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THE ANTIOXIDANT POTENTIAL OF SOME *Mespilus germanica* L. EXTRACTS

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Abstract

This study aimed to evaluate the antioxidant activity and mineral content of medlar fruit (Mespilus germanica L.*) extracts, using different solvents to identify the most effective extraction method. Extracts were prepared using acetone, ethanol, water, and chloroform. Polyphenol content was analyzed using HPLC, minerals were determined using inductively coupled plasma mass spectrometry (ICP-MS), and antioxidant activity was assessed using chemiluminescence assays to determine IC_{50} values. The acetonic extract (MP1) had the highest polyphenol content and strong antioxidant activity (AA% of 64%). The aqueous extract (MP2) was richest in minerals. The ethanolic extract (MP3) balanced polyphenol content and antioxidant activity (AA% of 51%). The chloroform extract (MP4) showed a pro-oxidant effect (AA% of -151%). Acetone is the best solvent for extracting polyphenols, while water is ideal for mineral extraction. The ethanolic extract provides a balanced option. Chloroform extracts should be used with caution due to potential pro-oxidant effects. These findings help optimize extraction methods for health benefits, with potential applications in food and pharmaceuticals.*

Key words*: antioxidant activity, chemical composition, Mespilus germanica* L.*, polar and non-polar extracts.*

INTRODUCTION

Mespilus germanica L., commonly known as medlar, is a fruit-bearing plant belonging to the *Rosaceae* family. Despite its long history of cultivation, its use has declined, making it an underutilized source of bioactive compounds. Recent studies have highlighted its potential as a significant source of antioxidants, which play a crucial role in preventing oxidative stress-related diseases (Popović-Djordjević et al., 2022; Voaides et al., 2021; Żołnierczyk et al., 2021).

The medlar fruit is rich in various bioactive compounds, including phenolics, flavonoids, and organic acids. These compounds contribute to its antioxidant capacity (Nistor et al., 2024; Yunusa, 2021). The study conducted by Katanić Stanković et al. (2022) have identified key phenolic compounds such as gallic acid, quercetin, and caffeic acid in medlar extracts. The total phenolic content (TPC) and total flavonoid content (TFC) in medlar fruits are comparable to other well-known antioxidant sources like blackthorn and hawthorn (Katanić Stanković et al., 2022).

Antioxidant activity of medlar extracts has been assessed using various *in vitro* assays, including DPPH and ABTS radical scavenging activities (Samarakoon et al., 2016). Using these analyses, it is possible to measure the ability of the extracts to neutralize free radicals, thus indicating their potential to decrease oxidative stress. Medlar extracts have shown significant scavenging activities, although they are often modest compared to synthetic antioxidants like BHT (butylated hydroxytoluene) (Rahimi-Nasrabadi et al., 2013).

The study of Katanić Stanković et al. (2022) reported that the DPPH radical scavenging activity of medlar extract was higher than that of several other natural antioxidants. This activity is attributed to the high concentration of phenolic compounds, which are effective hydrogen donors, stabilizing free radicals (Katanić Stanković et al., 2022). Additionally, the ABTS assay results corroborate these findings, further establishing the potent

antioxidant properties of medlar extracts (Görmez et al., 2024).

The antioxidant properties of *Mespilus germanica* extracts are not just limited to neutralizing free radicals but also extend to various health benefits. These extracts have shown potential antidiabetic and anti-inflammatory effects (Kızıltaş et al., 2021). The α-glucosidase inhibitory activity of medlar extracts suggests their possible use in managing diabetes by slowing down carbohydrate digestion and glucose absorption (Żołnierczyk et al., 2023).

In another study, medlar extracts demonstrated cytotoxic effects against cancer cell lines, indicating their potential in cancer prevention and therapy. The phenolic compounds in medlar are considered to induce apoptosis in cancer cells, thereby inhibiting their proliferation (Görmez et al., 2024).

The high antioxidant potential of *Mespilus germanica* extracts makes them suitable for various applications in the food and pharmaceutical industries (Tessa et al., 2021). They can be used as natural preservatives to extend the shelf life of food products by preventing oxidation. Moreover, their incorporation into functional foods and nutraceuticals can provide health benefits associated with their antioxidant and antidiabetic properties.

MATERIALS AND METHODS

1. Raw material

Medlar fruits were harvested in October 2022 from the Prahova region. Taxonomic identification was conducted by a team of agronomy specialists from the Faculty of Biotechnologies, USAMV of Bucharest. The *M. germanica* L. fruits were washed with distilled water, dried, and frozen at -20°C. Before use, the fruits were brought to room temperature and then ground in a ceramic mortar. The homogenized samples were used for obtaining polar (water, acetone, ethanol solvents) and non-polar (chloroform solvent) extracts.

