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Original Research

Assessment of Phenolic Composition, Antioxidant Potential, Antimicrobial Properties, and Antidiabetic Activity in Extracts Obtained from *Schinus molle* L. Leaves and Fruits

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Abstract

Background: The current research centers on exploring the antioxidant, antimicrobial, and antidiabetic features of *Schinus molle* L. grown in Turkey. **Methods**: Quantitative analysis of chlorogenic acid, caffeic acid, and hyperoside levels in leaf, ripe fruit, and raw fruit extracts was conducted using High-Performance Liquid Chromatography (HPLC) in a 70% methanol-water mixture. Among the extracts, the methanol extract from ripe fruits displayed the highest chlorogenic acid concentration, measuring at 2.040% ± 0.172% standard deviation (SD). Moreover, analysis of their total phenolic and flavonoid contents was carried out. Antioxidant power was assessed through different chemical assays, together with their antimicrobial and anti-diabetic properties. **Results**: The results of DPPH (2,2-Diphenyl-1-picrylhydrazyl), ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), and reducing power assays showed that leaf and ripe fruit alcoholic extract exhibited peak performance. While the MIC (minimum inhibitory concentration) values of the extracts were determined to have moderate bactericidal effects on *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* it was observed that none of the extracts displayed biofilm inhibition. The inhibition percentage of α-glucosidase enzyme activity for the methanol extract of raw fruits was determined to be 99.11 ± 1.61. In diabetic β-TC cells, glucose level was measured as 129 ± 2.03 mg/dL, and insulin amount was measured as 37.2 ± 0.02 mg/dL. **Conclusions**: The findings of our study seem to have important implications for future research, as *Schinus molle* L. may be a potential pharmaceutical candidate with important pharmacological activities.

Keywords: *Schinus molle*; Anacardiaceae; β-TC cells; biological activity

1. Introduction

Throughout history, the therapeutic properties of medicinal plants have played a significant role in people's lives. Because of their affordability and accessibility, these plants have been utilized for ages by several civilizations to cure a variety of ailments [1]. Worldwide, it is estimated that 422,000 plant species are used for therapeutic purposes [2]. *Schinus molle* belongs to the Anacardiaceae family and a native of South America, particularly the coast of Brazil, is one of these medicinal plants. Amenorrhea, bronchitis, gingivitis, gonorrhea, gout, tuberculosis, tumors, ulcers, urethritis, warts, and urogenital disorders are only a few of the ailments that have historically been treated with *S. molle* [3]. As a result of its antibacterial and antiseptic capabilities, it has also been employed for the treatment of wounds and pathogenic conditions. Rheumatism and ophthalmia have both been healed using the juice from the leaves [4,5]. Apart from its therapeutic use, *S. molle's* fruit and essential oil are occasionally substituted for "black pepper" in dishes despite having a milder flavor. In Peru, it is often drunk as a beverage during celebrations. This drink (chicha de molle) is made by soaking the *S.molle* fruit in water, then adding sugar and processing the combination in certain containers [6–8].

S. molle fruits and leaves are rich in essential oil and the oil contains α-phellandrene, β-phellandrene, α-terpineol, α-pinene, β-pinene, p-cymene, limonene, β-myrcene and elemol [9,10]. In addition, phenolic components including flavonoids, bioflavonoids, phenolic acids, anthocyanins, tannins, and catechins, as well as sesquiterpenes, triterpenes, and steroids were detected in the plant [11]. It is effective as an antifungal, antibacterial, antiviral, topical antiseptic, pain reliever, anti-inflammatory, spasmolytic, antioxidant, and antitumor agent, due to its bioactive secondary components [12].

As it is known, plants exhibit diverse responses to the geographical locations they inhabit, which greatly influences their phenolic content. The proportion of secondary

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Table 1. Total phenol/flavonoid content and quantitative determination of chlorogenic acid, caffeic acid, and hyperoside in *S. molle* extracts (n = 3).

Extracts	Total Phenol [mg_{GAE}/g_{extact}]	Total Flavonoid [$mg_{CA}/g_{extract}$]	Chlorogenic acid (% ± SD*)	Caffeic acid (% ± SD*)	Hyperoside (% ± SD*)
S.m L MeOH	231.32 ± 11.10	44.40 ± 2.71	0.325 ± 0.005	ND*	0.057 ± 0.0006
S.m L water	172.00 ± 5.84	40.32 ± 2.37	0.396 ± 0.024	0.006 ± 0.0003	0.068 ± 0.0002
S.m Ripe F MeOH	126.11 ± 13.62	26.02 ± 1.37	2.040 ± 0.172	UD*	UD*
S.m Ripe F water	59.54 ± 2.53	12.99 ± 0.67	0.306 ± 0.011	UD*	UD*
S.m Raw F MeOH	117.70 ± 11.84	20.16 ± 0.39	0.508 ± 0.007	UD*	UD*
S.m Raw F water	63.48 ± 1.57	15.18 ± 3.66	0.209 ± 0.014	UD*	UD*

*UD, Undetected; *SD, Standard Deviation; S.m L MeOH, *S. molle* leaf methanol extract; S.m L water, *S. molle* leaf water extract; S.m Ripe F MeOH, *S. molle* Ripe Fruit methanol extract; S.m Ripe F water, *S. molle* Ripe Fruit water extract; S.m Raw F MeOH, *S. molle* Raw Fruit methanol extract; S.m Raw F water, *S. molle* Raw Fruit water extract.

Table 2. Calibration values for standards.

