	0.084
Error	217.760	18	12.098		
Total	32667.506	27			
Corrected total	739.486	26			

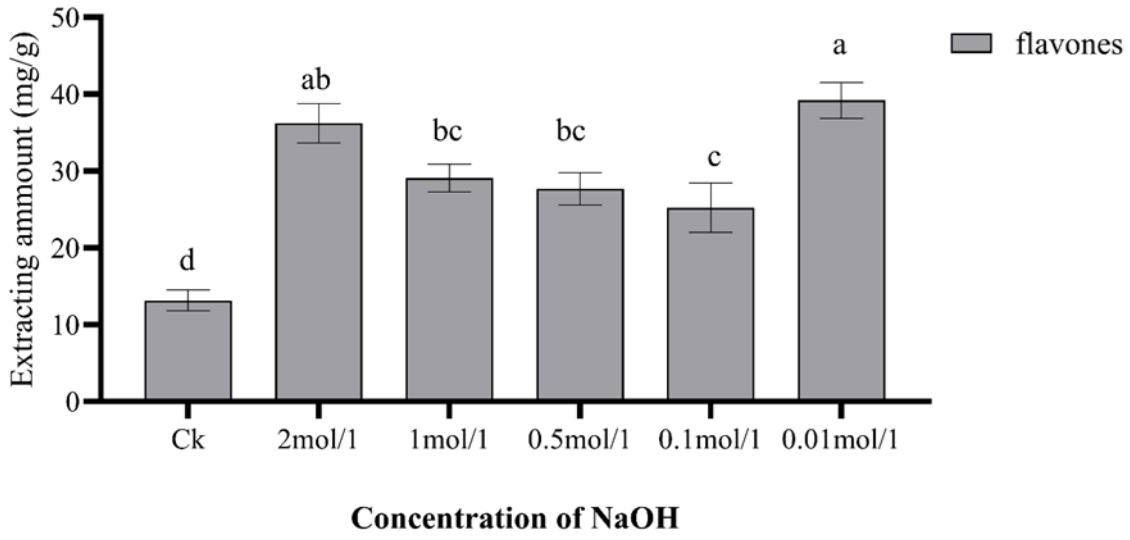

Figure 3 The total flavones extracted from the leaves of *E. Pungens* at different concentrations of NaOH.

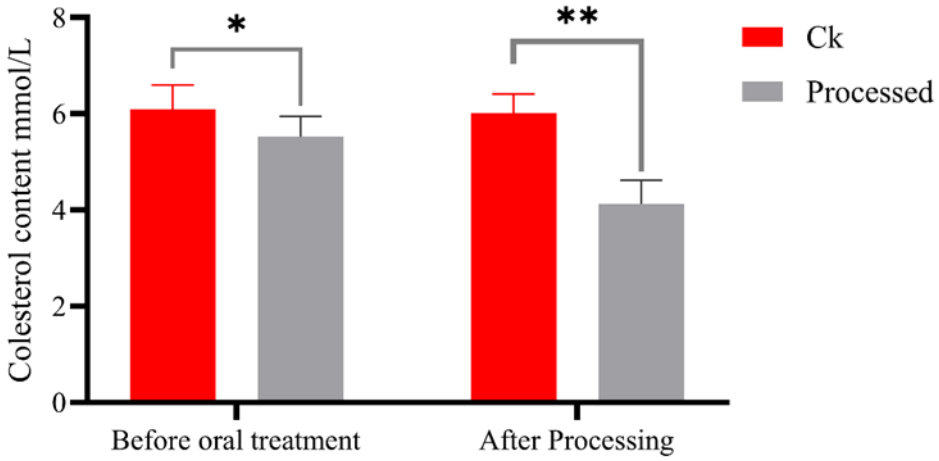

Figure 4 The cholesterol content before and after compared between different treatment groups.

Optimization of alkali alcohol extraction: The experimental results of alkali-alcohol extraction are shown in Figure 3. There was a significant difference between the 0.01mol/L sodium hydroxide treatment group and the 1mol/L, 0.5 mol/L, and 0.1 mol/L treatment groups. The result indicated that the extraction effect of the alkali-alcohol solution treated with a certain concentration of sodium hydroxide was much higher than that obtained by group CK. Moreover, the extraction effect was the best with the concentration of 0.01 mol/L, and the extraction concentration from 0.1 mol/L to 2 mol/L increases with the concentration. The maximum extraction amount of the experimental treatment group was 38.53 mg/g, and the extraction efficiency was 3 times higher than the CK group's extraction amount of 13.03 mg/g. The average efficiency of the experimental treatment group was 2 times higher than that of the CK group. It may be that the low concentration of alkali alcohol solution is enough to combine with some of the flavonoids to promote solubility, while the high concentration of alkali alcohol solution may be due to the change in the structure of flavonoids.

