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The Phenolic Content of Syrian *styrax officinalis* L. Fruits and its Antioxidant and Anti-Cholesterol Activities

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ABSTRACT

The objective of the current study was to determine the chemical constituents, total phenols, antioxidant activity, and hypocholesterol effect of the aqueous and ethanolic extracts of *Styrax officinalis* fruits.The chemical composition of the extracts was assessed by (GC) and (GC-MS). The extract contained various compounds, with styracitol (90.8-97.4%) being the major compound in the aqueous and ethanolic extracts, respectively. The total phenolic content was quantitatively determined using the Folin-Ciocalteu reagent, with gallic acid as the standard. Antioxidant activity was assessed by measuring the scavenging of 2,2-Diphenyl-1-Picryl-Hydrazyl (DPPH). The total phenolic content of the aqueous and ethanolic extracts was found to be 19.2 ± 0.75 mg GAE/g and 9.16 ± 0.23 mg GAE/g, respectively, in terms of gallic acid equivalent (GAE). The antioxidant activity has an IC50 of 1.076-2.49 mg/mL, respectively. The anti-cholesterol activity was determined using the CHOD-PAP method. The aqueous extract was more effective than the ethanolic extract at lowering cholesterol. The IC_{50} for the ethanolic extract was 6.4 mg/ml, while the IC_{50} for the aqueous extract was 0.54 mg/ml.

Keywords: *Styrax officinalis* ; Phenols ; Antioxidant ; Anti-cholesterol ; CHOD/PAP.

1. INTRODUCTION

Medicinal plants have been used in healthcare for many years. They play important roles in disease prevention and promotion of health. Medicinal plants contain natural compounds that are important sources of molecules with medicinal properties [(1)].

Styrax.officinalis L. is an important medicinal plant that grows in desert, temperate climate, subtropical, and Mediterranean Basin regions. It has been used for medicinal, cosmetic, and agricultural purposes [(2,3)]

Earlier studies on the pericarps of Styrax species have reported the isolation of a triterpene sapogenin and a triterpene saponin [(4)], Which has cholesterol-lowering efficacy [(5,6)]

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Phenolic compounds contain hydroxyl groups that directly contribute to antioxidant action, and also some of these compounds stimulate the synthesis of endogenous antioxidant molecules within the cell. After reviewing the literature, it was found that, phenolic compounds have been found to demonstrate free radical inhibition, metal inactivation, peroxide decomposition, or oxygen scavenging in biological systems, thereby aiding in the prevention of oxidative diseases [(7)] .Therefore, it is essential to investigate the phenolic content and antioxidant activity of different plants.

Hypercholesterolemia, known as high cholesterol levels, is responsible for one-third of cases of ischemic (coronary) heart disease worldwide.

Hypercholesterolemia causes 2.6 million deaths (4.5% of the total) and results in 29.7 million disability-adjusted life years (DALYs), accounting for 2.0% of the total DALYs. The worldwide prevalence of high total

cholesterol among adults in 2008 was 39%, with 37% in men and 40% in women [8].

The use of medicinal plants in combating non-communicable diseases has been extensively documented through field studies worldwide. However, a systematic compilation of these surveys for specific disease conditions, such as hypercholesterolemia, has not been carried out [9].

According to previous literature, there are four mechanisms through which bioactive compounds from herbal medicines reduce lipid levels. These mechanisms include inhibiting the absorption of cholesterol in enterocytes, reducing cholesterol synthesis, enhancing reverse cholesterol transport, and stimulating cholesterol excretion in the liver [9], so far, there is no study to determine the effectiveness of *S. officinalis* in lowering cholesterol.

2. MATERIALS AND METHODS

2.1. Chemicals

Gallic acid standard was purchased from AvonChem (United Kingdom); Folin–Ciocalteu reagent and ethanol (95%) were obtained from Sigma; 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Tokyo Chemical Industry (Japan); methanol from SHAM LAB; sodium carbonate from Scharlau; and the CHOD-PAP kit from Coral Clinical Systems (India).