Standards	Caffeic acid	Chlorogenic acid	Hyperoside
Calibration range (µg/mL)	5–200	5–200	5–200
Linear Equation	y = 53.607x + 14.135	y = 3.607x + 14.135	y = 50.62x + 32.131
Correlation factor (r^2 ± SD*)	0.999 ± 0.015	0.996 ± 0.001	0.999 ± 0.0005
Limit of Detection (µg/mL)	0.039	0.371	0.112
Limit of Quantitation (µg/mL)	0.131	1.237	0.375

*SD, Standard Deviation.

metabolites may vary due to a multitude of factors [13]. In a study conducted in 2023, it was noted that there was a remarkable variation in the substance of *S. molle* specimens collected from different locations [14]. Therefore, the analysis of the content of *S. molle* grown in our region and indirectly the investigation of its bioactivity are pioneering and make a significant contribution to the literature. While studies evaluating the antioxidant and antimicrobial effectiveness of the plant exist, our results in this research indicate that the content varies due to the reasons mentioned above and affects the activity outcomes. Furthermore, the antibiofilm activity of extracts from the plant has been evaluated for the first time by us. When it comes to the antidiabetic activity, especially its effects on β-TC cells, it shows promising results. From this perspective, it is believed that the plant has potential.

In light of the facts presented above, the aim of the article is to scrutinize the bioactivity and chemical profiles of extracts obtained from the leaves, ripe, and immature fruits of *S. molle*, thanks to its remarkable therapeutic properties. The chemical compositions of the extracts, as well as the presence and quantity of the plant's most common phenolic components, were determined through High-Performance Liquid Chromatography (HPLC). Comprehensive information regarding their biological properties, including antioxidant, antimicrobial, and antidiabetic activities, was examined. The most significant point derived from this data is that this plant, which is also used as a spice, holds promise as a potential source for various medicinal uses.

2. Materials and Methods

2.1 Plant Samples

Schinus molle L. utilized in experimental studies was collected from the Didim (37°20'32.1"N 27°15'39.2" E) district of Aydın province in August 2021. The leaves and ripe and raw fruits were dried in the shade to avoid direct sunlight. The herbarium sample of the plant, collected and diagnosed, is preserved in the Faculty of Pharmacy of Erciyes University (Sİ 175).

2.2 Extraction Procedure

Using alcohol-water mixtures to extract flavonoids and their conjugates from plant materials is a common application, and 70% methanol solution has proven to be an effective solvent in the extraction methods used [15]. For this reason, leaves (50 g), ripe (25 g), and raw (25 g) fruits from the collected plant parts were roughly powdered and extracted by adding a sufficient amount (approximately 500 mL) of 70% methanol. Water extract was prepared using distilled water. It was macerated at 37 °C using a shaking water bath. The same procedures were repeated 3 times for methanol and water extract on the same plant residue. After filtration, the filtrates were combined and the solvents were eliminated in vacuum rotavapor (37–38 °C). Concentrated extracts were lyophilized and kept at –18 °C for use in the experiment.

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Table 3. Precision data of the method.

	Amount (µg/mL)	Caffeic acid	Chlorogenic acid	Hyperoside
Intra-day precision (RSD*%)	5	2.284	3.402	0.490
	50	2.636	0.719	0.458
	200	1.244	1.477	0.836
Inter-day precision (RSD*%)	5	0.598	3.139	0.344
	50	2.609	3.568	0.450
	200	1.241	0.852	0.820

*RSD, Relative Standard Deviation.

Table 4. Recovery assay's statistical data of the method (n =3).

Standards	Concentration in a sample (mg/mL)	Amount spiked (mg/mL)	The mean amount found in the mixture (mg/mL)	Mean recovery (% ± SD*)	RSD** (%)
Caffeic acid	0.0002	0.0004	0.0003	101.629 ± 0.796	0.783
		0.0002	0.0002	97.514 ± 1.409	1.445
		0.0001	0.00015	104 ± 3.17	1.296
Chlorogenic acid	0.016	0.032	0.024	104.345 ± 0.526	0.504
		0.016	0.016	98.127 ± 1.050	1.070
		0.008	0.012	103.565 ± 1.591	1.536
Hyperoside	0.002	0.004	0.003	99.570 ± 0.503	0.505
		0.002	0.002	101.107 ± 0.649	0.642
		0.001	0.0015	100.593 ± 1.244	1.244

*SD, Standard Deviation; **RSD, Relative Standard Deviation.

2.3 Total Phenolic and Total Flavonoid Analysis

To calculate the amount of total phenolic content, distilled water (3.95 mL), Folin-Ciocalteu reagent (250 µL), and 20% Na_2CO_3 (750 µL) were added to the weighed extracts and left at room temperature (25 °C) for 2 hours. Then, measurements were made at 760 nm. Gallic acid was used as the standard [16].

To measure the total amount of flavonoids, 4 mL of distilled water and 0.3 mL of 5% $NaNO_2$ were added to the weighed extracts. After waiting for 5 minutes, 0.3 mL of 10% $AlCl_3$ solution was added and finally, 2 mL of 1 M NaOH was added to complete the total volume to 10 mL. Measurement of the samples was made at 510 nm. Catechin was used as the reference substance [17].

2.4 HPLC Analysis

The sample solution was prepared at a concentration of 4 mg/mL, and for the stock solution, caffeic acid, chlorogenic acid, and hyperoside were prepared at a concentration of 500 µg/mL. HPLC analysis (Agilent 1100 series with diode array detector, New York, USA) was conducted with WatersSpherisorb C18 column (25 cm 4.6 mm, 5 µm). The mobile phase consisted of 0.01% formic acid (A) and acetonitrile (B), provided in a gradient system at a flow rate of 1 mL/min each. The proportion of A was reduced from 90% to 60% over 30 minutes, then returned to the initial conditions in 5 minutes. A wavelength of 270 nm was utilized for the detection of all samples. Three injections of five different standard concentrations were made, and a calibration curve was constructed for each standard for quantification purposes. Method validation included the calculation of accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), recovery values, and other metrics [18,19]. Differences were demonstrated using the relative standard deviation (RSD) to assess the method's accuracy in terms of intra-day and inter-day variance. To determine LOD and LOQ values, ten injections of standards were performed, considering a signal-to-noise ratio of 3:1 for LOD and 10:1 for LOQ. For the recovery analysis, three different known concentrations of the standard were included in the sample, and the recovery percentage was computed. Minor adjustments to the flow rate, column temperature, mobile phase, and wavelength were made for the robustness study, and their effects were examined.

