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Variations in Quantitative Composition of Phenolic Compounds in Flowers, Leaves, and Fruits of *Mespilus germanica* L. During Harvesting Period

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Abstract

The medlar (*Mespilus germanica*) is a deciduous tree that belongs to the Rosaceae family. This plant has been valued throughout history for its culinary uses and medicinal applications. Medlar fruit contains a high amount of vitamin C, carbohydrates, and pectin, making it a valuable remedy for treating scurvy. In folk medicine, medlar is used to treat constipation and other digestive issues. This study investigates the quantitative composition and seasonal variation in phenolic compounds in flowers, leaves, and fruits of *Mespilus germanica* L. using high performance liquid chromatography, as well as total phenolic content and antioxidant activity. The predominant class of phenolics in flowers were flavonols, with 52.5% of the total phenolic content. On the other hand, dominant phenolics in fruits were flavan-3-ols, with procyanidin B2 and epicatechin being most abundant, whereas in leaves, hydroxycinnamic acids were the most prevalent phenolic compounds. Seasonal variations were noted for analyzed compounds in various plant parts. This study highlights significant changes in the phenolic profile of *M. germanica* during various harvesting periods and suggests that both fruits and leaves are rich sources of bioactive compounds. However, its nutraceutical potential might depend on harvesting time.

Keywords: *Mespilus germanica*; chemical composition; phenolic compounds; harvesting time

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1. Introduction

Phenolic compounds represent one of the most important groups of secondary metabolites in plants, exhibiting a broad range of biological activities, including antioxidant, antimicrobial, anti-inflammatory, and antimutagenic properties, as well as neuroprotective and cardioprotective effects. Due to their diverse bioactivities, these plant-derived compounds have demonstrated significant health-promoting potential and are considered key contributors in the prevention and management of various chronic conditions, such as cardiovascular diseases, cancer, diabetes, neurodegenerative disorders, depression, arthritis, infections, obesity, and metabolic syndromes [1–4]. Consequently, phenolic compounds have become the focus of extensive research in phytochemistry, cosmetics, and food science.

A comprehensive understanding of their composition and mechanisms of action within plant tissues is essential for optimizing their application across various industries [3,4].

Mespilus germanica L., commonly known as medlar in English, mušmula in Serbian, muşmula in Turkish, azgil tree in Persian, and Deutsche Mispel or simply Mispel in German, is a deciduous tree belonging to the Rosaceae family. It is naturally distributed across non-tropical regions, including the Balkans, the Black Sea area, Iran, and Turkey. The species grows naturally in the wild but is also widely cultivated, and flowers during May and June. The fruit of *M. germanica* is initially hard and green to reddish-brown. Due to their high tannin content, the fruits are astringent and not suitable for consumption when fresh. They become edible through a natural softening process known as bletting, which occurs either after exposure to frost or during storage under cool, dry, and dark conditions. As they ripen, the fruits turn brown, soften in texture, and develop a flavor reminiscent of stewed apples or fruit puree [4]. Medlar fruits are recognized for their commercial value as a nutritious food source for humans. In traditional medicine, various parts of the medlar—including its fruits, leaves, wood, and bark—have been used to treat a wide range of ailments affecting the digestive tract, kidneys, intestines, and metabolic disorders such as diabetes. Medlar is traditionally regarded as effective in alleviating conditions like diarrhea, cough, rheumatism, hemorrhoids, asthma, constipation, kidney and bladder stones, hepatitis, stomach bloating, and menstrual irregularities [4–6].

The fruit of *M. germanica* L. is rich in sugar, organic acids, amino acids, carotene, pectin, carbohydrates, minerals such as potassium, calcium, phosphorus, magnesium, and iron, vitamin C, polyphenols, and flavonoids [7]. There is limited data and significant uncertainties in the literature regarding the distribution and concentration of phenolic compounds, as well as the quantitative changes in phenolic compounds in their different organic parts, and their changes during the ripening phase and the harvesting period [4–6]. Generally, the composition and distribution of phenolic compounds are primarily influenced by genetic factors, but also vary depending on the stage of maturation, seasonal conditions, and the physicochemical properties of the soil [8]. The fruits and leaves of *M. germanica* are a great source of polyphenols, including flavonoids, coumarins, and phenolics [7,9,10]. The predominant phenolic constituents of medlar fruits include benzoic acids—such as gallic acid, protocatechuic acid, p-aminobenzoic acid, salicylic acid, 3,4-dihydroxybenzoic acid, and syringic acid—and cinnamic acids, including caffeic acid, p-coumaric acid, neochlorogenic acid, chlorogenic acid, and ferulic acid [8,11]. In addition, several flavonoids have been identified, such as epicatechin gallate, rutin, quercetin, quercetin-3-O-α-L-rhamnopyranoside, and luteolin [9,10,12]. Among the phenolic acids detected, chlorogenic acid was found to be the most abundant in multiple studies [4,9,13]. The ripening process is accompanied by complex biochemical transformations that affect both the concentration and composition of various bioactive compounds. A decrease in free phenolic acids has been observed during ripening, while ester-linked phenols tend to increase during intermediate stages of fruit maturity [14].

This study aimed to conduct a quantitative analysis of individual phenolic compounds in the flowers, leaves, and fruits of *M. germanica* grown in Serbia, and to evaluate their variation during the harvest period. Total phenolic content and antioxidant activity of medlar flower, leaves and fruits extracts were also analyzed. The findings contribute to the existing knowledge on phenolic profiles in fruit species and offer new insights into the phytochemical potential of medlar.

2. Materials and Methods

2.1. Chemical and Reagents

Formic acid, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu's phenol re-agent, iron(III)chloride, TPTZ, and acetonitrile were purchased by Merck (Darmstadt, Germany). The following standards were used for quantification of phenolic compunds: p-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, quercetin, quercetin 3-O-glucoside (isoquercetin), quercetin 3-O-rutinoside (rutin), kaempferol 3-O-glucoside, (-)-epicatechin, hyperoside, luteolin, apigenin, cyanidin 3-O-glucoside, procyanidins B1 and B2 (Sigma Aldrich, Steinheim, Germany). 6-Hydroxy- -2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Acros Organics (Morris Plains, NJ, USA). Solutions were prepared using deionized water (specific conductivity 0.05 μS/cm) which was produced using MicroMed high purity water systems (TKA Wasseraufbereitungsysteme GmbH, Niederelbert, Germany).