2.2. plant samples collection

Fruits of *s. officinalis* were harvested in September 2023 during maturation (seeds: dark brown

Color) from the coastal region (Tartous) of Syria.

The plant was identified by prof. G.TAYOUB (Tishreen university), The voucher specimens were deposited at the herbarium, Damascus University.

Figure 1. ***Styrax officinalis***

2.3. Sample preparation

The seeds from the fresh fruits samples were manually separated from pericarp, then the pericarp where dried on filter paper by oven at 50°C until constant weight.

2.4. Extraction

Pericarps of fruits (50 g) were grounded using a milling machine and divided into two equal samples.

The first was prepared as ethanolic extract using 200 ml of 70% ethanol; the second was dissolved in 200 ml of distilled water. An ultrasound path set 25°C, 2 h was used to dissolve the powder completely.

Followed maceration at room temperature for 48 h, the extract was filtered and dried. Then were stored at 2-8 °C until use.

2.5. Gas Chromatography

Extracts of fruits were analyzed using an Agilent GC system. The capillary column used was BPS (30 m x 0.25 mm) with helium as the carrier gas at 1 ml/min.

The initial temperature of the column was 50°C (held for 5 min), and then heated to 300°C at a rate of 0°C held for 5.5 min. The injector temperature was 300°C with a source temperature of 280°C.

2.6. Gas Chromatography- mass spectroscopy (GC-MS)

Constituents of the extracts were identified using GC-MS. The GC-MS analysis was carried out using an Agilent GC-MS model GC-6890, with an inert mass selective detector 5973.

The capillary column was BP5 (30x0.2 mm, film thickness 0.25 µm). The operating conditions were as follows: carrier gas, helium with a flow rate of 1 mL/min, injected volume was 1 µL of the extract.

The GC-MS system was operated under the following conditions: injection temperature 300°C.

The initial temperature of the column was 50°C (held for 2 minutes), then heated to 170°C at a rate of 2°C/min (held for 7 minutes), and finally heated to 300°C at a rate of 10°C/min (held for 5.5 minutes).

Identification of components in the extract was based on retention time (RT).

Individual components were identified by comparing mass spectra and their corresponding GC retention data. Identification was made by comparing the obtained mass spectra with those in data system libraries installed in the GC-MS system. The quantitative analysis of percentages was performed according to reference materials and standards obtained from Aldrich. Calculations were made using the gas chromatography ChemStation software.

2.7. Determination of phenolic content

The total phenolic content of the extracts was determined colorimetrically by the Folin-Ciocalteu method [10-12]. The F–C reagent was to a 1:10 ration distilled water just before the experiment.

Proceed by taking a 1 mL aliquot of extract and mixing it mixed with 1 mL of Folin-Ciocalteau phenol reagent. After the reaction, 1N Na_2CO_3 was added. The absorbance was measured at a wavelength (λ) of 740 nm using a UV-Vis spectrophotometer.

The standard gallic acid curve was determined by creating five standard solutions of gallic acid with concentrations ranging from 0.05 to 0.6 mg/ml, as shown in **Figure 2A**.

From each concentration of the standard gallic acid solution, 40 µL was taken and mixed with 3.16 ml of distilled water, and 600 µl of 1N Na_2CO_3. The mixture was then shaken and left to stand for 8 minutes and 30 seconds. Afterward, 200 µL of Folin-Ciocalteu reagent was added, and the solution was measured using a UV-Vis spectrophotometer at a wavelength 740 nm.

160 µL of sample, standard, or blank from the assay tube was transferred to a clear 96-well microplate (see **Figure 2B**) and the absorbance of each well was measured at 740 nm.

Figure 2. A- Standard series of gallic acid
B- the Microplate Contains samples and standards

2.8. Antioxidant activity

The sample was diluted into 5 concentrations: (6, 3.75, 2.5, 1.25, 0.5) mg/ml of aqueous extract and (6, 3.25, 2.5, 1.5, 1.25) mg/ml of ethanolic extract. The DPPH radical scavenging activity of *S. officinalis* was assessed using the method described by [13,14] with some modifications. Aliquots of 160 µL of DPPH in methanol were mixed with 40 µL of the extracts. The mixtures were vigorously shaken and then left to stand for 30 minutes at 25 °C under subdued light. The absorbance of each test sample and control sample was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm, as shown in (Figure 3A**)**.

