



Extraction of Sesquiterpene Lactones from *Inula helenium* Roots by High-Pressure Homogenization and Effects on Antimicrobial, Antioxidant, and Antiglycation Activities

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Abstract

The sesquiterpene lactones (SL) of *Inula helenium* (*I. helenium*) roots were extracted using high-pressure homogenization (HPH) and compared against those derived from maceration. The effect of process conditions on the extraction efficiency of bioactive compounds (alantolactone, isovalantolactone, total phenols, and flavonoids); the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity, ferric-reducing antioxidant power (FRAP), and antimicrobial activity of the extract; and the inhibition of advanced glycated end product (AGE) formation were assessed. The HPH of 90 MPa for 4 passes demonstrated the highest alantolactone (38.1 ± 0.7 mg/g) and isovalantolactone yields (34.4 ± 0.2 mg/g), respectively, which were significantly higher than the rates obtained using maceration (20.0 ± 0.2 mg/g and 18.9 ± 0.7 mg/g). Under optimal extraction conditions of 90 MPa for 4 passes, inhibition of AGE formation in the extract reached a rate of $97.16 \pm 1.86\%$, with the highest DPPH and FRAP of 231.63 ± 2.96 µg/mL and 949.43 ± 1.86 µmol TE/100 mL, respectively. HPH exhibited lethal activity against all tested bacteria and fungi strains (*Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Aspergillus niger*, *Fusarium oxysporum*, and *Penicillium expansum*) at a concentration of 15 µL. SEM results proved that HPH severely damaged the cell structures of roots, increased solvent permeability, and improved the extraction rate of bioactive compounds. Additionally, this study also demonstrated that HPH yields high recovery with a specific energy consumption of 0.22 kWh/kg, which is close to the recommended minimum energy demand for an extraction procedure within industrial production processes. Therefore, these findings showed that HPH can be used as an efficient extraction technique for the production of SL in related industries.

Keywords High-pressure homogenization · Sesquiterpene lactones · Antiglycation activity · Antioxidant capacity · Antimicrobial activity · LC-MS/MS

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Abbreviations

AL	Alantolactone
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EtOH	Ethanol
FRAP	Ferric reducing antioxidant power
HPH	High-pressure homogenization
IAL	Isoalantolactone
IHRE	<i>Inula helenium</i> Root extract
L	Liquid
LC–MS/MS	Liquid chromatography tandem mass spectrometry
MCF	Microfluidizer
S	Solid
SEM	Scanning electron microscope
SL	Sesquiterpene lactones
TFC	Total flavonoid content
TPC	Total phenolic content

Introduction

Inula helenium L., an important member of the Asteraceae family, is mainly distributed in East Asia and Europe and is one of the most popular plants in traditional medicine (Chun & Kim, 2018). *I. helenium* root extract (IHRE) is rich in sesquiterpene lactones (SL), primarily alantolactone (AL) and isoalantolactone (IAL), which are characteristically related to plant defense systems due to their antibacterial, antifungal, and antiviral properties. Furthermore, many studies over the last three decades have shown that sesquiterpenes have health-promoting properties against a variety of diseases, such as cardiovascular complications, diabetes, hyperlipidemia, neural disorders, and cancer (Zheng et al., 2021). *I. helenium* is a rich source of sesquiterpene lactones, which have gained attention due to their aforementioned numerous biological activities (Chun & Kim, 2018). Therefore, selecting an extraction process that minimizes environmental effects while maximizing bioactive extraction efficiency is critical. Thus, extraction approaches should attempt to maximize extraction yield with shorter operating durations for bioactive isolation while also lowering carbon emissions and energy usage, contributing to improved environmental performance (Chemat et al., 2017).

The high-pressure homogenization technique causes mechanical cell disintegration and the release of intracellular compounds and is easily adaptable to an industrial scale (Gali et al., 2020). Fluid is exposed to mechanical stress in the HPH process by being forced through a narrow gap under the influence of high pressure. HPH treatments cause the extraction of bioactive compounds from plant materials by reducing particle size, homogenous distribution, and mixing (Mesa et al., 2020). HPH treatment might be regarded as the better option for the recovery of bioactive

compounds since it is a solvent-free and non-thermal technique (Vichakshana et al., 2023; Yong et al., 2021). In previous studies, the HPH has been extensively used for the extraction of polysaccharides (de Oliveira et al., 2016; Li et al., 2023), phenolic compounds (Alexandre et al., 2017; Le-Tan et al., 2022; Lin et al., 2022), proteins (Chen et al., 2022), and carotenoids (Gosavi et al., 2019). However, to the best of our knowledge, no research has been reported until now regarding the extraction of sesquiterpene lactones by HPH. Based on all of these factors, this study provides a new approach for the extraction of sesquiterpene lactones. To reduce resource waste, especially concerning raw materials and energy resources in SL extraction, critical parameters were determined for this eco-friendly and efficient extraction method. In addition, this study aims to increase the effects of the HPH technique on the AL and IAL yields, antimicrobial, antioxidant, and antiglycation activities of extracts obtained from *I. helenium* roots.