2.5 Antioxidant Activity

2.5.1 DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

The extracts were mixed with a DPPH solution made in methanol (0.1 mM) and Tris-HCl buffer (50 nM, pH 7.4). A reagent mixture devoid of extract and chlorogenic acid was used as a control. The samples' absorption spectra at 517 nm were read after incubating them in the dark at room temperature for 30 minutes. The following equation was used to calculate inhibition % after three parallel runs of the experiment [20].

Table 5. DPPH radical scavenging activity of *S. molle* extracts.

Extract	% Inhibition			
	4 mg/mL	2 mg/mL	1 mg/mL	0.5 mg/mL
S.m L MeOH	80.57 ± 0.1^{a}	80.46 ± 0.06^{a}	80.19 ± 0.21^{a}	77.38 ± 4.27^{b}
S.m L water	$79.20 \pm 0.66^{a,b}$	78.51 ± 0.42^{b}	75.47 ± 2.25^{b}	69.30 ± 2.75
S.m Ripe F MeOH	81.32 ± 0.37^{a}	81.16 ± 0.14^{a}	80.92 ± 0.07^{a}	78.48 ± 3.97^{b}
S.m Ripe F water	74.52 ± 0.30^{c}	72.09 ± 1.98^{c}	37.94 ± 2.82^{f}	21.03 ± 2.74^{h}
S.m Raw F MeOH	$79.72 \pm 0.30^{a,b}$	66.65 ± 0.85^{d}	$40.14 \pm 2.23^{e,f}$	18.54 ± 0.05^{h}
S.m Raw F water	66.04 ± 1.41^{d}	48.24 ± 1.68^{e}	26.83 ± 1.37^{g}	$11.45 \pm 0.7^{h,i}$
Chlorogenic acid	85.11 ± 0.18^{a}	84.74 ± 0.05^{a}	84.28 ± 0.13^{a}	75.20 ± 7.68^{c}

Values expressed as mean ± standard error (n = 3), statistical analyses by Tukey comparison test. Bars with the same lower-case letters (a–i) are not significantly ($p > 0.05$) different. S.m L MeOH, *S. molle* leaf methanol extract.; S.m L water, *S. molle* leaf water extract; S.m Ripe F MeOH, *S. molle* Ripe Fruit methanol extract; S.m Ripe F water, *S. molle* Ripe Fruit water extract; S.m Raw F MeOH, *S. molle* Raw Fruit methanol extract; S.m Raw F water, *S. molle* Raw Fruit water extract.

Table 6. ABTS radical scavenging activity of *S. molle* extracts.

Extract	TEAC mmol/L Trolox			
	4 mg/mL	2 mg/mL	1 mg/mL	0.5 mg/mL
S.m L MeOH	2.58 ± 0.00^{a}	2.58 ± 0.01^{a}	2.58 ± 0.02^{a}	2.57 ± 0.01^{a}
S.m L water	2.56 ± 0.02^{a}	2.53 ± 0.00^{a}	$2.47 \pm 0.02^{a,b}$	2.36 ± 0.1^{b}
S.m Ripe F MeOH	2.59 ± 0.00^{a}	2.59 ± 0.01^{a}	2.58 ± 0.00^{a}	2.58 ± 0.00^{a}
S.m Ripe F water	2.39 ± 0.01^{b}	2.38 ± 0.24^{b}	2.19 ± 0.09^{c}	1.51 ± 0.21^{e}
S.m Raw F MeOH	2.58 ± 0.00^{a}	2.57 ± 0.00^{a}	2.53 ± 0.09^{a}	$1.73 \pm 0.22^{d,e}$
S.m Raw F water	2.41 ± 0.06^{b}	$2.30 \pm 0.14^{b,c}$	2.08 ± 0.22^{c}	1.17 ± 0.16^{f}
Chlorogenic acid	$2.32 \pm 0.08^{b,c}$	2.28 ± 0.16^{c}	1.91 ± 0.11^{d}	0.71 ± 0.03^{g}

Values expressed as mean ±standard error (n = 3), statistical analyses by Tukey comparison test. Bars with the same lowercase letters (a–g) are not significantly ($p > 0.05$) different. S.m L MeOH, *S. molle* leaf methanol extract; S.m L water, *S. molle* leaf water extract; S.m Ripe F MeOH, *S. molle* Ripe Fruit methanol extract; S.m Ripe F water, *S. molle* Ripe Fruit water extract; S.m Raw F MeOH, *S. molle* Raw Fruit methanol extract; S.m Raw F water, *S. molle* Raw Fruit water extract.

2.5.2 ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) Assay

An $ABTS^{+}$ radical (7 mM) was produced by exposing a watery solution of ABTS and $K_2S_2O_8$ (2.45 mM, final concentration) to darkness for 12–16 hours. At 734 nm, its absorbance was changed to 0.700 (0.030). 990 µL of the formulated radical solution and 10 µL of the extract samples were utilized to test and record the reaction kinetics at 734 nm every minute for 30 minutes. As a percentage of inhibition assessed against concentration, Trolox equivalents were derived (TEAC). Chlorogenic acid was utilized as a positive control. Three parallel runs of the experiment were conducted, and mean data were computed [21].

2.5.3 Iron (III) to Iron (II) Reduction Assay

1 mL of each extract was combined with 2.5 milliliters of a 1% (w/v) potassium hexacyanoferrate solution and 2.5 mL of a 0.2 M phosphate buffer (pH 6.6). 2.5 mL of 10% (w/v) trichloroacetic acid (TCA) was added after 30 minutes at 50 °C of incubation, and the mixture was subjected to centrifugation at 1000 g for 10 minutes. Subsequently, the absorbance at 700 nm was determined by mixing 2.5 mL of the upper layer with 2.5 mL of H_2O and 0.5 mL of a 0.1% (w/v) $FeCl_3$ solution. The reductive activities of the extracts were expressed in millimoles of ascorbic acid per gram of the sample (mmol ascorbic acid/g sample) equivalents [22].