The percentage of DPPH inhibition is calculated using the following equation:

IC (%) = (1 - (As / A0)) * 100

Where A0 represents the absorbance of the negative control, and as represents the absorbance of the sample. After calculating the percentage of inhibition using the above equation, each sample concentration will be determined. This assay is performed in micro centrifuge tubes and assessed using a 96-well plate reader, as shown in (Figure 3B).

Figure 3. A- Microplate contains sample,DPPH
B- UV-Vis-spectrophotometer (made in USA)

2.9. cholesterol-lowering activity

The cholesterol-lowering activity was determined using the CHOD-PAP method [15].

2 mL of a standard cholesterol solution (200 mg/dl) was taken and then added to 2 mL of the sample solution. The mixture was homogenized using ultrasound and then incubated at 37°C for 60-minutes.10 µL of each sample was taken, and then 1000 µL of the CHOD-PAP cholesterol reagent kit was added. The mixture was incubated at 37°C for 10 minutes.200 µL of sample, standard, and blank were

transferred from the assay tube to a clear 96-well microplate (see Figure 4B), and the absorbance of each well was measured at 500 nm using a UV-Vis spectrophotometer.

A separate blank was prepared for each concentration of plant samples. 20 µL of plant samples were mixed with 20 µL of distilled water, according to the experimental conditions. Subsequently, 10 µL was extracted from the mixture, and 1000 µL of reagent was added to eliminate any interference from the plant in the experiment. The inhibition ratio of cholesterol was calculated using the following equation: I% = ((A_STD – A_TEST) / A_STD) x 100

Where A_STD represents the absorbance of standard and A_Test represents the absorbance of Test.

Figure 4. A- Cholesterol SR Kit
B- the Microplate Contains tests and standards.

2.10. Statistical analysis

All tests were performed in three repetitions, and the measurement was repeated three times for each sample. The results were presented as mean ± standard deviation after being calculated using Microsoft Excel 2013. The statistical analysis was conducted using the Costat 6.4 program. The analysis included an analysis of variance (ANOVA) and post-hoc comparisons (LSD) test at a significance level of 0.05.

3. RESULTS AND DISCUSSION

3.1. Phytochemical analysis of the plants extracts

The yielded extracts obtained by ethanolic and aqueous extraction of fruit S.officinalis were about (35%-29%) w/w on dry weight basis, respectively.

The active constituents available in styrax officinalis fruits were identified using GC-MS.

Table 1: Chemical constituents of S. officinalis fruit aqueous extract identified by GC-MS.

Chemical Compound	*Molecular formula*	*Molecular weight*	RT	*Percent %*
Melezitose	***C18H32O16***	***342.30 g/mol***	15.3	0.9%
Paromomycin	***C23H45N5O14***	***615.6 g/mol***	25.52	0.3%
2-Myristynoyl pantetheine	***C25H44N2O5S***	***484.7 g/mol***	25.74	0.1%
DL-Arabinose	$C_5H_{10}O_5$	***150.13 g/mol***	12.95	0.3%
Melibiose	***C12H22O11***	***342.30 g/mol***	12.57	0.2%
Styracitol	***C6H12O5***	***164.16 g/mol***	22.69	90.8%
palmitic acid	***C16H32O2***	***256.42 g/mol***	24.07	0.9%
Vitamin E	***C31H52O3***	***472.7 g/mol***	34.11	0.1%

Table 2: Chemical components of *S. officinalis* fruit ethanolic extract identified by GC-MS.