2.2. Plant Material

Medlar (*Mespilus germanica* L.) leaves, flowers, and fruits were collected in Bela Palanka, Serbia. Samples were collected from May to October 2015 at about one-month intervals. Samples were collected at flowering stage, 23 days after flowering (DAF), 56 DAF, 96 DAF, 121 DAF, 162 DAF. Sampling dates were selected to cover key phenological and biochemical transitions of *Mespilus germanica*: full bloom (0 DAF), early fruit set (23 DAF), early fruit growth (56 DAF), mid development (96 DAF), late development/early ripening (121 DAF), and advanced ripening/bletting onset (162 DAF). Samples development in various period was as follows:

- 0 DAF (flowering; 19 May)—Full bloom: petals fully expanded, many open flowers per tree. No fruit yet. Leaves fully expanded and green.
- 23 DAF (11 June)—Fruit set/early development: petals mostly fallen; young fruitlets visible, small and green; leaves fully expanded and green.
- 56 DAF (14 July)—Early fruit development: fruit still green, skin smooth and firm; leaves green.
- 96 DAF (23 August)—Mid fruit development: fruit increasing in size; skin still predominantly green to pale green-yellow; pulp firm and pale; leaves remain green.
- 121 DAF (17 September)—Late development/early ripening: skin color begins to change (light brownish or yellow-brown in some fruits), pulp still mostly pale and firm—fruits are approaching consumption maturity for some cultivars but still firm. Leaves still present, mostly green.
- 162 DAF (28 October)—Advanced ripening, skin brown to brownish-orange, pulp softening and browning. Leaves still present (yellowing to reddish-brown),and starting to fall down.

Three trees were chosen randomly for the uniformity of samples, regarding tree development, health, and light exposure influence. After the harvest, fruits were frozen and stored at −18 °C until analysis, while leaves and flowers were air dried and homogenized.

2.3. Extraction

Plant material was extracted using maceration in combination with ultrasound-assisted extraction. Medlar fruits, flowers, and leaves (0.2 g each) were mixed with 15 mL of 80% acetone and kept at room temperature for 48 h. Then, the extract was filtered, while residual plant material was subjected to ultrasound-assisted extraction in ultrasound bath (Bandelin SONOREX Digital 10 P, Berlin, Germany) at room temperature for 15 min with 10 mL of 80% acetone, five times. The liquid after each ultrasound-assisted extraction step was collected and pooled together with the liquid obtained from maceration. Collected

extracts were evaporated using rotary evaporator (IKA RV 05 basic, Werke, Germany), and dissolved to final volume of 50 mL with 80% acetone.

2.4. High-Performance Liquid Chromatography (HPLC)

Qualitative analysis of individual phenolics was performed with the HPLC, employing a direct injection method. The analysis was performed using the HPLC system (Agilent 1200 series, Agilent Technology, Santa Clara, CA, USA). Chromatographic analyses were performed using 150 mm × 4.6 mm i.d., Zorbax Eclipse XDB C18 column (Agilent Technologies, Santa Clara, CA, USA). The column was thermostatted at 30 °C. The flow rate was 0.8 mL/min and the injection volume was 5 µL. The mobile phases were A: H_2O-HCOOH (95:5, v/v) and B: ACN-HCOOH-H_2O (80:5:15, $v/v/v$). The gradient procedure was 0–10 min with 0% B, 10–28 min gradually increases 0–25% B, from 28 to 30 min 25% B, from 30 to 35 min gradually increases 25–50% B, from 35 to 40 min gradually increases 50–80% B, and finally for the last 5 min gradually decreases 80–0% B. Eluate was monitored at 280 nm, 320 nm, 360 nm, and 520 nm with DAD, for compounds identification of flavones, flavonols, flavonol glycosides hydroxycinnamic acids, and anthocyanins. On the other hand, (-)-epicatechin, procyanidin B, procyanidin B5, and procyanidin B2, were monitored using FLD at 275/322 nm ($\lambda_{Ex}/\lambda_{Em}$). All chemicals and solvents were of analytical or HPLC purity. All analyses were performed in triplicate. Results were expressed on dry weight base for flower and leaves samples, whereas for fruit samples, all results were expressed on fresh weight base.

The phenolic compounds in the samples were identified by comparing their retention times and spectra with the retention times and spectra of the authentic standards for each component. The calibration curve, determination coefficient (R^2), limit of detection (LOD), limit of quantification (LOQ), recovery, and matrix effect values are shown in Table 1. The concentrations of the constituents in the samples were calculated using the equation obtained from the calibration charts prepared for each standard, while the quantification of constituents without the corresponding standard was performed based on a calibration chart prepared for structurally similar standards.

Table 1. Analytical parameters for phenolic compounds used for HPLC-DAD analysis.