2.6 Antimicrobial and Antibiofilm Activity

2.6.1 Minimum İnhibitory Concentrations (MIC) of the Extracts

The determination of the Minimum Inhibition Concentrations (MIC) of the extracts was performed using the broth microdilution assay, following the guidelines outlined in the Clinical Laboratory Standards Institute (CLSI) M100-S28 protocol for bacteria and CLSI M27-A3 protocol for fungi [23,24]. *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231 were utilized. Serial two-fold dilutions of each extract in the concentration range of 1–1024 g/mL were prepared in Mueller Hinton Broth (MHB) for bacteria and

Table 7. Reducing capacities of *S. molle* extracts and standards from iron (III) to iron (II).

	AscAE [mmol/g]
S.m L MeOH	1.283 ± 0.004^{d}
S.m L water	1.542 ± 0.017^{c}
S.m Ripe F MeOH	$1.997 \pm 0.001^{a,b}$
S.m Ripe F water	1.498 ± 0.020^{c}
S.m Raw F MeOH	1.833 ± 0.013^{b}
S.m Raw F water	1.832 ± 0.009^{b}
Chlorogenic acid	2.315 ± 0.001^{a}

Values expressed as mean ± standard error (n = 3), statistical analyses by Tukey comparison test. Bars with the same lower-case letters (a–d) are not significantly ($p > 0.05$) different. S.m L MeOH, *S. molle* leaf methanol extract; S.m L water, *S. molle* leaf water extract; S.m Ripe F MeOH, *S. molle* Ripe Fruit methanol extract; S.m Ripe F water, *S. molle* Ripe Fruit water extract; S.m Raw F MeOH, *S. molle* Raw Fruit methanol extract; S.m Raw F water, *S. molle* Raw Fruit water extract; AscE, Ascorbic Acid Equivalent.

RPMI-1640 for yeast. All extracts were dissolved in a 10% DMSO solution. Reference antimicrobials were also tested against these microorganisms. Concurrently, growth control for microorganisms and sterilization control for the media were conducted. Additionally, 10% DMSO, used as a solvent for the extracts in this test, was assessed for antimicrobial activity. Following incubation for 18–24 hours at 37 °C for bacteria and 48 hours for fungi, the last tube without any microbial growth was identified as the Minimum Inhibition Concentration (MIC) value (in g/mL). The experiment was repeated three times to ensure consistency and accuracy of results.

2.6.2 Minimum Biofilm Inhibition Concentration (MBIC) of the Extracts

To assess the antibiofilm activities of extracts, Nostro *et al*. (2007) [25] method was used. Firstly, 100 µL of microorganism suspensions prepared in Tryptic Soy Broth (TSB) medium involving 1% glucose were added to the 96-well microplate. 100 µL of each extract concentration at the range of 128–512 µg/mL were added into wells. The negative control consisted of the TSB medium, while the positive control consisted of microorganism cultures alone without any additional extract. After 24 hours of culturing for bacteria and 48 hours for yeast, the contents of the microplate were gently emptied. Immediately afterward, every well was washed with 300 µL of sterile Phosphate Buffered Solution (PBS). After waiting for half an hour in the open air, the wells were dyed with 0.1% (w/v) crystal violet for half an hour at room temperature. Washing with 200 µL PBS was repeated 3 times this time. The open-air waiting process was repeated. The crystal violet was subsequently dissolved using 95% (v/v) ethanol, and the optical density (OD) was quantified at 595 nm for *S. aureus* and *E. coli* and at 540 nm for *C. albicans,* employing a Microplate reader from Azure Biosystems. MBIC was set as the concentration of the extract at which the OD was less than or equal to that of the negative control [26]. The experiment was repeated 3 times.

2.7 Antidiabetic Activity

2.7.1 α-Glucosidase and α-Amylase Inhibition

The procedure outlined by Liu *et al.* [27] was followed. 200 µL of the extract or acarbose, along with 50 µL of a 2 U/mL α-glucosidase solution, were mixed with 1000 µL of phosphate buffer and incubated at 37 °C for 10 minutes. Afterward, 50 µL of 5 mM p-nitrophenyl-D-glucopyranoside (pNPG) was added, and the mixture was incubated at 37 °C for an additional 20 minutes. The reaction was halted by adding 2000 µL of 0.2 M sodium carbonate and 4700 mL of distilled water to the mixture, and the absorbances were measured at 405 nm.

A modified version of the Sigma-Aldrich method [28] was employed to determine inhibitory effects on the α-amylase enzyme. In a test tube, different volumes of 200 µL of α-amylase enzyme solution (EC 3.2.1.1, type VI, Sigma; 20 units/mL), 160 µL of 20 mM phosphate buffer (pH 6.9, containing 6.7 mM sodium chloride), and 40 µL of extract or acarbose were combined. Subsequently, 400 µL of starch solution (0.5% w/v) was introduced as a substrate, and the mixture was incubated at 25 °C for an additional 3 minutes after an initial 5-minute incubation. Following the incubation period, 200 µL of the dinitro salicylic acid reagent (composed of 96 mM 3,5-dinitro salicylic acid and 5.31 M sodium potassium tartrate in 2 M NaOH) was added to each tube. The tubes were then placed in an 85 °C water bath and maintained at this temperature for 15 minutes. After this time elapsed, all tubes were removed from the water bath, and their absorbance was measured at 540 nm, following the addition of 4000 µL of distilled water.