Chemical Compound	*Molecular formula*	*Molecular weight*	RT	*Percent %*
Melezitose	***C18H32O16***	***342.30 g/mol***	15.5	1.1%
Oleic Acid	***C18H43O2***	***282.5 g/mol***	25.93	0.1%
Paromomycin	***C23H45N5O14***	***615.6 g/mol***	27.35	0.3%
2-Myristynoyl pantetheine	***C25H44N2O5S***	***484.7 g/mol***	26.14	0.1%
DL-Arabinose	$C_5H_{10}O_5$	***150.13 g/mol***	12.95	0.3%
Melibiose	***C12H22O11***	***342.30 g/mol***	15.5	0.2%
Styracitol	***C6H12O5***	***164.16 g/mol***	22.73	97.45%
palmitic acid	***C16H32O2***	***256.42 g/mol***	35.03	0.9%
d-Mannose	***C6H12O6***	***180.16 g/mol***	23.84	0.2%
Vitamin E	***C31H52O3***	***472.7 g/mol***	34.11	0.1%

3.2. Determination of phenolic content

Table 3. Average absorbance values of a series of standard concentration of standard prepared from Gallic acid reference material.

concentration(mg/ml)	0.05	0.1	0.2	0.5	0.6
Average absorbance	0.002	0.016	0.039	0.102	0.124

When graphically representing the relationship between the previous values, the graph of Gallic acid was used to calculate the phenol content (Figure 5).

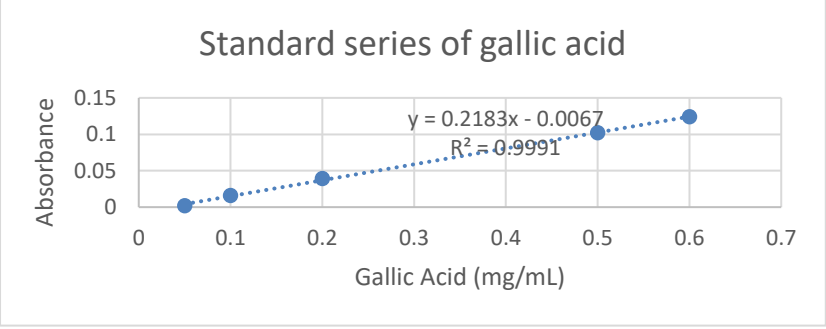

Figure 5. The curve of the standard gallic acid concentration

The data obtained showed that the phenols content of aqueous is higher than ethanol extract (19.2± 0.75, 9.16 ± 0.23) mg GAE/1g DE respectively, this is due to the difference in polarity of the two solutions. The polarity of the solution plays an essential role in the process of extracting phenols and liberating them from the plant material. The extraction is also influenced by the nature of the extracted phenolic extract. There are few studies on the fruits of the plant, as most of the research has focused on the leaves or used different sweeteners. However, a study

by Almutairi G [16] and colleagues confirms that the fruits have the lowest phenolic content.

3.3. The antioxidant activity

The antioxidant activity of *styrax* fruits is demonstrated in **figure 6**, where it shows the ability to scavenge 50% of free radicals (diphenylpicrylhydrazyl) and convert them into non-radical compounds (diphenylpicrylhydrazine). A linear relationship was also observed between the total phenolic content and antioxidant activity of *S. officinalis*. The antioxidant activity was expressed as IC_{50}, which is the amount of sample required to reduce the initial DPPH concentration by half.

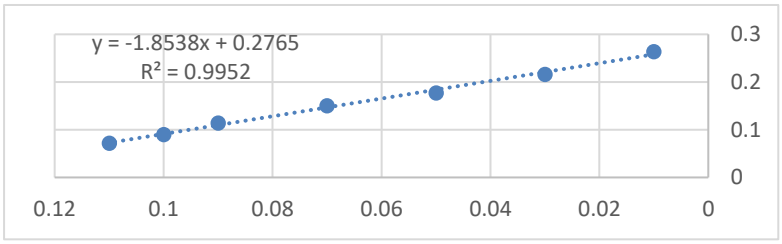

Figure 6. Free Radical Scavenging Activity of Gallic Acid

Table 4. Concentration of aqueous extract and percentage of DPPH inhibition

Concentration (mg/ml)	**0.5**	**1.25**	**2.5**	**3.75**	**6**
RSA% First iteration	46.48	47.89	56.34	64.08	77.46
RSA% Second repetition	49.3	51.05	59.86	67.25	79.23
RSA% Third repetition	47.53	49.65	58.45	66.2	78.52
Average RSA%±SD	47.77± 1.42	49.53 ± 1.58	58.22± 1.77	65.84± 1.61	78.40± 0.89