Compound	Calibration Curve	(R^2)	LOD [1] (µg/mL)	LOQ [2] (µg/mL)	Recovery (%)	Matrix Effect (%)
p-Hydroxybenzoic acid	$y = 9934.4x + 0.1$	0.9998	0.003	0.010	102 ± 1	−3.3
Caffeic acid	$y = 32{,}241.5x - 0.1$	0.9998	0.035	0.116	89.7 ± 0.8	1.55
Vanilic acid	$y = 10{,}781.0x - 0.5$	0.9995	0.003	0.010	93.1 ± 0.6	3.12
Chlorogenic acid	$y = 10{,}491.8x - 0.1$	0.9998	0.035	0.116	101 ± 1	2.65
Syringic acid	$y = 11{,}253.6x + 0.5$	0.9999	0.011	0.037	99 ± 0.9	−3.23
p-Coumaric acid	$y = 16{,}239.7x - 0.8$	0.9997	0.042	0.140	97.6 ± 0.5	−1.65
Ferulic acid	$y = 24{,}685.7x - 0.7$	0.9998	0.029	0.097	104 ± 1	2.53
Sinapic acid	$y = 13{,}332.5x + 1.1$	0.9998	0.032	0.106	98.4 ± 0.3	2.33
Rutin	$y = 4589.0x - 0.7$	0.9999	0.052	0.173	102.1 ± 0.8	4.21
Quercetin	$y = 10{,}336.2x + 0.3$	0.9996	0.033	0.110	96 ± 1	2.43
Kampferol	$y = 13{,}636.5x + 0.1$	0.9997	0.029	0.097	98.6 ± 0.9	−3.21
Luteolin	$y = 15{,}958.6x - 0.2$	0.9998	0.030	0.100	100 ± 1	1.45
Apigenin	$y = 14{,}797.5x - 0.9$	0.9999	0.055	0.183	94.4 ± 0.9	−1.76
B2	$y = 8105.0x + 0.6$	0.9998	0.032	0.106	100.3 ± 0.3	−2.96
Catechin	$y = 4600.1x + 0.3$	0.9998	0.011	0.037	97.9 ± 0.5	4.01
Epicatechin	$y = 4780.1x - 0.5$	0.9999	0.011	0.037	99.3 ± 0.5	−3.10

[1] LOD—limit of detection; [2] LOQ—limit of quantification.

2.5. Antioxidant Activity and Total Phenolic Content

Antioxidant activity and total phenolic content were performed by methods described by Nikolic et al. [15] All analysis were performed using UV/Vis spectrophotometer Agilent 8453 (Agilent Technologies, Santa Clara, CA, USA).

Total phenolic content was performed using Folin–Cicoalteu assay and the results were expressed as mg gallic acid equivalents per 1 g of dry weight (mg GAE/g dw) for flower and leaves samples and 1 g of fresh weight (mg GAE/g fw) for fruit samples.

DPPH method was performed by mixing plant extracts with DDPH radical solution and results were expressed as μmol of Trolox equivalents (TE) per 1 g of dry weight (mg TE/g dw) for flower and leaves samples and 1 g of fresh weight (mg TE/g fw) for fruit samples.

FRAP method was performed by mixing plant extracts with FRAP reagent and results were expressed as μmol of Trolox equivalents (TE) per 1 g of dry weight (mg TE/g dw) for flower and leaves samples and 1 g of fresh weight (mg TE/g fw) for fruit samples.

2.6. Statistical Analysis

All measurements were conducted in triplicate and results were reported as mean with the standard deviation (mean $\pm$ SD). Statistical analysis was carried out in Statistica 8.0 software (StatSoft, Tulsa, OK, USA). Analysis of variance with Tukey's multiple comparison test ($p < 0.05$) was performed to compare the mean values of the results.

3. Results

3.1. Quantitative Composition of the Phenolic Compounds in the Flowers and Fruits of M. germanica L. During the Harvest Period

The results of the quantitative composition of phenolic compounds revealed the presence of 24 compounds in the fruit samples of M. germanica. All compounds were classified as individual phenolic acids, flavonoids, and procyanidins. The content of phenolic compounds in the flowers and fruits of M. germanica, as well as the changes in the percentage distribution of phenolic compound classes during the harvest time, are presented in Table 2. The corresponding results obtained for the leaves of M. germanica are shown in Table 3. The predominant class of phenolic compounds in the flowers were flavonols (5.72 mg/g dry weight), representing 52.5% of the total phenolic content. Within this class, quercetin glycosides constituted 40%, while kaempferol glycosides made up 54.4%. Flavan-3-ols were the major class of phenolic compounds found in fruit samples. Their content during the harvest period ranged from 2.41 to 4.35 mg/g fresh weight, corresponding to 37.48–56.19% of the total quantified phenolic compounds. Procyanidin B2 and epicatechin were identified as the most abundant flavan-3-ols. Hydroxybenzoic acids were also significantly represented (1.96–3.36 mg/g fresh weight corresponding to 35.1–44.8% of total phenolics), while hydroxycinnamic acids were less prominent. When considered together as total phenolic acids (hydroxybenzoic and hydroxycinnamic acids), their combined contribution ranged from 43% to 52% over the harvesting period, making them—along with flavan-3-ols—the key contributors to the phenolic profile of the M. germanica fruits.

Table 2. Content of individual phenolic acids, flavonoids, and procyanidins in the flowers (mg/g dry weight) and fruits (mg/g fresh weight) of *Mespilus germanica* L. during the harvest period.

