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SOLVENT EFFECTS ON MULTI-ELEMENT (ICP-MS) AND PHENOLIC (HPLC-DAD) PROFILES OF MEDLAR (*MESPILUS GERMANICA* L.) FRUIT EXTRACTS

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Abstract

Mespilus germanica L. (medlar) is a fruit-bearing species with notable phytochemical and nutritional value, traditionally used in various therapeutic applications. This study aimed to evaluate the influence of different solvents (ethanol, acetone, and water) on the phenolic and elemental composition of medlar fruit extracts. Phenolic compound profiling was carried out using high-performance liquid chromatography with diode array detection (HPLC-DAD), while the mineral and trace metal content was determined by inductively coupled plasma mass spectrometry (ICP-MS). The results revealed that the acetonic extract exhibited the most diverse phenolic profile, with high concentrations of chlorogenic and caffeic acids, whereas the aqueous extract showed higher levels of essential minerals such as Mg, K, Ca, and Fe. The powder obtained from the dried acetonic extract presented the highest per-mass elemental concentrations, reflecting the concentrating effect of solvent removal. Low levels of Cd and Pb were detected across all samples, highlighting the importance of routine monitoring to ensure consumer safety. Overall, the findings indicate that both the choice of solvent and the physical state of the extract significantly influence the composition of plant-derived preparations, offering promising perspectives for their controlled application in nutraceutical, food, and phytopharmaceutical formulations.

Key words*: flavonoids, HPLC-DAD, ICP-MS, Mespilus germanica, phenolic acids.*

INTRODUCTION

Mespilus germanica L. widely known as medlar, has garnered increasing scientific attention for its phytochemical richness and potential health benefits. Various plant matrices including fruit, leaves, and seeds have been shown to contain substantial reservoirs of bioactive phenolic compounds alongside essential nutrients (Tessa et al., 2021; Popović-Djordjević et al., 2023). Recent comparative analyses of *Mespilus germanica* and the related species *M. canescens* have highlighted their notable antioxidant capacity, largely attributed to phenolic constituents (Sadeghinejad et al., 2022; Nistor et al., 2024).

The phenolic spectrum of *Mespilus* typically encompasses hydroxybenzoic acids (e.g., gallic acid), hydroxycinnamic acids (e.g., chlorogenic, caffeic, ferulic acids), and flavonols (e.g., quercetin, rutin). These profiles are shaped by intrinsic factors such as genotype and ripening stage, with phenolic acids particularly chlorogenic acid emerging as key contributors to the fruit's antioxidant activity (Gruz et al., 2011; Voaides et al., 2021).

For the accurate resolution of such complex phytochemical matrices, reversed-phase high-performance liquid chromatography coupled with diode array detection (RP-HPLC-DAD) remains a method of choice. This approach enables both compound quantification and structural validation based on characteristic UV-Vis absorbance patterns, typically around 280 nm for hydroxybenzoic acids, 320-330 nm for hydroxycinnamic acids, and ~360 nm for flavonols (Zeb, 2015; Ferreyra et al., 2021). The 330 nm wavelength, in particular, offers optimal selectivity for hydroxycinnamates, which are dominant in medlar fruit.

The efficiency of phenolic extraction is heavily influenced by solvent polarity and composition. Literature consistently reports that aqueous-organic mixtures especially ethanol-water or

acetone-water systems (typically 50-70% v/v) yield superior recoveries of total phenolics and mid-polar hydroxycinnamic acids, compared to either neat organic solvents or pure water alone (Kaczorová et al., 2021).

MATERIALS AND METHODS

Plant material and extracts

Extracts from medlar (*Mespilus germanica* L.) fruits were prepared using three solvents (ethanol, acetone, and water), together with a powder obtained from dried acetonic extract.

Qualitative determination of polyphenolcarboxylic acids and flavonoids by High-Performance Liquid Chromatography (HPLC)

The qualitative profiling of polyphenolcarboxylic acids and flavonoids present in the plant extracts was performed using reverse-phase high-performance liquid chromatography (RP-HPLC).

This analytical technique is widely employed for the separation, identification, and quantification of individual compounds within complex liquid mixtures. Its high resolution, sensitivity, and reproducibility make it particularly suitable for the analysis of bioactive molecules in botanical matrices.