Materials and Methods

Plant Sample Preparation

The roots of *I. helenium* were collected from Kırşehir, Turkey (39°18'26" N, 34°18'25" E, date: 31/07/2022), dried naturally, finely ground, and passed through a 0.15-mm stainless steel sieve. Identification and collection of the plant materials were performed by botanists Assoc. Prof. Ozlem Cetin and Dr. Mustafa Celik. Voucher specimens (voucher number: O. Cetin 2930& M. Celik (GAZI)) were deposited at Gazi University Herbarium (GAZI), Ankara, Turkey. The moisture content of the roots was 9.72% before extraction (AOAC 925.10).

High-Pressure Homogenization

A high-pressure homogenization (HPH) procedure was performed using a microfluidizer (MCF) (MP110P, Microfluidics, Westwood, MA, USA) for the extraction of SL from *I. helenium* roots with ethanol. A preliminary screening of liquid-to-solid (L:S) ratios was performed (1 pass at L:S ratios of 5:1, 10:1, 25:1, and 50:1 mL/g) for each suspension. The L:S ratio of 50:1 mL/g was chosen because the high solid content causes clogging in the device. First, each suspension was homogenized at various pressure levels (1 pass at pressures of 30 MPa, 60 MPa, 90 MPa, and 120 MPa) and then passed through a microfluidizer at various pass numbers (90 MPa at pass numbers ranged from 1 to 7). Each suspension was placed in an ice bath to keep the temperature constant (25 ± 0.1 °C) during the extraction. The extraction parameters were selected according to previous studies (Gali et al., 2020) and preliminary trials.

Conventional Extraction (Maceration)

Maceration was performed in a 50-mL flask filled with 1 g of sample and 50 mL of ethanol, according to Fernández-Ronco et al. (2013), with slight modifications. The flask was placed in a water bath (Nüve, ST 402, Turkey) to keep the temperature constant (25 ± 0.1 °C) during the extraction. After extraction, samples were filtered and taken into vials for LC–MS/MS analysis.

LC–MS/MS Determination of Sesquiterpene Lactones

The determination of SL was carried out according to Özcan et al. (2023) using liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Zivak Tandem Gold, Turkey) equipped with a C18 column (Zorbax SB, 3.0×100 mm I.D., particle size $3.5 \mu\text{m}$; Agilent, USA). The chromatographic conditions of the LC–MS/MS analysis are presented in Table 1. Under the detection conditions in Table 1, 29.79 and 30.11 min were the retention times of AL and IAL, respectively. The AL and IAL peaks were determined by comparing them to the retention times of the analytical standards, as shown in Fig. S1.

Scanning Electron Microscope

The scanning electron microscope (SEM) (SEM Quanta 250, FEI Company, Eindhoven, The Netherlands) was used to make microscopic observations on the morphological structure changes of the *I. helenium* roots following extraction, with magnifications of $\times 250$, $\times 500$, $\times 1000$, and $\times 2500$. The

samples were analyzed at various acceleration voltages ranging from 10 to 20 kV (Ying et al., 2011).

Antibacterial Activity

The qualitative antibacterial activities of HPH-IHRE and maceration-IHRE were investigated using the disk diffusion technique against strains of *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli*. Tryptic soy agar petri plates were seeded with diluted 10^2 CFU/mL quantities of *S. aureus*, *L. monocytogenes*, and *E. coli*. A 6.0-mm sterile filter paper disk was adsorbed with 5, 15, 25, and 50 μL of each extract and evaporated at room temperature for around 10 min. The plates were incubated for 24 h at 37 °C. Antibacterial extracts created distinct, visible, and circular zones of inhibition around the filter disks. The growth inhibition zone (mm) surrounding the disks after 24 h was measured to compute this positive activity (Corona et al., 2017). Chloramphenicol (25 g) was used as a control.