2.7.2 Determination of Glucose and INsulin Levels in Pancreatic β Cells

The β-TC cells (CRL-3237™) were sourced from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cell line was validated by STR (short tandem repeat) profiling and tested negative for mycoplasma. Cells cultured in a medium comprised of Dulbecco's Modified Eagle's Medium (DMEM Biochrom, Berlin, Germany) with 25 mM glucose, 10% fetal bovine serum, 1% gentamicin, and L-glutamine at 37 °C in a 5% CO_2 atmosphere. To assess toxicity in the β-TC cell line, the extracts were studied at concentrations ranging from 3.9 to 250 µg/mL using the MTT assay [29]. Based on the viability results, it was determined that there was no significant decrease in cell viability at a concentration of 62.5 µg/mL compared to the control, with a viability rate of 93.72%.

To evaluate the efficacy of the extracts on diabetes, pancreatic β-TC cells were seeded with 2.5×10^5 cells per

Table 8. Antimicrobial activity of *S. molle* extracts.

Extracts	Bacteria		Yeast
	S. aureus	*E. coli*	*C. albicans*
	ATCC 29213	ATCC 25922	ATCC 10231
S.m L MeOH	256 µg/mL^{c}	128 µg/mL*	128 µg/mL$^{\text{ı}}$
S.m L water	256 µg/mL^{c}	128 µg/mL*	128 µg/mL$^{\text{ı}}$
S.m Ripe F MeOH	256 µg/mL^{c}	128 µg/mL*	128 µg/mL$^{\text{ı}}$
S.m Ripe F water	256 µg/mL^{c}	128 µg/mL*	256 µg/mL$^{\text{ıı}}$
S.m Raw F MeOH	128 µg/mL^{b}	128 µg/mL*	128 µg/mL$^{\text{ı}}$
S.m Raw F water	256 µg/mL^{c}	128 µg/mL*	256 µg/mL$^{\text{ıı}}$
Ampicilin	2 µg/mL^{a}	16 µg/mL**	-
Gentamycin	1 µg/mL^{a}	1 µg/mL***	-
Vancomycin	1 µg/mL^{a}	-	-
Fluconazole	-	-	1 µg/mL$^{\text{ıı}}$

Values presented as mean ± standard error (n = 3), statistical analyses by Tukey comparison test. Bars with the same lower-case letters (a–c), superscripts (*–***), and symbols (ı–ıı) are not significantly ($p > 0.05$) different. S.m L MeOH, *S. molle* leaf methanol extract; S.m L water, *S. molle* leaf water extract; S.m Ripe F MeOH, *S. molle* Ripe Fruit methanol extract; S.m Ripe F water, *S. molle* Ripe Fruit water extract; S.m Raw F MeOH, *S. molle* Raw Fruit methanol extract; S.m Raw F water, *S. molle* Raw Fruit water extract.

well in 6-well plates and incubated for 24 hours. Afterward, the wells were divided into two groups and the study was carried out. The first group was the control group, while the second group was the diabetic group. No interventions were administered to the control group and only the initial glucose and insulin determinations were made. In the second group, cells were stimulated with Krebs-Ringer Bicarbonate (KRB) solution containing 25 mM glucose (119 mM NaCl, 4.7 mM KCl, 2.54 mM $CaCl_2$, 1.19 mM $MgSO_4$, 1.19 mM KH_2PO_4, 25 mM $NaHCO_3$). In the second group, those with a glucose level of >250 mg/dL were regarded as diabetic [30]. The dose to be applied to the extracts was determined as 2. 62.5 µg/mL to pancreatic β-TC cells, and glucose and insulin were determined from the samples taken before and after 24 hours of incubation (37 °C, 5% CO_2 incubator). The "Glucose Liquicolor" glucose kit and Mouse Insulin ELISA kit were used [31].

2.8 Statistical Analysis

For variance homogeneity, the Levene test was used. Several groups were examined with one-way ANOVA (Analysis of Variance). For pairwise comparison tests, the Dunnett and Tukey tests were used at the $p < 0.05$ level.

3. Results and Discussions

3.1 Total Phenol and Total Flavonoid Contents

The phenol and flavonoid composition of the plant was determined by spectrophotometric methods as stated in the material method section. The extract with the highest total phenolic content was identified as the S.m L MeOH extract, with a value of 231.32 ± 11.10 $mg_{GAE}/g_{extract}$. The extracts for which the lowest amount of phenolic substance was calculated were S.m Ripe F water and S.m Raw F water extracts (59.54 ± 2.53 $mg_{GAE}/g_{extract}$ and 63.48 ± 1.57 $mg_{GAE}/g_{extract)}$. The extracts with the highest total flavonoid content were calculated as S.m L MeOH, and S.m L water, respectively (Table 1).

In a study conducted in Tunisia, the highest total phenol and flavonoid contents of methanol extracts of *S. molle* fruits collected from two different localities were found to be 29.01 ± 1.18 mg GAE/g DW and 20.10 ± 0.85 mg QE/g DW, respectively [32]. Accordingly, it can be said that more phenolic content was detected in the species collected from Turkey. In addition, the locality where the plant is collected, climate, and several conditions have important effects on plant physiology. However, differences in experimental conditions and extraction methods may cause different results in terms of plant content.

3.2 HPLC Profiling of Extracts

HPLC analyses of the extracts were made for some phenolic compounds and results of analytical measurements are shown in Tables 1,2,3,4. Qualitative and quantitative analyses of phenolics such as chlorogenic acid, caffeic acid, coumaric acid, ferulic acid, rutin, hyperoside, and rosmarinic acid were performed. All extracts were analyzed and it was observed that chlorogenic acid was identified in all extracts. Caffeic acid was found only in S.m L Water, and hyperoside was detected in S.m L water together with S.m L MeOH. The highest quantity of chlorogenic acid was calculated in *Schinus molle* ripe fruit methanol extract (S.m Ripe F MeOH = 2.040% ± 0.172%, Fig. 1).