Phenolic Compounds	MFlo	MF 23 DAF	MF 56 DAF	MF 96 DAF	MF 121 DAF	MF 162 DAF
Caffeic acid	0.570 ± 0.002 ^{b}	0.620 ± 0.008 ^{a}	0.230 ± 0.001 ^{c}	0.110 ± 0.001 ^{d}	0.120 ± 0.001 ^{d}	0.090 ± 0.001 ^{d}
Chlorogenic acid	1.730 ± 0.009 ^{a}	1.65 ± 0.02 ^{b}	-	-	-	-
4-Caffeoylquinic acid	-	-	0.440 ± 0.004 ^{a}	0.380 ± 0.003 ^{b}	0.340 ± 0.003 ^{b}	0.280 ± 0.002 ^{c}
p-Coumaric acid	-	0.080 ± 0.001 ^{a}	0.030 ± 0.001 ^{c,d}	0.030 ± 0.001 ^{c,d}	0.050 ± 0.001 ^{b}	0.040 ± 0.002 ^{c}
Sinapic acid	0.290 ± 0.002 ^{a}	0.210 ± 0.002 ^{b}	0.150 ± 0.001 ^{c}	0.081 ± 0.003 ^{d}	0.050 ± 0.001 ^{e}	0.030 ± 0.001 ^{f}
Ferulic acid	0.290 ± 0.002 ^{a}	0.160 ± 0.001 ^{b}	0.050 ± 0.001 ^{c}	0.050 ± 0.001 ^{c}	0.040 ± 0.001 ^{c}	0.040 ± 0.001 ^{c}
4-Hydroxybenzoic acid	-	-	1.45 ± 0.01 ^{c}	1.55 ± 0.01 ^{c}	1.77 ± 0.02 ^{b}	2.68 ± 0.03 ^{a}
Vanillic acid	-	-	0.270 ± 0.002 ^{d}	0.320 ± 0.002 ^{c}	0.560 ± 0.006 ^{a}	0.470 ± 0.003 ^{b}
Syringic acid	-	-	0.240 ± 0.001 ^{b}	0.140 ± 0.002 ^{c}	0.390 ± 0.002 ^{a}	0.210 ± 0.001 ^{b}
∑Hydroxycinnamic acid	2.88 ± 0.010 ^{a}	2.72 ± 0.01 ^{a}	0.900 ± 0.005 ^{b}	0.650 ± 0.001 ^{c}	0.601 ± 0.003 ^{c}	0.480 ± 0.001 ^{d}
w(Hydroxycinnamic acid)	26.42	42.30	16.22	11.68	7.77	6.41
∑Hydroxybenzoic acid	-	-	1.96 ± 0.01 ^{c}	2.01 ± 0.01 ^{c}	2.72 ± 0.02 ^{b}	3.36 ± 0.02 ^{a}
w(Hydroxybenzoic acid)	-	-	35.31	36.08	35.13	44.80
Rutin	0.090 ± 0.001 ^{b}	0.090 ± 0.001 ^{b}	0.120 ± 0.001 ^{a}	0.140 ± 0.001 ^{a}	0.060 ± 0.003 ^{c}	0.050 ± 0.001 ^{c}
Hyperoside	0.060 ± 0.001 ^{a}	0.050 ± 0.003 ^{b}	-	-	-	-
Isoquercetin	0.780 ± 0.001 ^{a}	0.140 ± 0.001 ^{b}	-	-	-	-
Quercetin-3-rhamnoside	0.930 ± 0.005 ^{a}	0.160 ± 0.001 ^{b}	-	-	-	-
Quercetin-3-pentoside	0.320 ± 0.002 ^{a}	0.170 ± 0.001 ^{b}	-	-	-	-
Kaempferol-3-rutinoside	3.11 ± 0.03 ^{a}	0.160 ± 0.002 ^{b}	-	-	-	-
Quercetin-glucoside	0.120 ± 0.002 ^{a}	0.120 ± 0.001 ^{a}	-	-	-	
Quercetin	0.180 ± 0.001 ^{a}	0.080 ± 0.001 ^{b}	0.020 ± 0.001 ^{c}	0.020 ± 0.001 ^{c}	0.010 ± 0.001 ^{d}	0.010 ± 0.001 ^{d}
Kaempferol	0.130 ± 0.001 ^{b}	0.170 ± 0.002 ^{a}	-	-	-	
∑Flavonols and Flavonol Glycosides	5.72 ± 0.013 ^{a}	1.140 ± 0.001 ^{b}	0.140 ± 0.001 ^{c}	0.160 ± 0.001 ^{c}	0.070 ± 0.003 ^{d}	0.060 ± 0.003 ^{d}
w (Flavonols)	52.47	17.73	2.52	2.87	0.90	0.80
∑Quercetin glycosides	2.300 ± 0.007 ^{a}	0.730 ± 0.001 ^{b}	0.120 ± 0.001 ^{a}	0.140 ± 0.001 ^{a}	0.060 ± 0.003 ^{d}	0.050 ± 0.004 ^{d}
∑Kaempferol glycosides	3.11 ± 0.03 ^{a}	0.160 ± 0.001 ^{b}	-	-	-	-
Luteolin glycoside	0.80 ± 0.01 ^{a}	0.0600 ± 0.0001	-	-	-	-
Apigenin glycoside	0.450 ± 0.006 ^{a}	0.100 ± 0.001	-	-	-	-
∑ Flavones	1.25 ± 0.02 ^{a}	0.1600 ± 0.0004	-	-	-	-
w (Flavones)	11.47	2.49	-	-	-	-
Procyanidin B2	0.270 ± 0.002 ^{f}	0.610 ± 0.008 ^{e}	0.970 ± 0.014 ^{d}	1.28 ± 0.01 ^{c}	2.11 ± 0.02 ^{a}	1.81 ± 0.02 ^{b}
Procyanidin B5	-	0.450 ± 0.003 ^{b}	0.530 ± 0.001 ^{a}	0.160 ± 0.001 ^{d}	0.210 ± 0.002 ^{c}	0.520 ± 0.004 ^{a}
Epicatechin	0.780 ± 0.006 ^{d}	1.35 ± 0.01 ^{b}	0.810 ± 0.006 ^{d}	0.970 ± 0.008 ^{c}	1.93 ± 0.02 ^{a}	1.210 ± 0.004 ^{b}
Catechin	-	-	0.240 ± 0.002 ^{b}	0.340 ± 0.003 ^{a}	0.100 ± 0.001 ^{c}	0.060 ± 0.0005 ^{d}
∑ Flavan-3-ols	1.050 ± 0.004 ^{d}	2.41 ± 0.001 ^{c}	2.55 ± 0.01 ^{c}	2.75 ± 0.01 ^{c}	4.350 ± 0.003 ^{a}	3.60 ± 0.02 ^{b}
w (Flavan-3-ols)	9.63	37.48	45.95	49.37	56.19	48.00
∑ Individual phenolics	10.90 ± 0.004 ^{a}	6.43 ± 0.01 ^{c}	5.55 ± 0.01 ^{d}	5.57 ± 0.01 ^{d}	7.74 ± 0.02 ^{b}	7.50 ± 0.03 ^{b}

MFlo—Medlar flowers collected on 19 May; MF—Medlar fruits; (DAF)—days after flowering; values followed by different letters are significantly different ($p < 0.05$).