Antifungal Activity

In this work, strains of *Aspergillus niger*, *Fusarium oxysporum*, and *Penicillium expansum* were inoculated onto malt extract agar (MEA) plates from stock culture tubes and incubated at 28 °C in the dark for 5 days until sporulation. MEA mold spores were isolated, suspended in sterile physiological water, and adjusted to a concentration of 10^6 CFU/mL. The malt extract, which was generated from this saline and contained around 10^6 CFU/mL of mold spores, was used to inoculate the agar. On top of it, sterile filter paper disks were

Table 1 Chromatographic conditions of LC–MS/MS analysis

Parameters	Chromatographic conditions	
Mobil phase A	Deionized water and 0.1% formic acid	
Mobil phase B	MeOH:ACN (90:10)	
Pump program	00:00–04:00 min, 10% B	
	04:00–27:00 min, 55% B	
	27:00–36:00 min, 80% B	
	36:00–38:00 min, 10% B	
Ion spray voltage	5000 V	
Nebulizer gas (nitrogen)	35 psi	
Source temperature	50 °C	
Drying gas temperature	350 °C	
Analytes	Alantolactone	Isoalantolactone
Precursor ion	233.15	233.15
Daughter ion	105.06	105.07
Capillary voltage	30	30
Collision energy	25	25
Retention time	30.11	29.79

inserted. After impregnating a 6.0-mm sterile filter paper disk with 5, 15, 25, and 50 μL of each extract, it was kept for around 10 min (Ünal et al., 2012). Fluconazole (25 μg) was used as a positive control.

Total Phenolic and Flavonoid Content

The extract (0.5 mL) was mixed with 2 mL of Folin-Ciocalteu's reagent to determine the total polyphenol content. After 5 min, 7.5% Na_2CO_3 solution (2.5 mL) was added, and the mixture was kept in the dark for 90 min. The absorbance of the reaction mixture was measured at 760 nm. The total phenolic content (TPC) of the plant extract was quantified as gallic acid equivalents (mg GA/g) (Gedikoğlu et al., 2019). All samples were analyzed in triplicate.

The plant extract (1 mL) was mixed with a 2% AlCl_3 (1 mL) solution to determine the total flavonoid content (TFC). After 15 min of incubation, the absorbance of the reaction mixture was measured at 430 nm. The TFC was quantified as rutin equivalents (mg Trolox/g) (Gedikoğlu et al., 2019). All samples were analyzed in triplicate.

Antioxidant Capacity

DPPH radical scavenging activity (Molyneux, 2004) and the FRAP (ferric reducing antioxidant power) analysis (Hanganu et al., 2021) were performed, and their results were expressed as the IC_{50} value ($\mu\text{g/mL}$) and μmol of Trolox equivalents (TE) per 100 mL of extract, respectively.

Antiglycation Activity

In the presence or absence of the extract (25 $\mu\text{g/mL}$), 10 mg/mL of bovine serum albumin (BSA) was incubated in fructose (2 mol/L) and phosphate buffer (0.1 M, pH 7.4) with sodium azide (0.02%). The solutions were incubated at 37 °C for 7 days. At 350/450 nm (Ex/Em), the total advanced glycation end products (AGEs) produced by each extract were measured (Lee et al., 2022). Aminoguanidine (AG) was used as a positive control.

Calculation of Energy Consumption and CO_2 Emissions

The energy consumption was calculated using the following formula: Eq. (1) (Gałuszka et al., 2012).

$$\text{Energy consumption} = P \times t \quad (1)$$

where P indicates the extraction equipment's power (W) and t the time (min).

The microfluidizer and water bath used in the present study had powers of 2640 W and 1600 W, respectively. The

powers mentioned herein are used as a standard to calculate the energy consumption of high-pressure homogenization and conventional extractions of sesquiterpene lactones from the IHRE.

Energy consumption analysis is required for a technology that has the potential to be scaled up to the industrial level. The total energy consumption was computed based on each technology's power usage. The carbon emissions were calculated by assuming that 1 KWh produces 0.8 kg of CO_2 (Li et al., 2013).

Statistical Analysis

Statistical analysis was performed using SPSS Statistics Software (IBM version 23, USA). The experiments were conducted in triplicate, and the vvv mean $\pm$ standard deviation.

Results and discussion

High-Pressure Homogenization (HPH)

Effects of Pressure and Number of Passes

The experiment examined the impact of HPH with varying pressures and passes on AL and IAL yields as key influencing variables. As shown in Fig. 1, the yields of AL and IAL increased with increasing HPH pressure between 30 and 90 MPa and decreased significantly above 90 MPa. The most significant changes in the yield of AL and IAL with increasing HPH pressure were concentrated in the region from 60 to 120 MPa. The effect of HPH on the extraction was explained by two mechanisms: cell disruption caused by high mechanical force and increased mass transfer rates across granular walls under high dynamic pressure (Xing et al., 2019). The mean AL–IAL contents of samples under 30, 60, 90, and 120 MPa were 18.5 ± 0.6 mg/g– 15.7 ± 0.9 mg/g; 20.9 ± 0.4 mg/g– 19.1 ± 0.1 mg/g; 37.8 ± 0.2 mg/g– 33.0 ± 0.7 mg/g; and 20.4 ± 0.1 mg/g– 18.0 ± 1.0 mg/g, respectively.