2. Preparation of extracts

- 100 grams of homogenized medlar fruit sample were extracted three times consecutively with 200 ml of acetone each time. The combined acetone extracts were concentrated under vacuum and redissolved in 100 ml of 40% ethanol (w/v), yielding 100 ml of extract coded MP1;
- 100 grams of homogenized medlar fruit sample were extracted with 500 ml of distilled water using ultrasound bath extraction for 1 hour at 40°C. The mixture was filtered through filter paper, resulting in 410 ml of aqueous extract. This aqueous extract was concentrated to a residue and redissolved in 100 ml of 40% ethanol, yielding an extract coded MP2;
- 100 grams of homogenized medlar fruit sample were extracted with 500 ml of 70% ethanol using ultrasound bath extraction for 1 hour at 40°C. The mixture was filtered through filter paper, resulting in 460 ml of ethanolic extract. This extract was concentrated to a residue and redissolved in 100 ml of 70% ethanol, yielding an extract coded MP3;
- 100 grams of homogenized medlar fruit sample were extracted with 500 ml of chloroform. The mixture was filtered through filter paper, resulting in 500 ml of chloroform extract. This extract was concentrated to a residue and redissolved in 100 ml of concentrated chloroform, yielding an extract coded MP4.

3. Qualitative determinations

Qualitative analysis aimed to investigate the specific polyphenols from the acetonic and ethanolic extracts, proper for HPLC (high-performance liquid chromatography in the reverse phase) studies; it was used the Elite LaChrom liquid chromatograph equipped with a DAD L2455 detector, a stainless-steel column containing the octadecylsilane stationary phase as the reverse phase. There were used standard solutions at 0.005 g each test sample, as follows: the four standard solutions (gallic acid 95% - *Sigma-Aldrich*, chlorogenic acid >95% - *Sigma-Aldrich*, caffeic acid 99% - *Sigma-Aldrich* and transferulic acid >95% - *Sigma-Aldrich*) were accurately weighed, dissolved in 7 ml of ethanol and added to a 10 ml volumetric flask with the same solvent. The sample solution was diluted 1 ml to 5 ml volumetric flask with ethyl alcohol (Table 1 shows the chromatographic conditions).

Table 1. Chromatographic working conditions

Chromatographic Column	Inertsil ODS3 250 x 4.6 mm
Elution	Gradient
Column Temperature	40 ± 1°C
Flow Rate	1.0 mL/min
Detection	330 nm
Injection Volume	20 µL
Mobile Phase A	1 g/L ortho-phosphoric acid solution dissolved in 1000 mL water, adjusted to *p*H 2.8
Mobile Phase B	Methanol
Mobile Phase A Ratio	70:30
Column Equilibration	30 minutes
Acquisition Time	70 minutes

Table 2. Gradient used in experiments

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

After the chromatographic system has been balanced, i.e. the baseline is straight, the reference solution is injected (Table 2 shows the gradient used in experiments).
The test solutions are injected, and the chromatograms are recorded.
The content of the components of interest was calculated with the formula:
Component ″i″, mg/100 ml = Ap x Ce/Ae x 100, where:
Ap: Appearance of the peak of component ″i″ in the chromatogram of the test solution;
Ae: peak area of component ″i″ in the chromatogram of the standard solution;
Ce: the concentration of component ″i″ in the standard solution (mg/ml).

4. Quantitative determinations

Quantitative analyses were performed to investigate the total polyphenols content in the four test extracts: MP1, MP2, MP3, MP4.
Also, it was investigated the content of minerals and trace elements in the four extracts: there was measured the content of magnesium, phosphorus, potassium, calcium, chromium, manganese, iron, copper, zinc and the heavy metals arsenic, cadmium and lead; these determinations were carried out according to a method elaborated and developed in accordance with the general provisions by inductively coupled plasma mass spectrometry (ICP-MS), using the following isotopes: ^{23}Na, ^{24}Mg, ^{31}P, ^{39}K, ^{44}Ca, ^{52}Cr, ^{55}Mn, ^{57}Fe, ^{65}Cu, ^{66}Zn, ^{75}As, ^{114}Cd, ^{208}Pb. The optimal operating conditions are presented in Table 3.