Table 3. Content of individual phenolic acids, flavonoids, and procyanidins in the leaves (mg/g dry weight.) of *Mespilus germanica* L. during the harvest period.

Phenolic Compounds	ML_1	ML 23 DAF	ML 56 DAF	ML 96 DAF	ML 121 DAF
Neochlorogenic acid	0.470 ± 0.004 ^{a}	0.210 ± 0.002 ^{b}	0.130 ± 0.001 ^{c}	0.100 ± 0.001 ^{d}	-
Caffeic acid	0.450 ± 0.005 ^{c}	0.570 ± 0.007 ^{a}	0.370 ± 0.002 ^{d}	0.510 ± 0.006 ^{b}	0.310 ± 0.003 ^{e}
4-Caffeoylquinic acid	3.16 ± 0.02 ^{b}	3.85 ± 0.03 ^{a}	2.32 ± 0.02 ^{c}	1.93 ± 0.02 ^{d}	1.43 ± 0.02 ^{e}
Feruloylquinic acid	0.330 ± 0.002 ^{e}	0.420 ± 0.002 ^{d}	0.570 ± 0.004 ^{b}	0.520 ± 0.004 ^{c}	0.680 ± 0.006 ^{a}
Sinapic acid	0.400 ± 0.002 ^{b}	0.594 ± 0.006 ^{a}	0.400 ± 0.003 ^{b}	0.391 ± 0.007 ^{b}	-
Ferulic acid	27.96 ± 0.24 ^{a}	21.38 ± 0.16 ^{c}	23.17 ± 0.08 ^{b}	20.45 ± 0.14 ^{c}	18.18 ± 0.17 ^{d}
∑Hydroxycinnamic acid	32.77 ± 0.24 ^{a}	27.03 ± 0.13 ^{b}	26.96 ± 0.07 ^{b}	23.90 ± 0.13 ^{c}	20.60 ± 0.19 ^{c}
w (Hydroxycinnamic acid)	64.52	54.67	55.14	50.19	50.15
Rutin	0.390 ± 0.005 ^{c}	2.34 ± 0.02 ^{a,b}	2.61 ± 0.02 ^{a}	2.62 ± 0.02 ^{a}	2.06 ± 0.02 ^{b}
Hyperoside	0.430 ± 0.003 ^{d}	0.480 ± 0.004 ^{d}	1.261 ± 0.005 ^{c}	1.789 ± 0.006 ^{b}	2.16 ± 0.01 ^{a}
Isoquercetin	0.150 ± 0.001 ^{a}	0.110 ± 0.001 ^{b}	0.100 ± 0.001 ^{b}	0.120 ± 0.001 ^{b}	0.110 ± 0.001 ^{b}
Quercetin-3-pentoside	3.60 ±0.03 ^{b}	3.59 ± 0.03 ^{b}	3.41 ± 0.02 ^{b}	4.27 ± 0.03 ^{a}	2.37 ± 0.01 ^{c}
Kaempferol-3-rutinoside	3.69 ± 0.01 ^{a}	3.37 ± 0.03 ^{a}	2.68 ± 0.02 ^{b}	3.49 ± 0.01 ^{a}	2.70 ± 0.02 ^{b}
Quercetin-3-rhamnoside	0.460 ± 0.003 ^{a}	0.400 ± 0.004 ^{b}	0.470 ± 0.002 ^{a}	0.420 ± 0.002 ^{b}	0.400 ± 0.002 ^{b}
Kaempferol-glucoside	0.530 ± 0.007 ^{b}	0.340 ± 0.004 ^{a}	0.360 ± 0.003 ^{a}	0.540 ± 0.003 ^{b}	1.27 ± 0.01 ^{a}
Quercetin	0.530 ± 0.006 ^{a}	0.280 ± 0.003 ^{c}	0.350 ± 0.002 ^{b}	0.270 ± 0.002 ^{c}	0.270 ± 0.002 ^{c}
Kaempferol	0.090 ± 0.0005 ^{c}	0.040 ± 0.0001 ^{d}	0.119 ± 0.0001 ^{b}	0.102 ± 0.002 ^{b,c}	0.360 ± 0.003 ^{a}
∑Flavonols	9.87 ± 0.01 ^{d}	10.95 ± 0.02 ^{c,d}	11.36 ± 0.004 ^{b,c}	13.62 ± 0.02 ^{a}	11.70 ± 0.03 ^{b}
w (Flavonols)	19.43	22.10	23.26	28.60	28.48
∑Quercetin glycosides	5.03 ± 0,04 ^{d}	6.92 ± 0.01 ^{c}	7.85 ± 0.04 ^{b}	9.22 ± 0.02 ^{a}	7.10 ± 0.03 ^{c}
∑Kaempferol glycosides	4.22 ± 0.02 ^{a}	3.71 ± 0.03 ^{c}	3.04 ± 0.02 ^{d}	4.03 ± 0.01 ^{a,b}	3.97 ± 0.03 ^{b,c}
Luteolin glycoside	0.320 ± 0.001 ^{e}	0.360 ± 0.004 ^{d}	0.390 ± 0.003 ^{c}	0.520 ± 0.003 ^{a}	0.420 ± 0.004 ^{b}
Apigenin glycoside	0.0406 ± 0.0006 ^{e}	0.120 ± 0.001 ^{d}	0.230 ± 0.001 ^{c}	0.350 ± 0.002 ^{b}	0.440 ± 0.002 ^{a}
∑ Flavones	0.3606 ± 0.0001 ^{d}	0.480 ± 0.003 ^{c}	0.620 ± 0.004 ^{b}	0.870 ± 0.001 ^{a}	0.860 ± 0.002 ^{a}
w (Flavones)	0.71	0.97	1.27	1.83	2.09
PC	0.420 ± 0.006 ^{b}	0.520 ± 0.004 ^{a}	0.390 ± 0.002 ^{c}	0.380 ± 0.001 ^{c}	0.410 ± 0.003 ^{b}
Procyanidin B2	2.36 ± 0.01 ^{c}	3.09 ± 0.01 ^{a}	2.64 ± 0.02 ^{b}	2.25 ± 0.01 ^{c}	1.76 ± 0.01 ^{d}
Epicatechin	3.67 ± 0.04 ^{c}	4.88 ± 0.03 ^{a}	4.38 ± 0.04 ^{b}	4.40 ± 0.04 ^{b}	3.89 ± 0.02 ^{c}
Procyanidin B5	1.06 ± 0.01 ^{d}	1.790 ± 0.003 ^{b}	2.10 ± 0.02 ^{a}	1.84 ± 0.01 ^{b}	1.580 ± 0.002 ^{c}
Catechin	0.281 ± 0.002 ^{d}	0.790 ± 0.008 ^{a}	0.440 ± 0.001 ^{b}	0.360 ± 0.002 ^{c}	0.280 ± 0.002 ^{d}
∑ Flavan-3-ols	7.79 ± 0.05 ^{d}	11.07 ± 0.03 ^{a}	9.95 ± 0.04 ^{b}	9.23 ± 0.03 ^{c}	7.92 ± 0.01 ^{d}
w (Flavan-3-ols)	15.34	22.35	20.35	19.38	19.28
∑ Individual phenolics	50.80 ± 0.26 ^{a}	49.53 ± 0.14 ^{a,b}	48.89 ± 0.11 ^{b}	47.62 ± 0.09 ^{c}	41.08 ± 0.12 ^{d}