Additionally, the maximum release of AL (38.2 ± 0.7 mg/g) and IAL (34.4 ± 0.2 mg/g) was observed after 4 passes (Fig. 1). The yields of AL and IAL increased with an increasing number of passes from 1 to 4, but decreased with an increasing number of passes from 4 to 7. Briefly, the results indicate that the AL and IAL yields did not increase continuously with increasing pressure and number of passes (Fig. 1). This is in agreement with previous studies, where the observed decrease in bioactive compounds upon microfluidization treatment of some plants was attributed to the fact that the high-energy dispersion of free bioactives in the solvent might cause their degradation (Gali et al., 2020). In addition, intense interfacial shear stresses and inherent heating that occur when there is no efficient heat dissipation

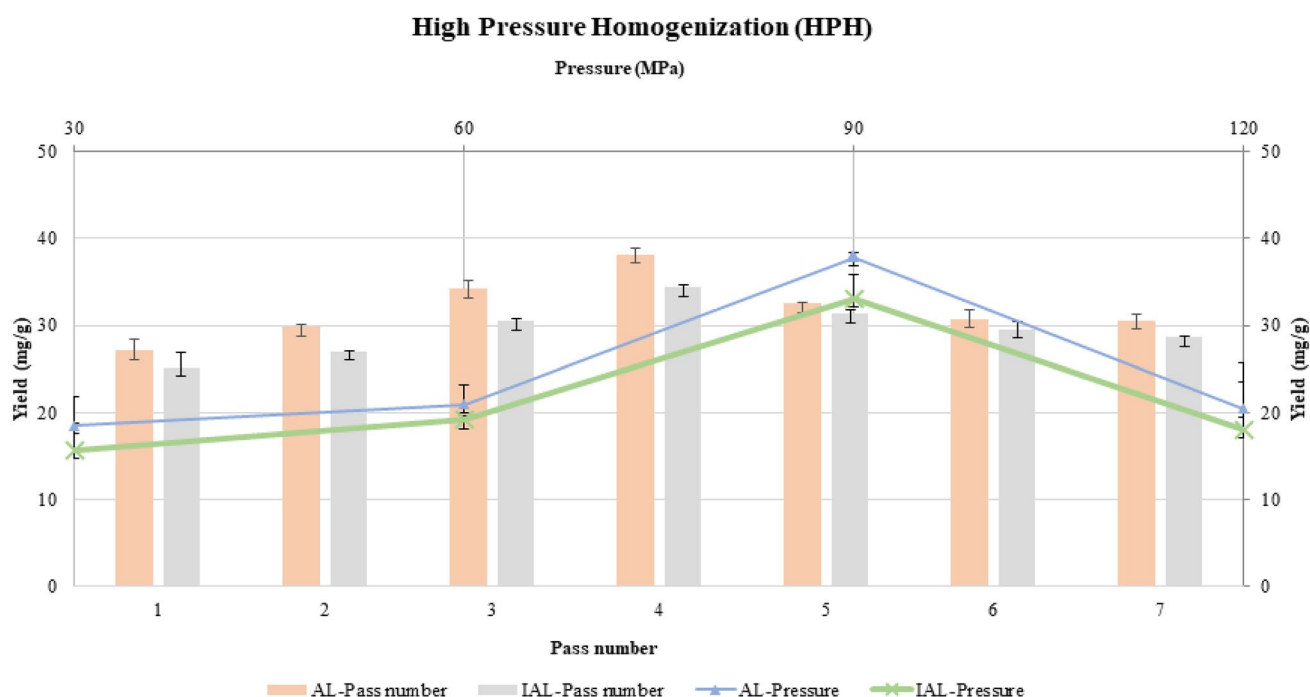


Fig. 1 The results of high-pressure homogenization-assisted extraction: The columns indicate the AL and IAL yields for pass number, and the curves indicate the AL and IAL yields for pressure

in the homogenization valve can cause the degradation of valuable compounds (Carullo et al., 2018).

Until now, various extraction techniques such as microwave-assisted extraction, ultrasound-assisted extraction with an ultrasonic water bath, and maceration have been used for the extraction of sesquiterpene lactones from *I. helenium* (Buza et al., 2022; Trendafilova et al., 2010; Wang et al., 2015; Zhao et al., 2015). No research has been reported until now on the extraction of sesquiterpene lactones by HPH. As a matter of fact, the obtained results demonstrated that HPH could be used to more efficiently extract sesquiterpene lactones from different plant materials compared to previous studies.