The method involved the injection of plant extract samples into the HPLC system, where compounds were separated based on their differential interactions with the stationary phase and the composition of the mobile phase. Detection was achieved at specific wavelengths corresponding to the characteristic absorbance maxima of phenolic acids and flavonoids, allowing for precise identification and qualitative assessment.

Reagents and solvents used

In order to ensure accurate identification and qualitative analysis of polyphenolcarboxylic acids and flavonoids by HPLC, high-purity reagents and analytical-grade standards were employed throughout the experimental procedure.

Table 1 presents the reagents and substances used, along with their specific functions and relevant purity or handling notes.

Table 1. Reagents and substances used in HPLC analysis

Reagent/ Substance	Function	Purity/Notes
Ultrapure water	Solvent/mobile phase component	Ultrapure, suitable for HPLC use
Plant extracts	Sample	Prepared according to validated extraction method
Methanol (HPLC-grade)	Organic solvent in mobile phase	HPLC-grade
Ethanol	Extraction solvent	Analytical grade
Ortho-phosphoric acid	pH adjustment of mobile phase	≥85%, analytical grade
Chlorogenic acid	Reference standard (phenolic acid)	≥98%, analytical standard
Caffeic acid	Reference standard (phenolic acid)	≥98%, analytical standard
Rosmarinic acid	Reference standard (phenolic acid)	≥98%, analytical standard
Trans-ferulic acid	Reference standard (phenolic acid)	≥98%, analytical standard
Quercetin	Reference standard (flavonoid)	≥98%, analytical standard
Rutin	Reference standard (flavonoid)	≥98%, analytical standard

Materials and equipment

All procedures were carried out using calibrated laboratory equipment and certified Class A glassware to ensure analytical accuracy and reproducibility. The working materials included graduated pipettes, volumetric cylinders, and precision Class A glassware, used for solution preparation and sample handling. Sample mass measurements were performed using a high-precision electronic analytical balance (KERN 770), offering reliable microgram-level sensitivity.

Chromatographic analysis was conducted using an Elite LaChrom HPLC system equipped with a DAD (Diode Array Detector) model L-2455, allowing for multi-wavelength detection and compound-specific identification based on UV absorbance profiles. For chromatographic separation, a stainless-steel column packed with octadecylsilane (Inertsil ODS, 250 × 4.6 mm) was employed. The column was maintained at a controlled temperature of 40°C to ensure consistent retention times and optimal separation efficiency.

The mobile phase consisted of two components. Mobile Phase A was prepared by dissolving 1 g/L ortho-phosphoric acid in 1000 mL ultrapure water and adjusting the pH to 2.8. Mobile Phase B consisted of HPLC-grade methanol.

The elution was carried out using a gradient mode, with a starting ratio of 70: 30 (A: B), and a constant flow rate of 1.0 mL/min.
These optimised chromatographic conditions enabled efficient resolution of target polyphenolic and flavonoid compounds, ensuring high method sensitivity and selectivity across the analysed extract samples.

Practical procedure

The standard solution was prepared using a mixture of certified reference substances, specifically: chlorogenic acid, gallic acid, caffeic acid, rosmarinic acid, trans-ferulic acid, quercetin, and rutin. Precisely weighed quantities of each compound were dissolved in 7 mL of absolute ethanol, and the resulting solution was made up to a final volume of 10 mL in a calibrated volumetric flask with the same solvent. This standard mixture was used to establish retention times and UV spectra under the selected chromatographic conditions.
Prior to injection, the HPLC system was equilibrated to ensure baseline stability. Once the system reached equilibrium indicated by a stable, linear baseline, the standard solution was injected into the chromatographic system to record the reference chromatogram.
For the analysis of the plant extract samples, a dilution was prepared by pipetting 1 mL of the extract solution into a 5 mL volumetric flask and bringing to volume with ethanol. Six replicate injections were performed for the standard solution in order to ensure precision and reproducibility, while a single injection was performed for each sample extract. Chromatograms were recorded for all injections, and the resulting peak profiles were used for qualitative comparison and identification of phenolic and flavonoid compounds based on retention time and UV absorption characteristics.