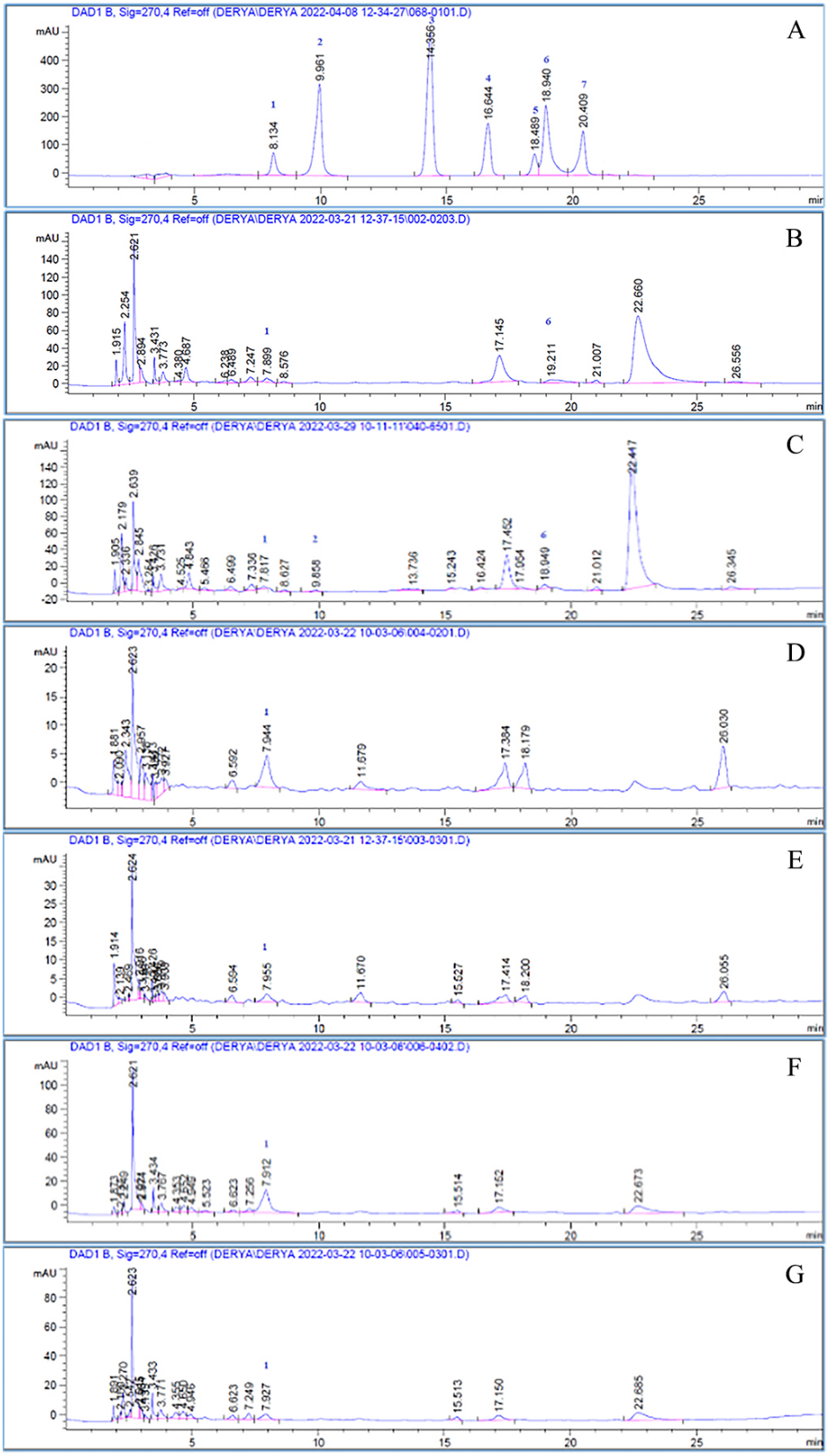

Fig. 1. High-Performance Liquid Chromatography (HPLC) chromatograms. A. Standards: (1) Chlorogenic acid, (2) Caffeic acid,(3) Coumaric acid, (4) Ferulic acid, (5) Rutin, (6) Hyperoside, and (7) Rosmarinic acid. B. Leaf methanol extract. C. Leaf water extract. D. Raw fruit methanol extracts. E. Raw fruit water extract. F. Ripe fruit methanol extracts. G. Ripe fruit water extracts.

Table 9. Antidiabetic activity of *S. molle* extracts.

	α-glucosidase	α-amylase		β-TC cell line	
	1 mg/mL	1 mg/mL		Glucose amount	Insulin amount
	Inhbition % $\pm$ SE*	Inhbition % $\pm$ SE*		(mg/dL)	(mg/mL)
S.m L MeOH	94.74 ± 5.61^{a}	n.d	S.m L MeOH	$144 \pm 3.52^{c,d}$	25.4 ± 0.2^{c}
S.m L water	89.64 ± 2.46^{b}	10.36 ± 2.93^{b}	S.m L water	147 ± 3.07^{d}	24.9 ± 0.5^{c}
S.m Ripe F MeOH	98.64 ± 1.00^{a}	19.04 ± 11.63^{b}	S.m Ripe F MeOH	128 ± 2.77^{b}	$33.7 \pm 0.3^{b,c}$
S.m Ripe F water	50.32 ± 0.74^{c}	34.07 ± 9.82^{c}	S.m Ripe F water	$133 \pm 2.66^{b,c}$	26.2 ± 0.17^{c}
S.m Raw F MeOH	99.11 ± 1.61^{a}	n.d	S.m Raw F MeOH	129 ± 2.03^{b}	37.2 ± 0.02^{b}
S.m Raw F water	n.d	n.d	S.m Raw F water	140 ± 10.09^{c}	21.9 ± 0.04^{d}
Acarbose	88.60 ± 0.64^{b}	78.4 ± 3.67^{a} (0.1 mg/mL)	Control	108 ± 8.03^{a}	50 ± 0.21^{a}

The values are exhibited as the mean $\pm$ standard error (SE, n = 3), and statistical comparisons were performed using the Tukey comparison test. Bars labeled with the same lowercase letters (a–d) do not exhibit significant differences ($p > 0.05$). S.m L MeOH, *S. molle* leaf methanol extract; S.m L water, *S. molle* leaf water extract; S.m Ripe F MeOH, *S. molle* Ripe Fruit methanol extract; S.m Ripe F water, *S. molle* Ripe Fruit water extract; S.m Raw F MeOH, *S. molle* Raw Fruit methanol extract; S.m Raw F water, *S. molle* Raw Fruit water extract.