Maceration

At the end of the 24-h maceration period, the AL yield was 20.0 ± 0.2 mg/g, and the IAL yield was 18.9 ± 0.7 mg/g. On the other hand, Trendafilova et al. (2010) determined AL and IAL yields of 17.5 mg/g and 11 mg/g, respectively. It is thought that the difference between the results obtained in these two studies is due to the ratio of ethanol used as the extraction solvent. Our results in this study were compared with the results of preliminary studies (Fig. S2) and Trendafilova et al. (2010), and it was proven that AL and IAL yields increased with the ethanol ratio.

LC–MS/MS Determination of Sesquiterpene Lactones

The internally developed LC-MS/MS method was used for the determination of sesquiterpene lactones. Mass spectrometry parameters were optimized using pure standard solutions. Chromatographic separation conditions were optimized using different columns and different mobile phase compositions to give the best separation and sensitivity. Optimized conditions are given in Table 1. As a result of the studies carried out with the developed method, the performance parameters of the method were determined as follows: The linearity of the method was between 0.1 and 1000 $\mu\text{g/L}$ for both ALs. The LOQ values for AL and IAL are 0.43 $\mu\text{g/L}$ and 0.54 $\mu\text{g/L}$, respectively. The interday precision, which is experienced as RSD% for AL and IAL, was 4.05 $\mu\text{g/L}$ and 4.13 $\mu\text{g/L}$, respectively.

Morphological Analysis

The extraction efficiency was related to morphological changes in the plant tissue (Ying et al., 2011). The morphological structure changes of different extraction approaches were characterized by SEM, and the images are shown in Fig. 2. The surface morphology of *I. helenium* roots was examined at different magnification levels, including untreated and treated with maceration and HPH. According to the results in Fig. 2, different extraction