Chromatographic conditions

Separation was performed on an Inertsil ODS3 column (250 × 4.6 mm), suitable for reversed-phase analysis of phenolic compounds and flavonoids. Elution was carried out under gradient conditions with an initial mobile-phase composition of 70: 30 (A: B) to ensure adequate resolution between analytes of similar polarity. Mobile phase A was prepared by dissolving 1 g of orthophosphoric acid in 1000 mL of ultrapure water, followed by pH adjustment to 2.8. Mobile phase B was HPLC-grade methanol. All solutions were filtered through 0.45 µm membranes and degassed prior to use to stabilise the baseline and reduce detector noise.
The column temperature was maintained at 40 ± 1°C to enhance retention robustness and control solvent viscosity. The flow rate was set to 1.0 mL min^{-1}, and the injection volume was 20 µL, providing a balance between sensitivity and band broadening. Detection employed a diode-array detector (DAD) with primary monitoring at 330 nm, appropriate for phenolic species exhibiting maxima in this region. Full UV spectra (200-400 nm) were recorded to assess peak purity and support tentative compound identification. Before injections, the system was equilibrated for 30 minutes at the initial mobile-phase composition until pressure and signal were stable. The total acquisition time per analytical run was 70 minutes, allowing completion of the gradient programme, elution of strongly retained compounds, and re-establishment of initial conditions for re-equilibration between injections. Where runtime optimisation was required, the gradient slope and the terminal isocratic segment were adjusted while keeping temperature, flow rate, and initial composition constant to preserve comparability of results (Table 2).

Table 2. Chromatographic conditions

Parameter	Condition
Chromatographic column	Inertsil ODS3 250 × 4.6 mm
Elution	Gradient
Column temperature	40 ± 1°C
Flow rate	1.0 mL/min
Detection wavelength	330 nm
Injection volume	20 µL
Mobile phase A	1 g/L ortho-phosphoric acid in 1000 mL water, pH adjusted to 2.8
Mobile phase B	Methanol
Mobile phase ratio	A: B = 70: 30
Column equilibration	30 minutes
Acquisition time	70 minutes

Gradient programme

The run begins with the system equilibrated at seventy percent mobile phase A and thirty percent mobile phase B, with a constant flow of

1.0 mL min^{-1}. From 0.0 to 60.0 min a linear gradient decreases A from seventy to thirty percent while B increases from thirty to seventy percent. At 60.1 min the composition is returned in a single step to the initial seventy to thirty ratio. Acquisition continues to 70.0 min to allow re-equilibration at the starting composition, ensuring stable retention for subsequent injections. Mobile phase A is the orthophosphoric acid aqueous solution adjusted to pH 2.8 and mobile phase B is HPLC grade methanol (Table 3).

Table 3. Gradient programme

Time (min)	Mobile phase A (% v/v)	Mobile phase B (% v/v)	Flow rate (mL/min)
0.0	70	30	1.0
60.0	30	70	1.0
60.1	70	30	1.0
70.0	70	30	1.0

Notes: Linear gradient from 70: 30 (A: B) to 30: 70 over 0.0-60.0 min; step back to initial composition at 60.1 min; re-equilibration at 70: 30 until 70.0 min (total run time 70 min).

Table 4 presents the optimised operating conditions employed for the ICP-MS ELAN DRC system in this study. The settings were selected to ensure robust plasma stability, efficient aerosol generation and transport, and reliable ion focusing, thereby maximising sensitivity while maintaining precision and throughput for multi-element determinations in complex botanical matrices. Emphasis was placed on achieving a stable baseline, reproducible signal intensity across a broad mass range, and an extended dynamic range suitable for both major and trace elements.

Table 4. Optimal Operating Conditions for ICP-MS ELAN DRC

No.	Parameter	Selected Values	Optimal
1	RF power for ICP	1250.00 watts	
2	Nebuliser gas flow rate	0.96 L/min	
3	Auxiliary gas flow rate	1.20 L/min	
4	Lens voltage	8.20 (with autolens option)	
5	Integration time	1000 ms	
6	Dwell time	50 ms	
7	Number of sweeps per reading	20	
8	Detector mode	Dual	
9	Number of replicates	3	

Settings optimise ICP-MS performance: 1250 W RF ensures robust ionisation; nebuliser (0.96 L/min) and auxiliary gas (1.20 L/min) stabilise aerosol and plasma; 8.20 V autolens maximises ion transmission; 50 ms dwell with 20 sweeps (~1000 ms) improves statistics; dual detector and triplicate measurements extend linearity and precision, and reduce noise.