A chemical examination of *S. molle* fruit was performed in research conducted in Tunisia, and when compared to the phenolic chemicals discovered in our study, the rutin compound was detected in fruits obtained from Tunisia, but not in fruits collected from Turkey [32].

3.3 Antioxidant Activity

The antioxidant properties of *S. molle* extracts were evaluated in terms of DPPH and ABTS radical scavenging activities and reducing power capacity, and the results are given in Tables 5,6,7. All extracts were studied at four different concentrations and chlorogenic acid was used as the standard. It was determined that the extracts with the highest DPPH radical scavenging capacity were S.m L MeOH and S.m Ripe F MeOH at 1mg/mL concentration. These extracts had an activity that was statistically equivalent to chlorogenic acid, according to the evaluation. When the ABTS radical scavenging activities were examined, it was confirmed that S.m L MeOH and S.m Ripe F MeOH extracts had the highest activity at 1 mg/mL concentration, and it was observed that they showed higher activity than chlorogenic acid at the same concentration. Only S.m. Ripe F MeOH from the extracts demonstrated comparable action with chlorogenic acid when the reduction power capabilities of Iron III to Iron II were assessed.S.m Raw F water extract had the lowest antioxidant capacity results in all antioxidant activity tests studied.

Most of the studies investigating the antioxidant properties of *S. molle* have used essential oils from various plant parts. The essential oils of *S. molle* grown in different countries were evaluated using different antioxidant activity determination methods [9,33,34]. The aqueous extract prepared from the woody branches of *S. molle* grown in Egypt demonstrated a DPPH radical scavenging action, with an IC_{50} value of 13.11 $\pm$ 3.00 mg/mL [35]. The antioxidant activity of the extracts of *S. areira* prepared with hexane, dichloromethane, ethyl acetate, methanol, and water were determined using the DPPH method, and the highest activity was noted in the methanol extract (EC_{50}: 476.560 µg/mL) [36]. The IC_{50} value was calculated as 0.14 mg/mL in ABTS radical scavenging effect experiments in methanol extracts of ripe and fresh fruits from S. molle fruits grown in Tunisia. The ability of this extract to scavenge ABTS radicals was studied at concentrations between 0.05 and 0.3 mg/mL [37]. In our study, the scavenging effect of ABTS radical at a concentration of 0.5 mg/mL was calculated as equivalent to Trolox and showed high activity with a TEAC mmol/L Trolox value of 2.58 $\pm$ 0.00.

Antioxidant capacities of water and methanol extracts of leaves, ripe and raw fruits of the plant were evaluated for the first time in this study according to DPPH, ABTS, and reducing power test.

3.4 Antimicrobial and Antibiofilm Activity

The extracts were tested for their *in vitro* antimicrobial activities against Gram-positive (*Staphylococcus aureus* ATCC 29213) Gram negative (*Escherichia coli* ATCC 25922) bacteria and yeast (*Candida albicans* ATCC 10231) by using broth microdilution assay. Ampicillin, gentamycin, and vancomycin for antibacterial and fluconazole for antifungal activity were used in this assay as reference antimicrobial agents. The MIC values determined for each extract and reference antimicrobial agent as a result of the experiment were presented in Table 8. 10% DMSO had no antimicrobial activity in this test.

According to the result of the antimicrobial assay, Minimum Inhibitory Concentrations (MICs) ranged from 128 to 512 µg/mL against all microorganisms in this study. Remarkably, the antimicrobial activity of the extracts on Gram-negative bacteria is relatively better. Therefore, it is observed that the antibacterial effects of the extracts are better than the antifungal effects. While the MIC values of the extracts in this study did not attain the levels of the reference antimicrobials, it's worth noting a moderate antibacterial effect.

In a study in which they investigated the antibacterial activity of *S. molle* L. ethanol extract on *S. mutans* ATCC 25175 strain, they concluded that some concentrations of the extract showed an antibacterial effect as good as chlorhexidine [38]. Another study, it was found that the MIC of leaf extracts of *S. molle* L. on *S. aureus* ATCC 25922 and *C. albicans* ATCC 10231 strains in the range of 16–400 µg/mL and 25–50 µg/mL, respectively [39].

The antifungal activity of the extracts of *S. molle* fruits grown in Syria prepared with hexane and petroleum ether was evaluated and it was found to be effective against *Botrytis cinerea* at a dose of 1000 ppm [40]. Aqueous extracts prepared from the woody branches of *S. molle* showed a notable antimicrobial effect against *Staphylococcus aureus* and *Escherichia coli*, which were also tested in our study. However, moderate activity was observed against the same strains in leaf, ripe, and raw water extracts [35].

The antibacterial and antifungal effects of the extracts of *S. lentiscifolius* leaves prepared with different solvents were tested and it was reported that the antifungal effects (MIC values 25, and 15.5l µg/mL) of the hexane extract were especially high. In our study, the MIC value of *S. molle* against *C. albicans* was calculated as 128 µg/mL, except for the water extracts of raw and ripe fruits [41].

It was stated that 70% methanol extract of *S. terebinthifolius* bark showed various effects on different strains of *Staphylococcus aureus*, while it showed a strong antibacterial effect against *Staphylococcus aureus* 6538, no effect was found against *Staphylococcus aureus* 25925. The identical extract did not demonstrate efficacy against *E. coli* 10536 [42].