RSA: scavenging the free radicals

Table 5. Concentration of ethanol extract and percentage of DPPH inhibition

Concentration (mg/ml)	**1.25**	**1.5**	**2.5**	**3.75**	**6**
RSA% First iteration	30.63	33.8	48,94	63.38	89.79
RSA% Second repetition	35.21	34.86	51.06	65.14	91.55
RSA% Third repetition	34.51	37.68	51.41	66.9	90.49
Average RSA%±SD	33.45± 2.47	35.44± 2	50.47± 1.34	65.14± 1.76	90.61± 0.088

The graph illustrating the relationship between concentrations and IC% from the previous values is shown in **Figure7**

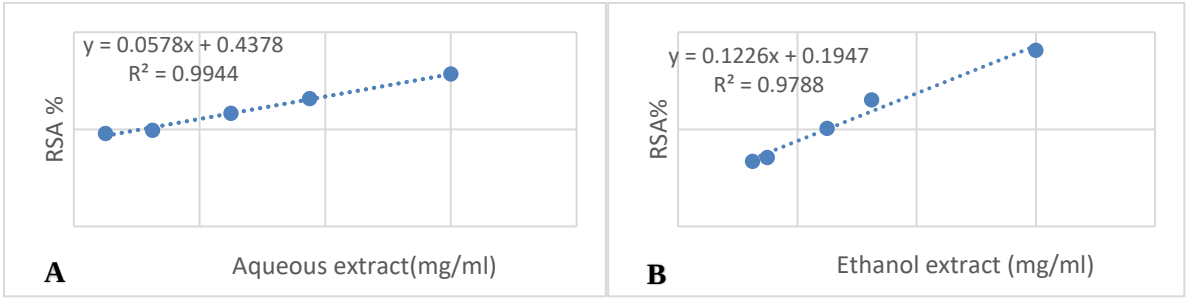

Figure 7. The linear relationship between concentration and IC (RSA) % measured at 517 nm for the aqueous extract (A) and the ethanol extract (B).

We obtained the IC_{50} values of Antioxidant activity from the linear equation for both extracts and the gallic acid standard, as shown in **Figure 8.**

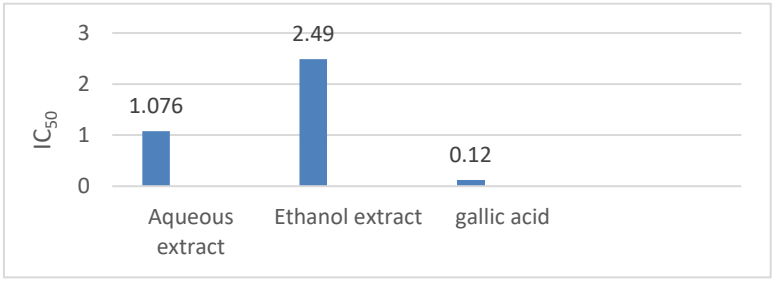

Figure 8. IC_{50} values of aqueous and ethanol extracts from S.officinalis pericarps and the gallic acid standard.

This study is consistent with the research conducted by silva et al [17] on the sensitization of Styrax camporum and S. ferrugineus fruits had weak free radical scavenging activity.

3.4. cholesterol-lowering activity

Table 6. Percentage reduction (IC%) of cholesterol for different concentrations of aqueous extract (three replicates).