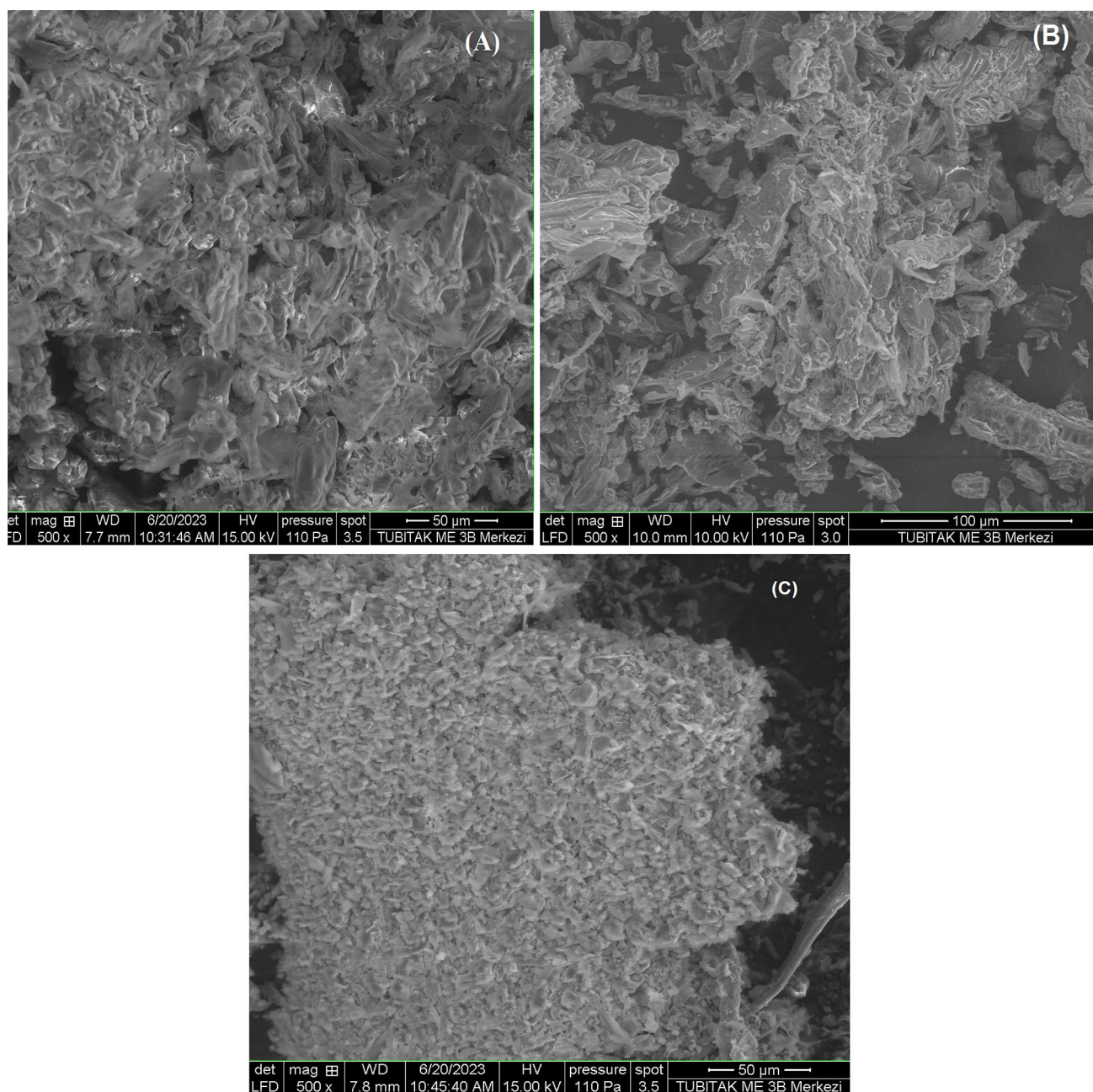


Fig. 2 SEM results of *I. helenium* root untreated (control) (A) and treated by maceration (B) and HPH (C)

procedures resulted in distinguishable physical changes. Compared with the untreated *I. helenium* roots displayed in Fig. 2A, the roots with maceration showed mild damage on the root surface (Fig. 2B). On the other hand, those treated with HPH were explosively damaged and showed obvious cell disruption (Fig. 2C). Similar results were observed with Okur et al. (2021) and Okur et al. (2023). HPH remarkably increased the solvent entrance into the matrix cells by disrupting the cell walls and membranes, increasing the mass transfer rate of bioactives (Okur et al.,

2023). Therefore, the SEM results showed that HPH can be a much more effective technique for the extraction of SL compared to others due to its drastic effect on the cell walls of *I. helenium* roots.

Antimicrobial Activity

Zheng et al. (2021) reported that *I. helenium* root extract (IHRE) is rich in sesquiterpene lactones, primarily AL and IAL, which are characteristically related to plant defense

systems due to their antibacterial, antifungal, and antiviral properties. Furthermore, plant extracts exhibit antibacterial and antifungal properties, which may be due to the presence of bioactive compounds, according to Gökbulut et al. (2013) and Flores et al. (2023). Therefore, in this paper, the extracts with the highest yields of AL and IAL obtained by HPH (HPH–IHRE) and maceration (Maceration–IHRE) were subjected to antibacterial and antifungal activity. For this purpose, the changes in the diameters of the zone inhibition of IHRE under the best extraction conditions (90 MPa, 4 passes for HPH) against gram-positive and gram-negative bacteria, fungi, and positive controls were investigated. The values are shown in Table 2. Accordingly, it was observed that the diameters of the zones of inhibition changed in a concentration-dependent manner. First of all, HPH–IHRE showed remarkable antimicrobial activity against all these pathogens compared to the antibacterial activity of chloramphenicol (25 µg) and the antifungal activity of fluconazole (25 µg). Additionally, HPH–IHRE registered the zones of inhibition of 25.25 ± 0.35 mm, 27.25 ± 0.35 mm, 25.75 ± 0.35 mm, 32.75 ± 1.06 mm, 28.25 ± 0.35 mm, and 34.25 ± 0.35 mm, respectively, against *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Aspergillus niger*, *Fusarium oxysporum*, and *Penicillium expansum*, respectively. Otherwise, maceration–IHRE recorded the zones of inhibition of 16.5 ± 0.71 mm, 18.75 ± 0.35 mm, 19.5 ± 0.71 mm, 21.25 ± 1.06 mm, 17.75 ± 0.35 mm, and 22.5 ± 0.71 mm, respectively, against all aforementioned organisms.

These results demonstrated that HPH–IHRE has higher potential. Despite the fact that the extract was produced

from the same *I. helenium* roots, the extraction technique played an important role in the recovery of bioactive compounds from the raw material. Moreover, it has been proven by Kenny et al. (2022) that AL and IAL are natural products with in vitro–observed antimicrobial activity. Therefore, HPH–IHRE has a higher antimicrobial activity, which is related to its higher AL and IAL yields. The results of the present investigation were in agreement with Kenny et al. (2022).

All extracts showed lethal activity against all tested bacteria and fungi at a concentration of 15 µL. Additionally, for all tested extracts, the most sensitive bacteria and fungi were *S. aureus* (50 µL concentration; Table 2) and *P. expansum* (50 µL concentration; Table 2), respectively.