Table 3. Optimal operating conditions of ICP-MS ELAN DRC-e

No. crt.	Parameter	Optimum values selected
1.	RF power for ICP	1250 Watts
2.	Nebulizer Gas Flow	0.96 L/min
3.	Auxiliary Gas Flow	1.20 L/min
4.	Lens Voltage	8.20 (with autolens option)
5.	Integration time	1000 m/s
6.	Dwell time	50 m/s
7.	No. sweeps/reading	20
8.	Mode detector	Dual
9.	No. replies	3

For the determination of the elements by ICP-MS, the samples must be brought to a liquid state; the digestion of the samples is done using a microwave oven, obtaining clear solutions, without loss of elements through evaporation and without contamination. The dry extract samples, accurately weighed, are separated with 8 ml of nitric acid and transferred into a digestion bottle.
The digestion program is one in two steps, for complete mineralization and is presented in Table 4.

Table 4. The digestion program

No. crt.	Power (W)	Preheating time (minutes)	Decomposition time (minutes)	Ventilation
1.	350	20	30	1
2.	550	10	45	3

At the end of the cycle, the mineralized solution is transferred into a 50 ml volumetric flask, this representing the base-stock solution. The sample solution represents the 3:50 ml dilution of the base solution.
- the reference solutions for the calibration curve are in the range: 0.001-1.000 µg/ml, which correspond to the range 1-1000 ppb for Ca, Mg, Zn, K, P, Cr, Mn, Fe, Cu (concentration measurement units given by the software of the ICP – MS device); 0.010-0.080 µg/ml, which corresponds to the range 10-80 ppb for Cd, Pb. Selective isotopes were chosen for each element, depending on the interferences present in the specific matrix of the products: Mg^{24}, P^{31}, K^{39}, Ca^{43}, Cr^{52}, Mn^{55}, Fe^{57}, Cu^{63}, Zn^{66}, Cd^{114}, Pb^{208}.

5. Determination of antioxidant activity

Studies on the antioxidant activity of the four active extracts obtained by processing *M. germanica* L. fruits were conducted using the chemiluminescent (CL) method. As resulted from the previous studies (Neagu et al., 2021; Pirvu et al., 2022; Pirvu et al., 2024), the chemiluminescence method is a fast, sensitive and reproducible chemical method, by which the free radical scavenger potential of a test vegetable extract can be determined, precisely the effectiveness in annihilating the hydroxyl radical from the test environment generated by oxygenated water from the environment; the CL method used is based on the property of luminol to emit (chemi)luminescence in the presence of oxygen free radicals, emitted by oxygenated water from the environment at basic pH (8.6) in the environment provided by the TRIS buffer. The intensity of the chemiluminescence reaction, measured as activity units (u.a.), is recorded every 4 seconds for 60 seconds and is proportional to the amount of free radicals generated by oxygenated water in the environment. The addition of an antioxidant reagent (test sample), for example a plant extract, which contains free radical scavengers leads to the annihilation of a share of hydroxyl free radicals from the environment, therefore a decrease in the intensity of the chemiluminescence reaction (u.a.) is recorded proportional to the concentration of active compounds and their antioxidant effectiveness. The effectiveness of the reaction is measured by comparing it with the negative control sample, which contains the solvent of the test sample.

Thus, by means of the chemiluminescent method, it is possible to analyze:

- the dynamics of the chemiluminescence reaction during 60 seconds, by comparing the undiluted sample with the negative control sample, which contains the solvent of the sample in which the sample was made and involves the serial dilutions;
- the maximum intensity of the antioxidant effect of the test extract (AA%) through comparative measurements 5 seconds after the initiation of the chemiluminescence reaction) - see the formula below:

$$AA\% = \frac{\text{Control sample activity units (a.u.)} - \text{Test sample activity units (a.u.)}}{\text{Control sample activity units (a.u.)}} \times 100$$

- at the same time, the IC_{50} of the test sample by studying a series of serial dilutions from the test sample to capture the inflection point of the chemical reaction, meaning the moment when a maximum antioxidant activity is obtained for a minimum concentration (punctual, the concentration at the halfway point of inflection from which the CL intensity starts to decrease).

Given the ability of the CL method to determine both the antioxidant effect and the pro-oxidant potential of a test sample, this can be considered a pre-pharmacological testing method as it provides an indication of the beneficial or toxic potential of a test product/extract; thus, the method allows a precise selection of the active extracts resulting from the phase of technological works in extractive biotechnologies, which has the effect of reducing the costs and time of subsequent pharmacological testing, allowing to obtain from the very beginning a product with maximized biological potential.