ML_1—Medlar leaves collected on 19 May; ML—Medlar leaves; values followed by different letters are significantly different ($p < 0.05$).

Phenolic Profile Changes in Fruits During the Harvest Period

Hydroxybenzoic acids, together with flavan-3-ols, represented the most abundant phenolic compounds in the fruits of *Mespilus germanica* L. throughout the entire ripening period. The content of hydroxybenzoic acids (ranging from 1.96 to 3.36 mg/g) showed a continuous increase over the sampling period, rising from 35.5% to 44.8% of the total phenolic content. 4-Hydroxybenzoic acid was the predominant compound within this class, with concentrations increasing from 1.45 to 2.68 mg/g.

Flavan-3-ol content in fruit samples increased over the sampling period from 2.41 mg/g to reach its peak at 4.35 mg/g on day 121 after flowering, followed by a decrease

observed at 162 days after flowering. Epicatechin and procyanidin B2 were the predominant flavan-3-ols, exhibiting variation patterns similar to those of the total phenolic compounds.

3.2. Quantitative Composition of the Phenolic Compounds in Leaves of M. germanica L. During the Harvest Period

The results of the quantitative composition of phenolic compounds revealed the presence of 22 compounds in *M. germanica* leaves. The analysis confirmed that hydroxycinnamic acids constitute the predominant class of phenolic compounds. During different leaf maturity stages, the content of hydroxycinnamic acids ranged from 32.77 to 20.60 mg/g dry weight, representing 64.5% to 50% of the total individual phenolic compounds throughout the sampling period. Flavonols and flavan-3-ols were present in approximately similar concentrations. Depending on the harvest time, the flavonol content varied from 9.87 to 13.62 mg/g (19.4–28.5% of the total phenolic content), while the flavan-3-ols content ranged from 7.79 to 11.07 mg/g (15.3–22.4% of the total phenolic content) over the maturation period.

Phenolic Profile Changes in Leaves During the Harvest Period

Both the total hydroxycinnamic acid content and the concentration of ferulic acid, the dominant compound within this class, showed a declining trend during fruit ripening. Despite this decrease, ferulic acid remained the major constituent throughout the sampling period, accounting for over 80% of the total hydroxycinnamic acids and more than 45% of the overall phenolic content.

The contents of flavonols and flavan-3-ols remained within similar ranges throughout the leaf senescence. In medlar leaves, flavonols were predominantly present in the form of flavonol glycosides, primarily quercetin and kaempferol derivatives. The total content of flavonol glycosides increased during the sampling period (ranging from 9.87 to 13.62 mg/g, corresponding to 19.4–28.6% of total phenolics), reaching the highest level at the later stage of development (96 days after flowering). While quercetin glycosides showed an increasing trend, the content of kaempferol glycosides declined as maturation progressed.

The content of flavan-3-ols increased by approximately 42% at 23 days after flowering, followed by a gradual decline during the subsequent stages. At the final developmental stage (ML 121 days after flowering), flavan-3-ol levels were nearly equal to those recorded at the initial stage. A similar variation trend was observed for the dominant flavan-3-ols: procyanidin B2, procyanidin B5, and epicatechin. According to Table 3, procyanidin B2 and epicatechin reached their highest level at 23 days after flowering, with a 30% and 36% increase, respectively. In both cases, the concentration gradually declined toward the final sampling point. In contrast, procyanidin B5 exhibited a delayed maximum at 96 DAF, nearly doubling its initial concentration (98% increase), followed by a steady decrease thereafter.

The sum of individual phenolic contents during the sampling period ranged from 5.55 to 7.74 mg/g in fruits and from 41.08 to 50.8 mg/g in leaves. Since hydroxycinnamic acids were the predominant class of phenolic compounds in *M. germanica* L. leaves, changes in total phenolic content closely reflect the trends observed for this subclass. Hydroxycinnamic acids content gradually decreased during the sampling period. Total flavonols and flavan-3-ols were present in nearly equal amounts during harvest time. The highest concentration of flavonols was recorded 96 days after flowering, while flavan-3-ols peaked at 23 days after flowering, followed by a slight decrease in their levels until the final sampling stage. At the final stage of leaf development, their levels remain slightly higher than those recorded at the beginning of the maturation period. The content of flavan-3-ols, as a major class of phenolic compounds found in the fruit samples, increased and reached its maximum at 121 days after flowering, followed by a slight decrease at the final stage of sampling.

3.3. Antioxidant Activity and Total Ohenolic Content

The content of total phenols determined, as well as the antioxidant activities determined by DPPH and FRP methods, of the acetone extracts of the flowers, fruits, and leaves of the *M. germanica* L. are presented in Table 4.