According to Buza et al. (2022), the ethanolic extract of *I. helenium* obtained by conventional extraction indicated significant antibacterial activity against *E. coli* (10.67 ± 0.47 mm) and *S. aureus* (16.33 ± 0.47 mm). On the other hand, in our study, HPH–IHRE showed higher antibacterial activity against these two bacteria (25.25 ± 0.35 mm and 27.25 ± 0.35 mm, respectively). The results showed that *I. helenium* roots are a promising source of antimicrobial agents and that this activity is directly related to AL and IAL contents.

As seen above, although there are studies on the antimicrobial activity of sesquiterpene lactones in the literature, there is no comparison data on the antimicrobial effects based on extraction efficiencies as a result of comparing HPH with maceration. As a consequence of our study, it was determined that the HPH was more effective in terms of bioactive compound extraction than classical extraction

Table 2 Antibacterial and antifungal activities of IHREs

		Zone of inhibition (mm)				CAP (25 µg)	FLC (25 µg)
		Concentration (µL)					
		5	15	25	50		
<i>E. coli</i>	Maceration	3.75 ± 0.35 ^B	8.50 ± 0.71 ^B	11.75 ± 0.35 ^B	16.5 ± 0.71 ^B	17.5 ± 0.35	-
	HPH	8.40 ± 0.14 ^A	16.25 ± 0.35 ^A	20.00 ± 0.00 ^A	25.25 ± 0.35 ^A		
<i>S. aureus</i>	Maceration	3.25 ± 0.35 ^A	9.25 ± 0.35 ^B	11 ± 0.00 ^B	18.75 ± 0.35 ^B	17.75 ± 0.71	-
	HPH	-	19.50 ± 0.71 ^A	21.15 ± 0.21 ^A	27.25 ± 0.35 ^A		
<i>L. monocytogenes</i>	Maceration	8.75 ± 0.35 ^B	11.5 ± 0.71 ^B	16 ± 0.00 ^B	19.5 ± 0.71 ^B	21.00 ± 0.35	-
	HPH	8.00 ± 0.00 ^A	17.25 ± 1.06 ^A	19.75 ± 0.35 ^A	25.75 ± 0.35 ^A		
<i>A. niger</i>	Maceration	4 ± 0.00 ^B	15.5 ± 0.71 ^B	19.5 ± 0.71 ^B	21.25 ± 1.06 ^B	-	20.5 ± 0.71
	HPH	10.25 ± 0.35 ^A	18.5 ± 0.71 ^A	28.5 ± 0.71 ^A	32.75 ± 1.06 ^A		
<i>F. oxysporum</i>	Maceration	3.75 ± 0.35 ^B	7.75 ± 0.35 ^B	13 ± 0.00 ^B	17.75 ± 0.35 ^B	-	13.3 ± 1.06
	HPH	10.25 ± 0.35 ^A	17.0 ± 0.00 ^A	26.25 ± 0.35 ^A	28.25 ± 0.35 ^A		
<i>P. expansum</i>	Maceration	6.5 ± 0.71 ^B	15 ± 0.00 ^B	18.25 ± 0.35 ^B	22.5 ± 0.71 ^B	-	19.8 ± 1.06
	HPH	11.5 ± 0.71 ^A	21.25 ± 0.35 ^A	24.75 ± 0.35 ^A	34.25 ± 0.35 ^A		

Values are mean $\pm$ SD. Superscript uppercase letters on the same column indicate differences in the inhibition diameter produced by *I. helenium* extracts; A to B, highest to lowest ($p < 0.05$). Gram-positive bacteria: *S. aureus*, *L. monocytogenes*; Gram-negative bacteria: *E. coli*

CAP chloramphenicol, FLC fluconazole, HPH high-pressure homogenization

(maceration), and these results were confirmed by the antimicrobial activity test.

Total Phenolic, Total Flavonoid Contents, and Antioxidant Capacity

The results of the TPC, TFC, and antioxidant capacity of the extracts are shown in Table 3. The HPH-IHRE had significantly higher TPC and TFC than the maceration-IHRE ($p < 0.05$). The TPC of HPH-IHRE and maceration-IHRE were 3.16 ± 0.08 g GAE/100 g dry weight (dw) and 1.67 ± 0.04 g GAE/100 g dw, respectively. On the other hand, the TFC of HPH-IHRE and maceration-IHRE were 1.00 ± 0.05 g GAE/100 g dw and 0.46 ± 0.04 g GAE/100 g dw, respectively. Our results were in agreement with Buza et al. (2022), who used the maceration technique for 10 days and reported the TPC of IHRE as 3.06 ± 0.04 GAE/100 g dw, and the TFC of IHRE as 0.60 ± 0.02 GAE/100 g dw. Furthermore, Spiridon et al. (2013) determined the TPC of the root extract as 4.22 ± 0.02 g GAE/100 g dw in their study.