6. Procedure

The test plant extracts are processed as serial dilutions of 6-10 samples (e.g., x1, x2, x4, x8, x16, x32), compared to the negative control series represented by 8-10 serial dilutions of the solvent used to obtain the plant extracts test; the aim of the studies is to calculate the antioxidant activity of the test vegetal extracts, as well as their IC_{50} value, for the quantitative comparison of their effectiveness needed further in the biological and pharmacotoxicological testing; work with samples in triplicate for each point in the serial dilution (n = 3). Punctually, three aliquots of 50 µL of test plant extract (in this case ethanol 50%, v/v) related to each point in the test series are treated (freshly prepared reagent in bi-distilled water); the negative control series is carried out identically, replacing the plant extract with 50 µL ethanol 50% (v/v). The CL reaction is initiated by adding H_2O_2 to the medium, therefore the samples are quickly placed in the measuring device of the chemiluminometer, and the emitted fluorescence, measured as arbitrary units (a.u.), is recorded every 4 seconds for 60 seconds in total, during which it was demonstrated that a constant minimum plateau is reached, which means that the chemiluminescence reaction is consumed.

The results obtained, a.u. during the 60 seconds, respectively a.u. along the series of dilutions, it indicates the effectiveness of the antioxidant activity, scavenger of free radicals of the test plant samples, by comparison with the values obtained for the negative control sample; the sweep of the dilutions of the same series leads to the establishment of the IC_{50}, respectively the moment of inflection in which a maximum antioxidant activity is obtained at the minimum concentration of the test extract is highlighted.

RESULTS AND DISCUSSIONS

1. Analytical results

Polyphenols content

The quantitative content in total polyphenols is presented in Table 5.

Table 5. Quantitative content in total polyphenols

Extract	GAE mg%
ACETONIC MP1 extract	26.70
AQUEOUS MP2 extract	4.90
ETHANOLIC MP3 extract	11.9
CHLOROFORM MP4 extract	1.0

The quantitative analysis of total polyphenols in various extracts is summarized in Table 5. The acetonic extract (MP1) shows the highest concentration of polyphenols, with a value of 26.70 GAE mg %. The ethanolic extract (MP3) has a moderate polyphenol content of 11.9 GAE mg %. The aqueous extract (MP2) contains a lower amount of polyphenols, measuring 4.90 GAE mg %. The chloroform extract (MP4) exhibits the lowest polyphenol content, with only 1.0 GAE mg %.

This data indicates that the acetonic extraction method is the most effective for extracting polyphenols from the samples, while the chloroform method is the least effective.

The qualitative content of polyphenol-carboxylic acids and flavonoids (HPLC) are presented in Table 6.

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Table 6. Qualitative content of polyphenol-carboxylic acids and flavonoids (HPLC)

Compound mg/100 ml	**ETHANOLIC Extract MP3**
Gallic acid	0.2727
Chlorogenic acid	0.381
Compound mg/100 ml	**ACETONIC MP1 Extract**
Gallic acid	0.2727
Chlorogenic acid	0.381
Caffeic acid	0.960
Transferulic acid	0.0467

Both extracts contain gallic acid and chlorogenic acid in the same amounts. The acetonic extract (MP1) also includes caffeic acid and ferulic acid, which are not present in the ethanolic extract (MP3).

The presence of increased amounts of caffeic acid and chlorogenic acid was found in the extracts standardized in 40% ethanol.

This data indicates that the acetonic extract (MP1) has a more diverse profile of polyphenol-carboxylic acids and flavonoids compared to the ethanolic extract (MP3).

Mineral and trace elements content

The mineral and trace elements content in the ethanolic, acetonic, and aqueous extracts of medlar (*Mespilus germanica* L.) reveal significant variations in the concentration of these elements across different extraction methods in Table 7.

Table 7. Mineral and trace elements content in the ethanolic, acetonic, and aqueous extracts of medlar

No. crt.	Elements	MEDLAR ACETONIC EXTRACT (MP1)	MEDLAR AQUEOUS EXTRACT (MP2)	MEDLAR ETHANOLIC EXTRACT (MP3)
1.	Mg µg/g)	288.33	386.09	298.27
2.	P (µg/g)	1017.04	1342.20	1121.02
3.	K (µg/g)	3403.08	5683.61	4698.06
4.	Ca (µg/g)	170.45	414.13	404.81
5.	Cr (µg/g)	3.82	3.74	4.55
6.	Mn µg/g)	1.29	2.23	1.805
7.	Fe (µg/g)	88.07	112.15	57.80
8.	Cu (µg/g)	2.48	3.41	3.25
9.	Zn (µg/g)	72.73	21.99	62.165
10.	Cd (µg/g)	0.142	0.10	0.10
11.	Pb (µg/g)	0.27	0.22	0.14

Magnesium (Mg) shows the highest concentration in the aqueous extract at 386.09 µg/g, followed by the ethanolic extract at 298.27 µg/g and the acetonic extract at 288.33 µg/g.