Table 4. Total phenolic content and antioxidant activity of *M. Germanica* flower, leaves, and fruit at various harvesting times.

Sample	TPC (mg GAE g^{-1})	DPPH (μmol TE g^{-1})	FRAP (μmol TE g^{-1})
MFlo	74.59 ± 0.39 ^{a}	527.62 ± 6.60 ^{a}	416.11 ± 3.90 ^{a}
ML1	109.26 ± 0.94 ^{b}	687.79 ± 19.02 ^{b}	563.44 ± 30.53 ^{b}
ML 23DAF	120.72 ± 0.85 ^{c}	790.67 ± 10.21 ^{c}	701.34 ± 7.81 ^{c}
ML 56DAF	122.03 ± 0.37 ^{c}	762.35 ± 16.74 ^{c}	777.02 ± 3.43 ^{d}
ML4 96DAF	123.93 ± 0.57 ^{c}	816.53 ± 4.83 ^{d}	797.34 ± 4.67 ^{d}
ML5 121DAF	144.45 ± 1.79 ^{d}	918.91 ± 3.18 ^{e}	883.31 ± 5.68 ^{e}
MF 23DAF	10.27 ± 0.09 ^{a}	17.95 ± 0.09 ^{a}	69.97 ± 2.29 ^{a}
MF 56DAF	12.23 ± 0.09 ^{b}	19.84 ± 0.09 ^{a}	85.18 ± 1.05 ^{b}
MF 96DAF	17.71 ± 0.15 ^{c}	30.90 ± 0.09 ^{b}	106.39 ± 2.53 ^{c}
MF 121DAF	41.63 ± 2.46 ^{d}	34.13 ± 0.08 ^{b}	304.32 ± 0.64 ^{d}
MF 162DAF	32.92 ± 0.68 ^{e}	35.21 ± 0.46 ^{b}	556.82 ± 6.60 ^{e}

Values followed by different letters are significantly different ($p < 0.05$).

The total phenolic content in fruits and leaves during the harvest season ranged from 10.27 to 41.63 mg g^{-1} and 109.26–144.45 mg g^{-1}, respectively. The antioxidant activity of fruits and leaves, determined by the DPPH and FRAP methods, also increased during the whole harvesting season, along with the increase in the total phenolic content.

4. Discussion

The results of the quantitative composition of phenolic compounds revealed the presence of 24 compounds in the fruit extract of *M. germanica*, and 22 compounds in the leaves' acetone extract obtained by ultrasound-assisted extraction. Phenolic compounds are a diverse group of non-nutrient plant metabolites with important roles in tissue development, plant defense, and the sensory qualities of food. They are of particular interest due to their strong antioxidant properties and potential protective effects against oxidative stress, inflammation, cancer, and cardiovascular diseases [14]. In addition, phenolic compounds play a significant role in determining fruit quality and nutritional properties by altering sensory characteristics such as color, taste, aroma, and flavor [9].

Most of the previously published studies have predominantly focused on the phenolic profile of medlar fruit, since it is the edible part of the plant. The phenolic composition of medlar fruit presented in this study is similar to that reported in previous studies [4,10,16–18]. The findings of this study identified flavan-3-ols as the predominant phenolic compounds in the fruit samples, comprising 37.48–56.19% of the total phenolic content, with procyanidin B2 and epicatechin being the most abundant representatives. A similar result was reported by Mikulić-Petkovšek et al., who found that flavan-3-ols accounted for 40% of the total phenolic content in medlar fruit [18]. The epicatechin content observed in our samples (0.81–1.93 mg/g of dry extract) differs from previously published data, being substantially higher than the levels reported in Serbian samples (7.29–25.31 μg/g fresh weight), yet lower than the concentrations found in Polish samples

(0.42–7.87 mg/g of dry extract) [13,17]. The observed discrepancy may be attributed to differences in sample preparation (fresh vs. dried material), extraction methods, or cultivar and environmental factors influencing phenolic biosynthesis [19].

The second most abundant flavan-3-ol identified in our samples was procyanidin B2, with concentrations ranging from 0.61 to 2.11 mg/g fresh weight. These values are lower compared to those reported in the literature, where procyanidin B2 content ranged from 2.93 to 3.52 mg/g in dry weight samples [13]. In this study, the observed trend in flavan-3-ol content indicated dynamic changes occurring during fruit maturation. The initial increase from 2.41 mg/g to a peak of 4.35 mg/g at 121 days after flowering may reflect active biosynthesis of flavan-3-ols during the early to mid-ripening stages, likely associated with their roles in defense and antioxidant protection. The subsequent decrease in later stages (at 162 days after flowering) is consistent with the literature reports and may be attributed to metabolic conversion, polymerization into proanthocyanidins, or degradation as the fruit approaches full ripeness. Similar declining patterns have been observed in other fruits; for instance, flavan-3-ols such as epicatechin and catechin decrease significantly as fruit matures in blueberries and raspberries [20,21]. Literature indicated that regular consumption of flavan-3-ol-rich foods contributes to various health benefits, including protection and restoration of gut barrier integrity, as well as support for cardiometabolic health [22,23]. Based on a review of human clinical data, the expert panel of the Academy of Nutrition and Dietetics developed intake guidelines and concluded that there is moderate evidence supporting the cardiometabolic benefits of consuming 400–600 mg of flavan-3-ols per day [22].

While previous studies on fruits of plants belonging to the Rosaceae family reported hydroxycinnamic acids (especially chlorogenic and neo-chlorogenic acid) as dominant, our results showed an opposite trend, with hydroxybenzoic acids being more abundant and hydroxycinnamic acids less prominent [23]. During the fruit ripening period, a continuous increase in hydroxybenzoic acid content was observed, while the concentration of hydroxycinnamic acids gradually decreased until the final sampling point. This decline in hydroxycinnamic acids is consistent with the findings of Gruz et al., who also reported a reduction in these compounds during the ripening of medlar fruit [14].