Determination of plasma cholesterol: In this study, the total cholesterol index in blood plasma was measured.

The mice were fasted for 12 h, and the blood was collected to measure the total cholesterol in plasma before and after the treatment. The total cholesterol content in the CK group was 3.97 mmol/L, and the total cholesterol content of the experimental group was 4.04 mmol/L. After the treatment, the total cholesterol content of the CK group was 3.44 mmol/L, and that of the experimental group was 3.26 mmol/L, as shown in Figure 4. This result showed that the reduction level of total cholesterol in the experimental group was significantly stronger than that of the CK group. The flavonoid extract of *E. pungens* could play a role in reducing the total cholesterol in blood plasma (Fig. 4). A paired analysis within each group confirmed that flavonoid supplementation significantly reduced cholesterol levels compared with baseline.

Discussion

Phytonutrients cannot be synthesized by humans and animals. Nonetheless, all mammals consume between 60-850 mg of them daily, though they are an essential component of their diets (Routray and Orsat, 2012; Kumar and Pandey, 2013). Owing to their capability to neutralize and inhibit the production of free radicals, these flavonoids are regarded as possible natural antioxidants. Additionally, one of the flavonoids' other properties is the chelation of metal ions to prevent lipid peroxidation (Fernando and Soysa, 2014; Guo *et al*., 2023). Flavonoids are primarily derived by chemical synthesis and biological extraction. Flavonoid extraction techniques and outcomes from a variety of plants, including safflower, lotus leaf, honeysuckle, scutellaria, etc., are now documented by numerous researchers. However, there is a paucity of studies on the extraction and isolation of flavonoids from Elaeagnus plants (Shang *et al*., 2017), and there is no further research on their significance in reducing plasma lipid cholesterol. Consequently, in this study, single-factor and orthogonal tests were initially performed on each factor of the extraction conditions. As indicated in Table 4, the orthogonal table serves as the foundation for the design of the orthogonal experiment. The orthogonal test technique is a type of developing approach to examine numerous factors and levels. It selects an appropriate number of representative test cases from a large set of test data that have neatly comparable, evenly distributed properties to run tests (Wang *et al*., 2010). According to the results, the temperature and the solid-to-liquid ratio factor have a larger impact on the extraction yield. The extraction yield increased significantly as the temperature and the solid-to-liquid ratio rose, and their changing association demonstrated a clear affirmative correlation. Similarly, the impact of extraction time on the extraction ratio also increased with the extension of time, but this change gradually decreased with the continuous extraction time. Previous research revealed that the extraction yield rose quickly as the solid-to-liquid ratio was enhanced from 20 to 40ml (Wang *et al*., 2018). These observations suggested that the concentration gradient that rises at decreasing solid-liquid ratios is directly correlated with the rate of diffusion (Al-Dhabi *et al*., 2017). The ability of solvents to solubilize analytes is increased by using higher temperatures. Higher temperature promotes the disintegration of the hydrogen bonding and van der Waals forces that bind bioactive molecules to plant matter. Moreover, it is well known that liquid solvents become less viscous at higher temperatures, which improves the solvent's ability to enter the plant matrix's pores and improve extraction (Richter *et al*., 1996; Viganó *et al*., 2020). The extraction performance of the procedure can be enhanced by raising the number of extraction rotations (Chua, 2013; Gupta *et al*., 2021). However, there are certain researchers who have observed a high recovery ratio of biological substances by limited extraction duration (Lama-Muñoz *et al*., 2020). The extraction time is a parameter that needs to be tuned in every experiment because there is no noticeable trend. To investigate a more effective extraction procedure, *E. pungens* leaves with a greater amount of flavonoid content were chosen for this experiment. We observed that, under ideal circumstances, the extraction yield has reached 4.1%, and that the additional extraction impact of alkali alcohol can cause the extraction rate to double. A recent study indicated that *E. pungens* leaves have a comparatively high flavonoid concentration when compared to other plants from which 2% and 3% of total flavonoids were isolated (Yu *et al*., 2019; Pham *et al*., 2020; Selvakumar *et al*., 2022). Li *et al*. (2014) enhanced the isolating parameters for entire flavonoids from the leaves of *Dimocarpus longan* at the maximum extraction rate of 2.35%. The majority of flavonoids are weakly acidic due to their structure; they react with alkali and weakly acidic phenolic hydroxyl groups, and their solubility rises when the flavonoid core is opened (Feng *et al*., 2017). Because of this, using an alkaline solution may enhance the impact of extraction by increasing the binding energy between the extract and the flavonoid. As a result, the present research used the alkali alcohol extraction method, based on the optimization conditions of the alcohol extraction method, which has an important effect on solubility. There is no relevant scientific research on the extraction of flavonoids using the alkali-alcohol extraction method that is discussed in this paper. However, the maximal extraction rate by alkali alcohol solution was projected to reach 8% under ideal conditions. In this experiment, the yield of flavonoids from Elaeagnus leaves reached up to 4.07% using alcohol extraction. Wang *et al*. (2010) also demonstrated that an alkaline solution could result in a notable increase in the efficiency of barley cytoplasmic and endosperm protein extraction. Alkaline solution has been generally recognized as a useful technique for extracting protein from plant sources (Shen *et al*., 2008). High concentrations of alkali aid in the dissociation of hydrogen bonds and remove hydrogen from carbolic and sulfate groups. In the present study, the alkali-alcohol solution's extraction efficiency was up to three times greater than that of the alcohol solution, whereas the orthogonal test findings indicated that the ethanol content had the least impact on the extraction results. Li *et al*. (2014) performed an alkaline extraction and acid precipitation method to extract all the flavonoids from the longan seed. His findings indicated that the extraction yield was 46.86 mg/g, and lye significantly increased the rate of