Phosphorus (P) is most abundant in the aqueous extract, containing 1342.20 µg/g, with the ethanolic extract having 1121.02 µg/g and the acetonic extract 1017.04 µg/g. Potassium (K) also has its highest concentration in the aqueous extract at 5683.61 µg/g, followed by the ethanolic extract at 4698.06 µg/g and the acetonic extract at 3403.08 µg/g.
Calcium (Ca) is found in the highest amount in the aqueous extract, with 414.13 µg/g, compared to 404.81 µg/g in the ethanolic extract and 170.45 µg/g in the acetonic extract. Chromium (Cr) has similar concentrations across all extracts, with the highest in the ethanolic extract at 4.55 µg/g. Manganese (Mn) is most concentrated in the aqueous extract at 2.23 µg/g, followed by the ethanolic extract at 1.805 µg/g and the acetonic extract at 1.29 µg/g.
Iron (Fe) is most abundant in the aqueous extract at 112.15 µg/g, with the acetonic extract containing 88.07 µg/g and the ethanolic extract 57.80 µg/g. Copper (Cu) shows the highest concentration in the aqueous extract at 3.41 µg/g, followed by the ethanolic extract at 3.25 µg/g and the acetonic extract at 2.48 µg/g.
Zinc (Zn) is most concentrated in the acetonic extract at 72.73 µg/g, with the ethanolic extract containing 62.165 µg/g and the aqueous extract 21.99 µg/g.
Both cadmium (Cd) and lead (Pb) are found in low concentrations across all extracts, with slight variations.
This data highlights the varying effectiveness of different extraction methods in concentrating specific minerals and trace elements from medlar fruits. The aqueous extract generally shows higher concentrations of most elements, making it potentially more beneficial for mineral extraction.

2. Antioxidant activity

Figures 1-4 (a, b) show the dynamics of the chemiluminescence reaction and the free radical scavenger effect of the active extracts obtained in the phase of technological studies on medlar fruits.

The results of the research on MP1 - Extract in ethanol 40% from acetonic extract

Figure 1.a illustrates the dynamics of the chemiluminescence reaction for the MP1 test extract (represented by the blue bars) in comparison to the negative control sample (represented by the green line).
The negative control sample shows a decreasing trend in chemiluminescence intensity over time, starting from around 15.000 a.u. and gradually declining to about 900 a.u. in 60 seconds. This indicates a reduction in reactive species over the duration of the test.

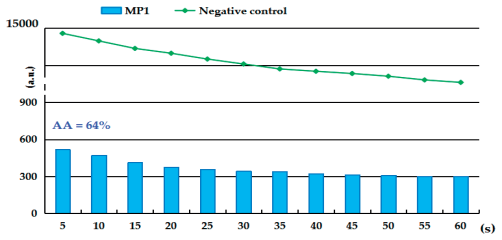

Figure 1.a. The dynamics of the chemiluminescence reaction of the MP1 test extract compared to the negative control sample (green line); AA% calculation

In contrast, the MP1 extract shows a significantly lower and relatively stable chemiluminescence intensity, starting at around 600 a.u. and maintaining values between 300 and 600 a.u. throughout the 60-second period. The consistent low intensity for the MP1 extract suggests it has a strong ability to inhibit the chemiluminescence reaction, indicating potential antioxidant properties.
The antioxidant activity (AA%) of the MP1 extract is calculated to be 64%. This percentage represents the inhibition of chemiluminescence by the extract compared to the negative control. The high AA% value further supports the effectiveness of the MP1 extract in neutralizing reactive species.
This analysis highlights the potential of the MP1 extract as an effective antioxidant agent, capable of significantly reducing the presence of reactive species in the chemiluminescence reaction.