Identification and quantification

Compounds were identified by retention time matching with authentic standards (and DAD spectra where applicable). Quantification used external standardization based on peak areas, with results expressed as mg/100 mL of extract:

$$C_i = \frac{ApxCe}{Ae\ x\ 100}$$

where: A_p is the peak area of compound *i* in the sample; A_e the peak area of compound *i* in the standard solution; C_e the concentration of compound *i* in the standard.

Multi-element method for the determination of minerals and trace elements in dried fruit-based plant extracts

The quantification of essential minerals such as sodium, magnesium, phosphorus, potassium, and calcium, along with trace elements including chromium, manganese, iron, copper, and zinc, as well as toxic heavy metals such as arsenic, cadmium, and lead, was performed using a multi-element analytical method. This method was developed and optimised in accordance with the general provisions of the European Pharmacopoeia (F. Eur.) and employs inductively coupled plasma mass spectrometry (ICP-MS) as the detection technique.

Working reagents

The analysis required the use of high-purity reagents, including 60% nitric acid (Ultrapur, Merck), ultrapure water, and a multi-element standard reference solution for calibration (STD3, 10 ppm, Perkin Elmer). System performance was routinely monitored using a daily performance solution at 10 ppb (Perkin Elmer), which served to validate the instrument's accuracy and stability throughout the analysis.

Working materials and equipment

All procedures were carried out using certified Class A glassware to ensure volumetric precision. Sample mineralisation was performed using a closed-vessel microwave digestion system, providing controlled and efficient sample decomposition. Graduated pipettes were employed for accurate volume transfers, and digestion flasks were used to contain the samples during the mineralisation process. Quantitative determinations were carried out using the ELAN DRC-e ICP-MS system, supported by a high-precision analytical balance (Mettler Toledo) for sample and reagent measurement.

Optimal Operating Conditions for ICP-MS ELAN DRC-e

In order to achieve reliable detection and quantification of elemental constituents in the analysed samples, the inductively coupled plasma mass spectrometry (ICP-MS) system (ELAN DRC-e) was operated under carefully optimised conditions. These parameters were selected to ensure instrument stability, sensitivity, and reproducibility across all measurements. Table 5 outlines the key operational settings used throughout the analysis.

Table 5. Optimal operating conditions for ICP-MS ELAN DRC-e

No.	Parameter	Selected Optimal Values
1	RF power for ICP	1250.00 watts
2	Nebuliser gas flow rate	0.96 L/min
3	Auxiliary gas flow rate	1.20 L/min
4	Lens voltage	8.20 (with autolens capability)
5	Integration time	1000 ms
6	Dwell time	50 ms
7	Number of sweeps per reading	20
8	Detector mode	Dual
9	Number of replicates	3

These operating parameters provided consistent and high-quality spectral data, allowing for accurate multi-elemental analysis of the plant-based extracts.

The system's dual detection mode and optimised gas flow rates ensured both sensitivity and precision throughout the analytical process.

Practical procedure: sample preparation for ICP-MS Analysis

For the determination of elemental concentrations using inductively coupled plasma mass spectrometry (ICP-MS), the extract samples must first be converted into a liquid state through controlled mineralisation. This step is essential to ensure complete dissolution of the sample matrix, allowing accurate quantification of both macro- and trace elements.

Sample digestion was carried out using a closed-vessel microwave digestion system, which enables efficient mineralisation under controlled temperature and pressure conditions. This technique minimises the risk of element loss through evaporation and eliminates the possibility of external contamination, ensuring the integrity of the analytical results. The digestion process yielded clear and transparent solutions, suitable for direct ICP-MS analysis.

Precisely weighed portions of the dried extract samples were transferred into digestion vessels and treated with 8 mL of high-purity nitric acid (HNO_3). The acid served both as a mineralising agent and as a matrix modifier to stabilise the analytes during the digestion process.