The available literature on the phenolic composition of medlar flowers and leaves is scarce. To the best of our knowledge, only one study has investigated the total phenolic and flavonoid content of *M. germanica* flowers, whereas the phenolic composition of the leaves has been examined in three published studies [6,24,25]. The authors of these studies reported that medlar flower bud and leaf extracts contain significantly greater amounts of total phenolics and flavonoids than the fruit extract [6,24,25]. A comprehensive quantification of the phenolic compounds of medlar flowers is reported in this study for the first time. Our results demonstrated that flavonols represented the dominant class of phenolic compounds in the flower samples, accounting for over 50% of the total phenolic content. Quercetin glycosides and kaempferol glycosides were recognized as the most abundant flavonols (Table 2).

Yunusa et al. [24] identified epicatechin, epigallocatechin, protocatechuic, caffeic, ferulic, and sinapic acids in various leaf medlar extracts, which is consistent with the findings of our study (Table 3). Our analysis confirmed that hydroxycinnamic acids were the dominant phenolic compound class in the leaf samples, comprising up to 64.5% of total phenolics during early stages of maturity. Although their overall content declined over time (from 32.77 mg/g to 20.60 mg/g DW) ferulic acid consistently remained the major compound throughout the sampling period. It is confirmed by Yunusa et al. [24] that ferulic acid was a significant constituent in leaf phenolic profiles; however, direct comparison with

literature data remains limited due to the scarcity of published studies on leaf phenolic profiles in *M. germanica*.

Our findings indicated that throughout the senescence of *M. germanica* leaves, flavonols and flavan-3-ols were found in comparable concentrations. As observed in the phenolic profile of medlar flowers, flavonols in leaves also predominantly existed as glycosides, mainly quercetin and kaempferol derivatives. An increase in total flavonol glycosides was observed during sampling (from 9.87 to 13.62 mg/g dry weight; 19.4–28.6% of total phenolic content), with peak levels at 96 days after flowering. Interestingly, quercetin glycosides showed a rising trend, whereas kaempferol glycosides declined as maturation progressed. These trends align with observations in phenolic composition of other Rosaceae species leaves, where quercetin and kaempferol glycosides were the primary flavonols, and total glycoside content remained or increased throughout the growing season, with kaempferol derivatives decreasing over time [26,27]. These parallels suggest that age- and season-dependent regulation of flavonol glycosides, favoring quercetin derivatives over kaempferol, is a consistent pattern across Rosaceae leaves.

According to the results of this study, an early accumulation of flavan-3-ols was observed in *M. germanica* leaves, with a 42% increase at 23 days after flowering. However, their levels declined progressively during maturation, returning to baseline values by 121 days after flowering. Among individual compounds, procyanidin B2 and epicatechin peaked moderately early (23 DAF), whereas procyanidin B5 showed a delayed maximum at 96 DAF. This temporal pattern mirrors observations in other Rosaceae species, particularly apple (*Malus domestica* Borkh), where flavan-3-ols accumulate early in leaf tissue during development and then decline in later stages. Catechin, epicatechin, and procyanidin B2 were prominent throughout the growing season, but overall flavan-3-ol content typically declines with maturation [28,29]. Such dynamic accumulation is often attributed to active biosynthesis during early developmental stages, mediated by enzymes like leucoanthocyanidin 4-reductases and anthocyanidin reductases, and subsequent metabolic conversion, polymerization, or catabolism of these compounds as tissues mature [30].

Overall, the total individual phenolics in *M. germanica* varied notably between organs and sampling stages. In this study, leaves contained significantly higher total individual phenolics (41.08–50.8 mg/g DW) than fruits (5.55–7.74 mg/g DW), which is consistent with previous reports [6,24,25]. This is largely attributed to the biological role of leaves in plant defense. As the primary site of photosynthesis, leaves are constantly exposed to environmental stressors such as UV radiation, pathogens, and herbivory, which stimulate the accumulation of phenolic compounds acting as protective antioxidants and chemical defense agents [31,32]. In contrast, fruit tissues are more transient and subject to phenolic degradation during maturation. Increased activity of polyphenol oxidase and shifts toward sugar synthesis reduce phenolic levels as ripening proceeds, further explaining the lower and more variable phenolic content observed in fruits compared to leaves [12]. As demonstrated by Voaides et al., the decline in phenolic content during the ripening stages remained unaffected by the choice of extraction solvent, showing a consistent decreasing trend across all tested solvents, including acetone, methanol, 80% ethanol, and water [12].

When comparing the total phenol content in different parts of the plant, it is observed that there is higher total phenol content in the flowers and leaves compared to the fruits, where the fruit (121 DAF) in the ripe stage has the highest total phenol content. Rop et al. [23] monitored the total phenol content of medlar fruit during various periods after flowering. They found that the total phenol content in medlar fruit 134 days after flowering was 1.70 mg g^{-1} of fresh weight. However, after 134 days, the total phenolic content decreases. The decline in total phenol content in medlar fruit after full ripeness, when the fruit enters an overripening phase, may be associated with the activity of polyphe-

nol oxidase [33]. Good antioxidant activity of medlar fruits was already described in previous studies [34].

5. Conclusions

The findings of this study highlight significant changes in the phenolic profile of *M. germanica* throughout different harvest periods and suggest that both fruits and leaves are rich sources of bioactive compounds. Among the identified phenolics, total phenolic acids, flavones and flavan-3-ols, were identified as the primary contributors to the overall phenolic composition. Moreover, leaves were shown to be a richer and more stable source of phenolics compared to fruits. However, the phenolic content of both leaves and fruits was influenced by the stage of maturity. Additionally, this study contributes by providing the first comprehensive quantification of phenolic compounds in medlar flowers.

These findings provide valuable insights for future research focused on the exploitation of *Mespilus germanica* as a functional food and a source of bioactive compounds, and it underscores the necessity of considering both organ type and maturity stage in phenolic evaluations.

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Abbreviations

The following abbreviations are used in this manuscript:

DAF	Days after flowering
FLD	Fluorescence detector

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