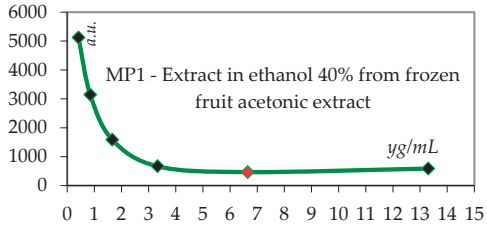

Figure 1.b. The antioxidant activity of the MP1 extract along the dilution series (A); IC_{50} calculation

Figure 1.b shows the antioxidant activity of the MP1 extract across a dilution series. The graph demonstrates how the chemiluminescence intensity decreases as the concentration of the extract increases, indicating its antioxidant activity.
The data points on the graph indicate a sharp decline in chemiluminescence intensity from 5000 a.u. to around 500 a.u. as the concentration of the extract increases from 0 to approximately 7 µg/mL. Beyond this point, the intensity levels off, suggesting that higher concentrations of the extract do not significantly further decrease the chemiluminescence intensity. This plateau indicates that the maximum antioxidant effect has been reached.
The IC_{50} value, represented by the red diamond on the graph, is the concentration of the extract at which the chemiluminescence intensity is reduced by 50% compared to the control. The IC_{50} value provides a quantitative measure of the extract's potency as an antioxidant.
This analysis highlights the effectiveness of the MP1 extract in inhibiting chemiluminescence, with its IC_{50} value serving as a key indicator of its antioxidant capacity.

The results of the research on MP2 - Extract in ethanol 40% from aqueous extract

Figure 2.a shows the dynamics of the chemiluminescence reaction for the MP2 extract (represented by the orange bars) in comparison to the negative control sample (represented by the green line).

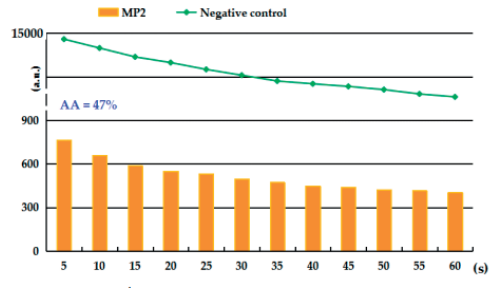

Figure 2.a. The dynamics of the chemiluminescence reaction of the MP2 extract compared to the negative control sample (green line); AA% calculation

The negative control sample shows a decreasing trend in chemiluminescence intensity over time, starting at around 15.000 a.u. and gradually declining to approximately 900 a.u. in 60 seconds. This decrease indicates the natural decay of reactive species over time.
The extract MP2 shows a significantly lower and relatively stable chemiluminescence intensity compared to the control. The intensity starts around 900 a.u. at 5 seconds and remains between 300 and 600 a.u. throughout the 60-second period. This stability and lower intensity suggest that the MP2 extract has a moderate ability to inhibit the chemiluminescence reaction, indicating its antioxidant properties.
The antioxidant activity (AA%) of the MP2 extract is calculated to be 47%. This percentage represents the inhibition of chemiluminescence by the extract compared to the negative control.

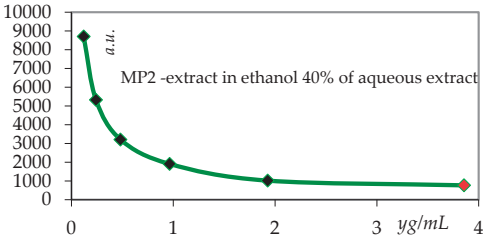

Figure 2.b. The antioxidant activity of the MP2 extract along the dilution series (A); IC_{50} calculation

Figure 2.b illustrates the antioxidant activity of the MP2 extract along a dilution series. The graph shows how the chemiluminescence intensity decreases as the concentration of the extract increases, indicating its antioxidant activity.
The data points on the graph show a steep decline in chemiluminescence intensity from about 9000 a.u. to approximately 1000 a.u. as the concentration of the extract increases from 0 to around 3 µg GAE/mL. This suggests that the extract has a strong ability to reduce reactive species at higher concentrations.
The IC_{50} value, represented by the red diamond on the graph, is the concentration at which the chemiluminescence intensity is reduced by 50% compared to the control.

Research results on MP3 - 40% ethanol extract from 70% ethanol extract

Figure 3.a illustrates the dynamics of the chemiluminescence reaction for the MP3 extract (represented by the purple bars) in comparison to the negative control sample (represented by the green line).