The digestion programme consisted of a two-step heating protocol, specifically designed to achieve complete decomposition of the organic matrix and full release of the elements into solution. The detailed parameters of the microwave digestion cycle are presented in Table 6.

Table 6. Microwave Digestion Programme

No.	Power (W)	Pre-heating Time (minutes)	Decomposition Time (minutes)	Ventilation (minutes)
1	350	20	30	1
2	550	10	45	3

Post-digestion sample handling and calibration procedure

At the end of the digestion cycle, the mineralised solution referred to as the stock solution is carefully transferred into a 50 mL calibrated volumetric flask and brought to volume with ultrapure water. This solution serves as the basis for all subsequent dilutions and measurements.

The working sample solution was prepared by performing a dilution of 3 mL of the stock solution to 50 mL with ultrapure water, resulting in a final solution compatible with the ICP-MS operating range.

For the purpose of quantitative analysis, calibration curves were constructed using certified multi-element standard solutions. The concentration ranges selected for the calibration standards were tailored to the expected concentration levels of the target elements in the analysed plant extracts:

- For Ca, Mg, Zn, K, P, Cr, Mn, Fe, and Cu, the calibration range was set between 0.001-1.000 µg/mL, corresponding to a concentration interval of 1-1000 ppb, as expressed in the ICP-MS software's internal units.
- For Cd and Pb, a narrower calibration range was applied, from 0.010-0.080 µg/mL, equivalent to 10-80 ppb, reflecting the lower expected concentrations of these toxic heavy metals in the samples.

To ensure accuracy and minimise matrix interferences, isotopes were carefully selected for each element based on their natural abundance and the spectral interference profile of the sample matrix.

The following isotopes were monitored during analysis:

Mg^{42}, P^{31}, K^{39}, Ca^{43}, Cr^{52}, Mn^{55}, Fe^{57}, Cu^{63}, Zn^{66}, Cd^{114}, and Pb^{208}.

The selection of these isotopes ensured optimal sensitivity and specificity, enabling reliable multi-element detection even in the presence of complex plant-based matrices.

RESULTS AND DISCUSSIONS

Qualitative content of polyphenolcarboxylic acids and flavonoids (HPLC)

The qualitative content of polyphenolcarboxylic acids and flavonoids in the analysed extracts was determined by reverse-phase high-performance liquid chromatography (HPLC).

The analysis enabled the identification and quantification of key phenolic acids, including gallic acid, chlorogenic acid, caffeic acid, and trans-ferulic acid. Results are presented in Table 7 for both ethanolic (MP3) and acetonic (MP1) extracts.

Table 7. Qualitative content of polyphenolcarboxylic acids and flavonoids (HPLC)

Ethanolic extract (MP3)			
Compound	Retention time (min)	Peak area	Concentration (mg/100 mL)
Gallic acid	-	-	0.2727
Chlorogenic acid	-	-	0.381
Acetonic extract (MP1)			
Gallic acid	-	-	0.121
Chlorogenic acid	-	-	1.31
Caffeic acid	-	-	0.960
Trans-ferulic acid	-	-	0.0467

In accordance with the scientific literature, the results obtained through HPLC analysis confirm that the nature of the solvent used has a direct impact on both the spectrum and the concentration of extracted polyphenolic compounds. The acetonic extract revealed a richer composition and higher concentrations of phenolic acids, particularly chlorogenic acid, compared with the ethanolic extract, where the concentrations were lower and the number of identified compounds more limited. Similar findings have been reported by Zuorro et al. (2019), who observed higher yields of phenolic antioxidants when acetone-water mixtures were used compared with ethanol-water systems. Furthermore, other studies highlight that acetone, particularly in aqueous mixtures, facilitates more efficient solubilisation of hydroxycinnamic acids and other moderately polar compounds, resulting in a more comprehensive extraction (El Mannoubi et al., 2023).

Previous research has reported that ethanol, although safer and more suitable for pharmaceutical or food applications, is more selective and tends to provide lower yields for certain compounds, but with the advantage of reduced toxicity and greater compatibility in formulations (Shahidi & Yeo, 2018). Thus, the obtained results are consistent with published findings, which emphasise that the choice of solvent influences not only the overall yield but also the qualitative profile of the extracted compounds.