Average IC%	IC%	Test absorbance	Aqueous extract concentration mg/ml	Standard absorbance
66.66 ± 2.7	64.42	0.053	2.5	0.149
	69.8	0.045		
	65.77	0.051		
61.29 ± 3.93	65.77	0.051	1.67	
	58.38	0.062		
	59.73	0.06		
55.35 ± 3.37	58.38	0.062	1.25	

Average IC%	IC%	Test absorbance	Aqueous extract concentration mg/ml	Standard absorbance
	55.7	0.066		
	51.68	0.072		
53.47 ± 1.69	51.68	0.072	1	
	55.03	0.067		
	53.69	0.069		
49.89 ± 3.03	46.98	0.079	0.5	
	49.66	0.075		
	53.02	0.07		

The graph of the previous data is used to derive the linear equation and calculate the IC_{50} (Figure 9).

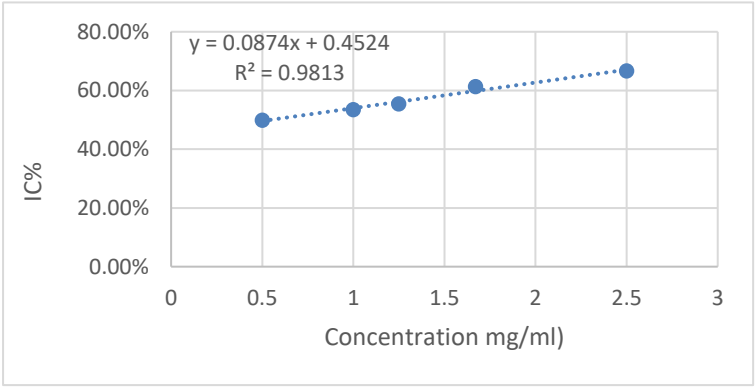

Figure 9. Significant correlation between different concentrations of aqueous extract and the percentage of cholesterol.

Table 7. Percentage reduction (IC%) of cholesterol for different concentrations of ethanolic extract (three replicates).

Average IC%	IC%	Sample absorbance	Ethanol extract concentration mg/ml	Standard absorbance
68 ± 3.38	71.14	0.043	16	0.149
	64.42	0.053		
	68.46	0.047		
58.61 ± 1.68	57.05	0.059	12	
	58.38	0.062		
	60.40	0.064		
51.9 ± 1.69	53.69	0.074	8	
	51.67	0.072		
	50.33	0.069		
47.2 ± 3.02	50.33	0.083	6	
	46.98	0.079		

Average IC%	IC%	Sample absorbance	Ethanol extract concentration mg/ml	Standard absorbance
	44.29	0.074		
44.74 ± 2.36	42.28	0.086	2	
	44.96	0.082		
	42.28	0.079		

The graph of the previous data is used to derive the linear equation and calculate the IC_{50} (**Figure 10**).

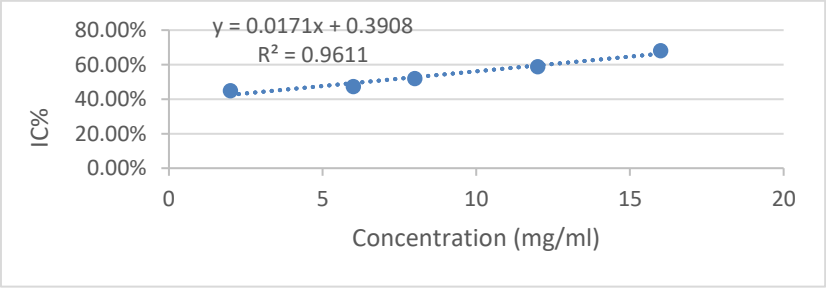

Figure 10. Significant correlation between the ethanolic concentrations of *S.officinalis* and reduction in cholesterol percentage.

Table 8. The IC_{50} values calculated from the linear correlation for both extracts.

IC_{50}	Extracts
0.54 mg/ml	Aqueous extract
6.4 mg/ml	Ethanolic extract

3.4. Statistical analysis

The results of the one-way ANOVA test (one-way analysis of variance) revealed significant differences in the phenol content between the ethanoic and aqueous extracts. The significance level for the mean differences was determined to be $P < 0.05$.

There is a significant correlation between the values of RSA% and IC% in the aqueous extract, where $p < 0.005$.