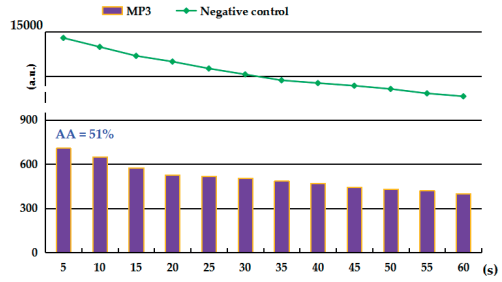

Figure 3.a. The dynamics of the chemiluminescence reaction of the MP3 extract compared to the negative control sample (green line); AA% calculation

The negative control sample shows a decreasing trend in chemiluminescence intensity over time, starting at around 15.000 a.u. and gradually declining to approximately 900 a.u. in 60 seconds. This decrease indicates the natural decay of reactive species over the course of the experiment.

In contrast, the MP3 extract maintains a significantly lower and relatively stable chemiluminescence intensity. The intensity starts around 600 a.u. at 5 seconds and remains between 300 and 600 a.u. throughout the 60-second period. This stability and lower intensity suggest that the MP3 extract has a good ability to inhibit the chemiluminescence reaction, indicating its antioxidant properties.

The antioxidant activity (AA%) of the MP3 extract is calculated to be 51%. This percentage represents the inhibition of chemiluminescence by the extract compared to the negative control.

Figure 3.b shows the antioxidant activity of the MP3 extract along a dilution series. The graph indicates how the chemiluminescence intensity decreases as the concentration of the extract increases, demonstrating its antioxidant activity.

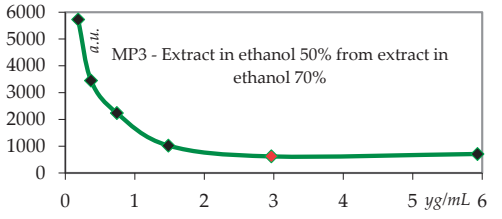

Figure 3.b. The antioxidant activity of the MP3 extract along the dilution series (A); IC_{50} calculation

The data points on the graph show a steep decline in chemiluminescence intensity from about 5000 a.u. to approximately 1000 a.u. as the concentration of the extract increases from 0 to around 3 µg GAE/mL. Beyond this point, the intensity levels off, suggesting that higher concentrations of the extract do not significantly further decrease the chemiluminescence intensity. This plateau indicates that the maximum antioxidant effect has been reached.

The IC_{50} value, represented by the red diamond on the graph, is the concentration at which the chemiluminescence intensity is reduced by 50% compared to the control.

The results of the research on MP4 - Extract in ethanol 40% of the extract in chloroform

Figure 4.a illustrates the dynamics of the chemiluminescence reaction for the MP4 extract (represented by the red bars) in comparison to the negative control sample (represented by the green line).

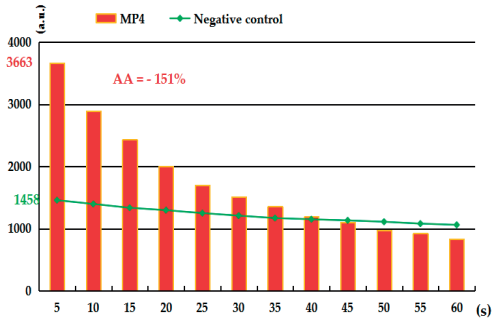

Figure 4.a. The dynamics of the chemiluminescence reaction of the MP4 extract in comparison with the negative control sample (green line); AA% calculation

The negative control sample shows a consistent decrease in chemiluminescence intensity over time, starting at approximately 1458 a.u. and gradually declining to around 1000 a.u. in 60 seconds. This decrease indicates the natural decay of reactive species over time.

In contrast, the extract MP4 shows a significantly higher chemiluminescence intensity initially, starting at around 3663 a.u. at 5 seconds. The intensity decreases over time but remains substantially higher than the negative control throughout the 60-second period. The MP4 extract exhibits a different/specific behavior where its intensity starts high and gradually declines, which suggests it may be acting as a pro-oxidant rather than an antioxidant in this specific setup.

The antioxidant activity (AA%) of the MP4 extract is calculated to be -151%. This negative percentage indicates that the MP4 extract actually increases the chemiluminescence intensity compared to the negative control, suggesting a pro-oxidant effect rather than an antioxidant effect.

Figure 4.b illustrates the antioxidant activity of the MP4 extract along a dilution series. The graph shows how the chemiluminescence intensity decreases as the concentration of the extract increases, indicating its antioxidant activity.