extraction. Moreover, the alkali-alcohol extraction method may greatly increase the extraction rate and be less harmful to the environment than other approaches. Furthermore, the low concentration of alkali alcohol solution used in the extraction procedure was stable, so it wouldn't alter the structure of flavonoids and could better preserve their activity. In addition to optimizing the extraction process, this study also conducted a preliminary identification of the physiological activities of flavonoid compounds in the extract. Previous research demonstrated that plant polyphenol molecules can scavenge free radicals, indicating their potential uses in the diet and health sector (Ratnam *et al*., 2006). The risk of cardiac disease is significantly increased via an increased amount of low-density lipoprotein (LDL) and a decreased amount of high-density lipoprotein (HDL). *In vitro* investigations by Hasnat (2024) revealed that flavonoids can prevent LDL oxidation by scavenging radicals. This research also showed that the extract had a significant down-regulating effect on the level of total cholesterol in the blood. The total cholesterol content of the CK group was 3.44 mmol/L, and that of the experimental group was 3.26 mmol/L. Therefore, the flavonoid extract of *E. pungens* could play a significant role in reducing the total cholesterol in blood plasma. Still, more research is required on the isolation, purification, and structural analysis of particular flavonoids, and whether the cholesterol-lowering effect was due to a specific flavonoid needs further identification. Certain in vitro studies have shown that flavonoids can lower the levels of triglycerides and cholesterol while also improving the absorption of cholesterol. Previous research has shown that plant flavonoids have antioxidant and free radical scavenging properties, so they have a positive impact on lipid metabolism, therefore reducing and preventing cardiovascular diseases (Bao *et al*., 2016). Multiple studies also showed the biological impacts of polyphenols in reducing cholesterol in the blood and lipid levels (Ling *et al*., 2020). Consequently, the biological impact of *E. pungens* in reducing blood lipids and cholesterol has greater research significance. All extraction experiments, including those in Figure 2, were conducted in triplicate, and results are expressed as mean ± standard deviation.

In conclusion, the present research describes the process of alkali alcohol precipitation utilized to isolate flavonoids from *E. pungens* leaves. The optimal extraction efficiency of 40.72 mg/g was achieved when the liquid ratio was 1:40, the concentration of ethanol was 50%, the extraction temperature was 80℃, the extraction time was five h, and the concentration of alkali ethanol solution was 0.01 mol/L. Currently, there is still limited research, development, and use of flavonoids from *E. pungens*. It should be noted that baseline cholesterol levels differed slightly between groups before treatment, which may influence the interpretation of post-treatment results. Future studies should use stratified randomization or larger sample sizes to minimize baseline differences. However, the outcome of this research recommends that the flavonoid-rich *E. pungens* plants have a lot of potential for further research and that their extract could be a novel source of natural antioxidants and medicinal foods with a lot of commercial potential in the pharmaceutical and food industries. Yet, more research is required to recognize and extract the phytochemicals from this genus of plants, as these specific phytochemicals belong to bigger families and must be separated to identify the active molecules and to fully understand the therapeutic mechanisms of isolated phytochemicals.

Conflict of interest statement: The authors declare that the research was carried out without any commercial or financial relationships that could be construed as a potential conflict of interest.

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CRediT authorship contribution statement

Qunqing Lu: Investigation, Project administration, Software. **Haozhe Liu**: Investigation, Project administration, Software. **Xinxin Zhang**: Investigation, Project administration, Software. **Nourhan Nassar**: Validation, Writing - Review & Editing. **Ibrar Muhammad Khan**: Formal analysis, Visualization, Writing - Original Draft. **Haji Gul**: Validation, Writing - Review & Editing. **Zaigui Wang**: Conceptualization, Supervision, Resources.

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