4. CONCLUSION

The study confirmed the presence of the sugar Styracitol in *S. officinalis* fruits [18], consistent with previous findings in related species [19] Additionally, the antibiotic compound Paromomycin was detected in both extracts. Notably, this study, for the first time, determined the chemical composition of *S. officinalis* fruits using GC-MS and found no toxic compounds.

Furthermore, the study revealed that the aqueous extracts of *S. officinalis* fruits exhibited higher antioxidant activity compared to the ethanolic extract, with IC50 values of 1.076 mg/ml and 2.49 mg/ml, respectively. The phenolic content in the two extracts was directly related to the IC50 value, with values of 19.2 mg GAE/1g DE and 9.16 mg GAE/1g DE for aqueous and ethanolic extracts, respectively.

It is important to note that there is a lack of reference studies on the fruits of *S. officinalis*. Most studies have focused on leaves or used different solvents compared to those used in the current study [8]. One study indicated that the fruits are considered to have the lowest phenolic content compared to other parts of the plant [16]. This study is the first to investigate the cholesterol-lowering

effectiveness of *S. officinalis* fruits. The study also highlighted the potential of *S. officinalis* as a valuable natural antioxidant resource.

The study also identified a linear relationship between extract concentrations and their efficacy in reducing cholesterol levels. Notably, the IC50 values for anti-cholesterol activity were 0.54 mg/ml for the aqueous extract and 6.4 mg/ml for the ethanolic extract, indicating that the aqueous extract was more effective at lowering cholesterol due to its superior ability to extract saponins and higher phenolic content [5,6]. As in many previous studies, there is a direct relationship between the phenolic content in extracts and their antioxidant and cholesterol-lowering abilities [20,21]. In conclusion, while this study provides valuable insights, further in vivo studies are recommended to understand the exact mechanism of cholesterol reduction and to isolate the active substances responsible for this effect.

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Conflict of Interest*:* The authors declare that there are no conflicts of interest.

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المحتوى الفينولي والفعالية الكاسحة للجذور الحرة والخافضة للكولستيرول لنبات الاصطرك الطبي المنتشر في الساحل السوري

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ملخص

الهدف من الدراسة: تحليل التركيب الكيميائي لثمار نبات الإصطرك الطبي *Styrax officinalis* باستخدام جهاز (GC-MS)، مع تحديد محتواها الكلي من الفينولات، وقدرتها الكاسحة للجذور الحرة، وفعاليتها الخافضة للكوليسترول.

الأساليب: طُبق المنهج التجريبي عبر تحديد المحتوى الفينولي الكلي بطريقة فولين–سيوكالتو (باستخدام حمض الغاليك كمعيار)، وقياس القدرة المضادة للأكسدة عبر كسح جذر (DPPH) الحر، بينما دُرست الفعالية الخافضة للكوليسترول لأول مرة بالطريقة الأنزيمية (CHOD/PAP method).

النتائج: كشفت النتائج عن وجود مركبات متنوعة، كان الـ(Styracitol) المركب الأساسي في المستخلصين المائي والإيثانولي.، تفوق المحتوى الفينولي في المستخلص المائي (19.2 ± 0.75 مغ/غ) على نظيره الإيثانولي (9.16 ± 0.23 مغ/غ) ، وفعالية مضادة للأكسدة بمتوسط قيم IC_{50} بلغت 1.076 مغ/مل (المائي) مقابل 2.49 مغ/مل (الإيثانولي) إضافة إلى تفوق ملحوظ للمستخلص المائي في خفض الكوليسترول (IC_{50} = 0.54 مغ/مل) مقارنة بالإيثانولي (IC_{50} = 6.4 مغ/مل).

الاستنتاجات: يُستنتج أن ثمار الإصطرك مصدر واعد للمركبات الفينولية ذات الخصائص المضادة للأكسدة والخافضة للكوليسترول، مما يدعم توظيفها في التطبيقات الطبية.

الكلمات الدالة: الفينولات الكلية، الإصطرك الطبي، فولين–سيوكالتو، مضادات الأكسدة، خافضات الكوليسترول

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