In a study on *S. molle* L., the MIC of volatile oils extracted from its leaves and fruits on *S. aureus* ATCC 29213 strain were found to be 125 and 500 µg/mL, sequentially. In the same research, the MIC of *S. molle L.* leaf and fruit essential oils on *Escherichia coli* LFG03 strain were determined to be 1000 and 1000 µg/mL, respectively [9].

In the literature, the number of studies investigating the antimicrobial activity of extracts and some other products of *S. molle* L. is limited, and the findings obtained in these studies are briefly mentioned. Considering the findings obtained in our study and these literature data, it seems that *S. molle* L. is a plant that should be focused on in pharmacy and medicine in terms of its antimicrobial activity.

Surprisingly, we discovered that the extracts had no antibiofilm activity when tested. It was observed that the plant, which is also used in traditional treatment due to its antimicrobial activity, did not have strong effects in our study. In the literature, it has been found that the aqueous alcohol extract of *S. terebinthifolius* has a remarkable antiadhesion activity against the biofilms produced by *Streptococcus mutans* UA159 and *Candida albicans* ATCC10231 [43].

3.5 Antidiabetic Activity

The antidiabetic impact was determined using α-glucosidase and α-amylase enzyme inhibition tests. Also, by comparing glucose and insulin levels after the administration of the extracts in the β-TC cell line, which was turned diabetic by glucose induction, antidiabetic activity was evaluated. Table 8 shows the results.

In the α-glucosidase enzyme inhibition experiment in which acarbose was used as a standard, S.m L MeOH, S.m Ripe F MeOH, and S.m Raw F MeOH extracts were found to be significantly active with statistical significance. While no activity was monitored in the water extracts of the raw fruits, the % inhibition was calculated as 50.32% $\pm$ 0.74% in the mature fruit juice extract at 1 mg/mL concentration. Leaf water extract significantly inhibited α-glucosidase with an inhibition value of 89.64 $\pm$ 2.46% compared to other water extracts. When the α-amylase inhibition activity of the extracts was evaluated, % inhibition in S.m L water S.m Ripe F MeOH, and S.m Ripe F water extracts were found to be very weak with the values of 10.36 $\pm$ 2.93, 19.04 $\pm$ 11.63 and 34.07 $\pm$ 9.82, respectively. No effect was detected in S.m L MeOH, S.m Raw F MeOH, and S.m Raw F water extracts. In research testing the biological activities of polysaccharide-rich extracts of the fruits of *S. molle,* the enzyme inhibition potentials of α-amylase, and α-glucosidase were assessed. Accordingly, the α-amylase inhibition IC50 value of the extract was determined as 0.16 mg/mL, while the α-glucosidase inhibition IC50 value was determined as 0.17 mg/mL [44]. The IC50 value was calculated as 0.19 mg/mL for α-amylase and 0.18 mg/mL for α-glucosidase in a study investigating the antidiabetic efficiency of methanol extracts of *S. molle* fruits. While our study found substantial discrepancies in the percentage values of α-glucosidase and α-amylase enzyme inhibition, other studies that evaluated the antidiabetic effects of the plant's fruit extracts using two separate enzyme inhibition assays reported numerically equivalent IC50 values [37].

The main function of pancreatic β-TC cells is the release of adequate bioactive insulin to maintain the physiological ratio of glucose in plasma in response to nutrients, hormones, and neural stimulation for optimal function of all tissues. The antidiabetic activity of the extracts was evaluated after the glucose-induced diabetes model of β-TC cells was established. Compared to the control, the glucose amount was 129 $\pm$ 2.03 mg/dL and the insulin amount was 37.2 $\pm$ 0.02 mg/dL in the diabetic β-TC cells of the S.m Raw F MeOH extract. The water extracts of the plant parts, especially S.m L water, S.m Ripe F water, and S.m Raw F water, showed low antidiabetic activity with high glucose and low insulin amount measurements. When the results were compared with the results of enzyme activity, it was observed that S.m Raw F MeOH, which showed high α-glucosidase activity, also showed antidiabetic activity in β-TC cells (Table 9).

We conducted the first-ever assessment of the impacts of leaf and fruit extracts from the plant on diabetic β-TC cells, making a novel contribution to the literature.

4. Conclusions

Extracts of leaves and raw and ripe fruits of *S. molle* prepared with water and 70% methanol were investigated in terms of antioxidant, antimicrobial, and antidiabetic activities. As a consequence, further research into the use of the plant's fruit and leaves for pharmaceutical purposes and in the food industry may encourage the use of this plant, which can be grown in many countries, as a potential nutraceutical. However, additional research is necessary to fully understand the plant's secondary metabolites and its pharmacological characteristics and biological actions. The results obtained from this study can be considered preliminary data necessary for the elaboration of the study. This intriguing strategy may compel scientists to thoroughly examine the possibilities of these natural products for the development of secure, environmentally responsible, and powerful medicines.

Availability of Data and Materials

The data utilized and/or examined in the present study can be obtained from the corresponding author upon a reasonable request.

Author Contributions

GŞK, EKA, and Sİ designed the research study. Sİ, DÇP, EKŞ, HÖU, and Uİ performed the research. EKA, ÇY and GŞK provided help and advice on analysis. GŞK, EKŞ, Uİ and ÇY analyzed the data. GŞK and Sİ wrote the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work. All authors contributed to editorial changes in the manuscript.

Ethics Approval and Consent to Participate

Schinus molle plant was used in this study. The plant was collected from Didim, Aydın Turkey by Gökçe Şeker Karatoprak (Erciyes University Faculty of Pharmacy, Kayseri, Turkey) and brought to our research laboratory.

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Conflict of Interest

The authors declare no conflict of interest. Esra Küpeli Akkol was one of the Guest editors of this journal. We declare that Esra Küpeli Akkol had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Daniela Rigano.

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