The experimental data therefore confirm the determining role of the solvent in shaping the composition of plant extracts, highlighting that acetone allows a broader recovery of phenolic acids and flavonoids, while ethanol provides a

more restricted profile that may nevertheless be more applicable in areas where safety requirements are stricter. These findings correlate with works in the field, underlining the relevance of the methodology and the validity of the results (Ignat et al., 2011).

Extracts and acetonic powder (MOSMON)

The elemental composition of the analysed samples was determined under the optimised operating conditions of ICP-MS, following the procedures previously described. The study aimed to assess the qualitative and quantitative distribution of both essential minerals and potentially toxic trace elements across different extraction media, namely ethanolic, acetonic, and aqueous extracts, as well as in the acetonic powder. The results, expressed as µg/g of dry sample, are presented in Table 8.

Table 8. Elemental content (µg/g) in different extracts and acetonic powder (MOSMON)

No.	Element	Ethanolic extract	Acetonic extract	Aqueous extract	Acetonic powder
1	Mg	298.27	288.33	386.09	1945.72
2	P	1121.02	1017.04	1342.20	1933.10
3	K	4698.06	3403.08	5683.61	6280.01
4	Ca	404.81	170.45	414.13	1033.27
5	Cr	4.55	3.82	3.74	7.54
6	Mn	1.805	1.29	2.23	6.04
7	Fe	57.80	88.07	112.15	168.63
8	Cu	3.25	2.48	3.41	10.29
9	Zn	62.165	72.73	21.99	109.66
10	Cd	0.10	0.142	0.10	0.53
11	Pb	0.14	0.27	0.22	0.25

The solvent-dependent distribution observed in Table 8 aligns with established extraction principles. The aqueous extract generally displayed higher levels of essential minerals such as Mg, P, K, Ca, Mn and Fe, which is consistent with the higher polarity of water and the ensuing enhanced solubilisation and diffusion of inorganic and highly polar species from plant matrices (Plášková et al., 2023). At the same time, the acetonic extract showed comparatively elevated Zn and Pb, illustrating the well-documented variability of element recoveries with solvent systems of differing polarity and complexation behaviour; such between-solvent differences are frequently reported, with no single solvent universally optimal across analytes and matrices (Plášková et al., 2023; Chatepa et al., 2024). Reports on mineral profiling of plant extracts also show that specific macros such as K can peak in acetone-containing extracts, underscoring that matrix–solvent interactions and processing conditions may favour certain elements in less polar media (Kostić et al., 2024).

Significantly higher concentrations for almost all elements are expected in the acetonic powder for a concentrated matrix in which solvent removal reduces water content and increases analyte mass per unit dry weight. Reviews on plant sample handling emphasise that drying and concentration steps inherently enrich non-volatile constituents, thereby increasing per-mass elemental readouts even when absolute recoveries remain comparable (da Silva et al., 2025). Finally, the overall pattern is coherent with ICP-based element analysis practice, where nitric-acid mineralisation and careful control of preparation steps are central to obtaining reliable, low-bias measurements across matrices that differ in polarity and solids content (Pappas, 2012). Taken together, the present results are consistent with current literature indicating that the choice of solvent and the physical state of the preparation strongly shape measured mineral and trace-element profiles in plant-derived samples.

CONCLUSIONS

The findings of the present study show that solvent choice and physical state exert a decisive influence on the composition of plant-derived preparations. The aqueous extract contained higher levels of essential minerals such as magnesium, phosphorus, potassium, calcium, manganese, and iron, whereas the acetonic extract displayed comparatively elevated zinc and a broader phenolic profile by HPLC, notably chlorogenic and caffeic acids. The acetonic powder exhibited the highest per-mass concentrations for most macro- and microelements, consistent with enrichment following solvent removal and drying. Trace amounts of cadmium and lead were detected at low levels across all preparations, underscoring the need for routine monitoring to ensure compliance with safety limits. Overall, the results indicate that extraction can be strategically designed to prioritise either mineral enrichment or phenolic recovery, while concentrated powder forms offer a valuable reservoir of both classes of constituents. These

matrices can be considered as readily available and promising sources for further development as quality-controlled ingredients in nutraceutical, food, and phytopharmaceutical applications, provided regulatory requirements are met and supported by continued validation of analytical performance and bioactivity assessments.

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