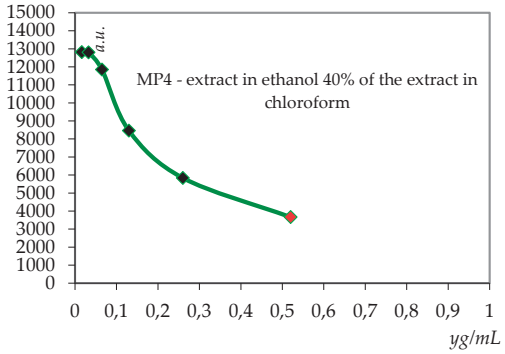

Figure 4.b. The antioxidant activity of the MP4 extract along the dilution series (A); IC_{50} calculation

The data points on the graph show a steep decline in chemiluminescence intensity from about 13.000 a.u. to approximately 4000 a.u. as the concentration of the extract increases from 0 to around 0.5 µg GAE/mL. This decline suggests that the extract has some ability to reduce reactive species at higher concentrations, but the initial high values and the form of the curve may still reflect pro-oxidant activity, as indicated in Figure 4.a.

The IC_{50} value, represented by the red diamond on the graph, is the concentration at which the chemiluminescence intensity is reduced by 50% compared to the control.

Overall, the results of our study on medlar fruit extracts align well with existing literature on the antioxidant activity and polyphenol content of plant-based extracts. The high polyphenol content found in our acetonic extract (MP1) and its diverse profile of polyphenolic compounds, including caffeic and ferulic acids, are consistent with findings from other studies that highlight acetone as an effective solvent for extracting bioactive compounds from plant materials (Katanić Stanković et al., 2022).

Moreover, our study's observation that the acetonic extract demonstrates strong antioxidant activity is supported by similar research on polyphenolic plant extracts. Studies have shown that polyphenols, particularly those extracted with organic solvents like acetone, possess significant antioxidant properties due to their ability to neutralize free radicals and inhibit oxidative stress (Stagos, 2020; Nieto, 2020).

Neagu et al. (2021) investigated the antiproliferative activity of ethanolic extracts from *Stokesia laevis*, highlighting that ethanolic extracts, due to their high content of bioactive compounds, are effective in therapeutic applications. This supports our conclusions regarding the ethanolic extract (MP3), which demonstrated significant antioxidant activity and a high polyphenol content, suggesting potential applications in health-related fields.

In contrast, the pro-oxidant effect observed in our chloroform extract (MP4) is notable and emphasizes the need for careful selection of extraction solvents. This phenomenon, while less commonly reported, is not unprecedented and suggests that certain solvents might extract compounds that can either neutralize or exacerbate oxidative stress, depending on the conditions (Stanković et al., 2022; Stagos, 2020).

Furthermore, the higher mineral content found in the aqueous extract (MP2) of medlar fruit supports the idea that water is particularly effective in extracting minerals, as corroborated by studies on mineral and trace element extraction from plant materials (Stanković et al., 2022).

Pirvu et al. (2022) investigated the effects of laser irradiation on the antioxidant activity of *Plantago lanceolata* aqueous extracts. This study can be related to our findings on the aqueous extract (MP2) of medlar fruits, especially regarding the antioxidant properties. Both studies emphasize the influence of different extraction and treatment methods on the antioxidant potential of plant extracts, providing a broader context for your results.

Pirvu et al. (2024) explored the chemical and biological attributes of fruits after their shelf life, showing that although the bioactivity of some compounds may decline, certain treatments can

maintain or even enhance their stability. These findings are relevant for discussions on the use of medlar fruit extracts in food and pharmaceutical products, highlighting the importance of optimising storage and processing conditions to maximise antioxidant benefits.
These findings reinforce the importance of selecting appropriate solvents based on the desired bioactive compounds and the intended application, whether for antioxidant purposes or mineral extraction in food and pharmaceutical industries.

CONCLUSIONS

The study investigated the antioxidant activity and extraction efficiency of medlar fruit extracts using various solvents. Polyphenol content, mineral composition, and antioxidant capacity were analyzed across acetonic, ethanolic, aqueous, and chloroform extracts. The acetonic extract (MP1) had the highest polyphenol content and showed the most diverse range of polyphenolic compounds, making it the most effective solvent for polyphenol extraction. The aqueous extract (MP2) was richest in essential minerals like magnesium and potassium. Antioxidant activity tests revealed that the acetonic extract had the strongest activity, while the chloroform extract exhibited a pro-oxidant effect, raising concerns about its use. The ethanolic extract offered a balanced profile of polyphenol content and antioxidant activity, making it suitable for potential health applications. The study recommends acetone for polyphenol extraction and water for mineral extraction, while advising caution with chloroform due to its potential pro-oxidant effects. These findings contribute to optimizing extraction methods for medlar fruits in the food and pharmaceutical industries.

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