In this study, HPH-IHRE (231.63 ± 2.96) showed a significantly higher IC_{50} value than maceration-IHRE (125.67 ± 2.18) ($p < 0.05$). Furthermore, the FRAP of the HPH-IHRE (949.43 ± 1.86 μ mol TE/100 mL) was significantly stronger than that of the maceration-IHRE (474.87 ± 3.29 μ mol TE/100 mL) ($p < 0.05$) (Table 3). Accordingly, the results in this study showed that the antioxidant capacity, TFC, and TPC of the extracts increased with HPH application. Our results were in agreement with Buza et al. (2022), who reported the FRAP value as 629.04 ± 2.07 μ mol TE/100 mL and IC_{50} value of IHRE as 173.2 ± 3.40 μ g/mL.

On the other hand, Jurić et al. (2019) found that after applying HPH treatment at 100 MPa for 10 cycles, antioxidant activity improved by 23.3%, and total polyphenols increased by 32.2% in tomato peel suspension. Jurić et al. (2019) revealed that the HPH technique might promote the release of intracellular substances, particularly phenolic

compounds, to the surrounding liquid phase by mechanically disrupting and opening plant cells.

Phenolic compounds in plants are natural antioxidants that can be utilized as food additives, coatings, or active packaging to slow down food product oxidation. TPC and TFC both provided information about the antioxidant capacity, since higher phenolic content was related to higher antioxidant activity (Gedikoglu et al., 2019). Moreover, oxidative stress, characterized by the production of reactive oxygen species (ROS) and free radicals, is a key factor in the progression and complications of various diseases, including diabetes. On the other hand, the plants are rich in polyphenols, which are strong antioxidants due to their electron-donating ability. These polyphenols have been shown to inhibit free radical generation in iron and diabetes-induced oxidative stress (Chukwuma et al., 2020).

Antiglycation Activity

In diabetics, glycemic control can assist in decreasing hyperglycemia-induced protein and lipid glycation, a process that results in the production of advanced glycated end products (AGEs), which increases diabetes symptoms owing to oxidative damage in organs and tissues (Chukwuma et al., 2020). Interestingly, HPH-IHRE showed a high antiglycation activity, with $97.16 \pm 1.86\%$ inhibition at 25 μ g/mL (Table 3). On the other hand, maceration-IHRE resulted in moderate antiglycation activity of $56.93 \pm 0.78\%$. The comparative results of the antiglycation activity test depending on the concentration used are shown in Fig. 3.

The above results showed that HPH-IHRE could effectively inhibit AGE formation. In a previous study, pomegranate peel extract significantly exhibited an inhibitory effect on AGE formation in the glucose-BSA model at 37 °C for 3 weeks (Chukwuma et al., 2020). To our best knowledge, there was no report about the inhibitory effect of SL extracted from *I. helenium* roots on AGE formation. As a result, the inhibitory mechanism on AGE formation of SL from *I. helenium* roots is still unclear. It has been reported that free radicals are involved in the generation of AGEs, and the ability of scavenging free radicals of ingredients could effectively inhibit the production of AGEs (Jia et al., 2023). Therefore, with this study, SL in *I. helenium* roots were confirmed to be an important source of antioxidants, and they could be used to inhibit the formation of AGEs. Additionally, HPH technique could significantly enhance the inhibition activity of AGE formation ($p < 0.05$). The different AGE formation inhibitory activity between HPH-IHRE and maceration-IHRE might be attributed to differences in the release of antioxidants caused by the extraction process. Consequently, these findings showed that *I. helenium* roots may contain bioactive compounds that can be used to control or prevent diabetes complications.

Table 3 The total phenolic (TPC), flavonoid (TFC), DPPH, FRAP, and glycation inhibition values of HPH-IHRE and maceration-IHRE

	HPH	Maceration
TPC (g GAE/100 g)	3.16 ± 0.08^A	1.67 ± 0.04^B
TFC (g rutin/100 g)	1.00 ± 0.05^A	0.46 ± 0.04^B
DPPH (IC_{50} (μ g/mL))	231.63 ± 2.96^A	125.67 ± 2.18^B
FRAP (μ mol TE/100 mL)	949.43 ± 1.86^A	474.87 ± 3.29^B
Glycation inhibition (%)	97.16 ± 0.36^A	56.93 ± 0.78^B

Mean $\pm$ SD. Different letters in the same row denote a significant difference ($p < 0.05$)

DPPH 2,2-diphenyl-1-picrylhydrazyl, FRAP ferric reducing antioxidant power

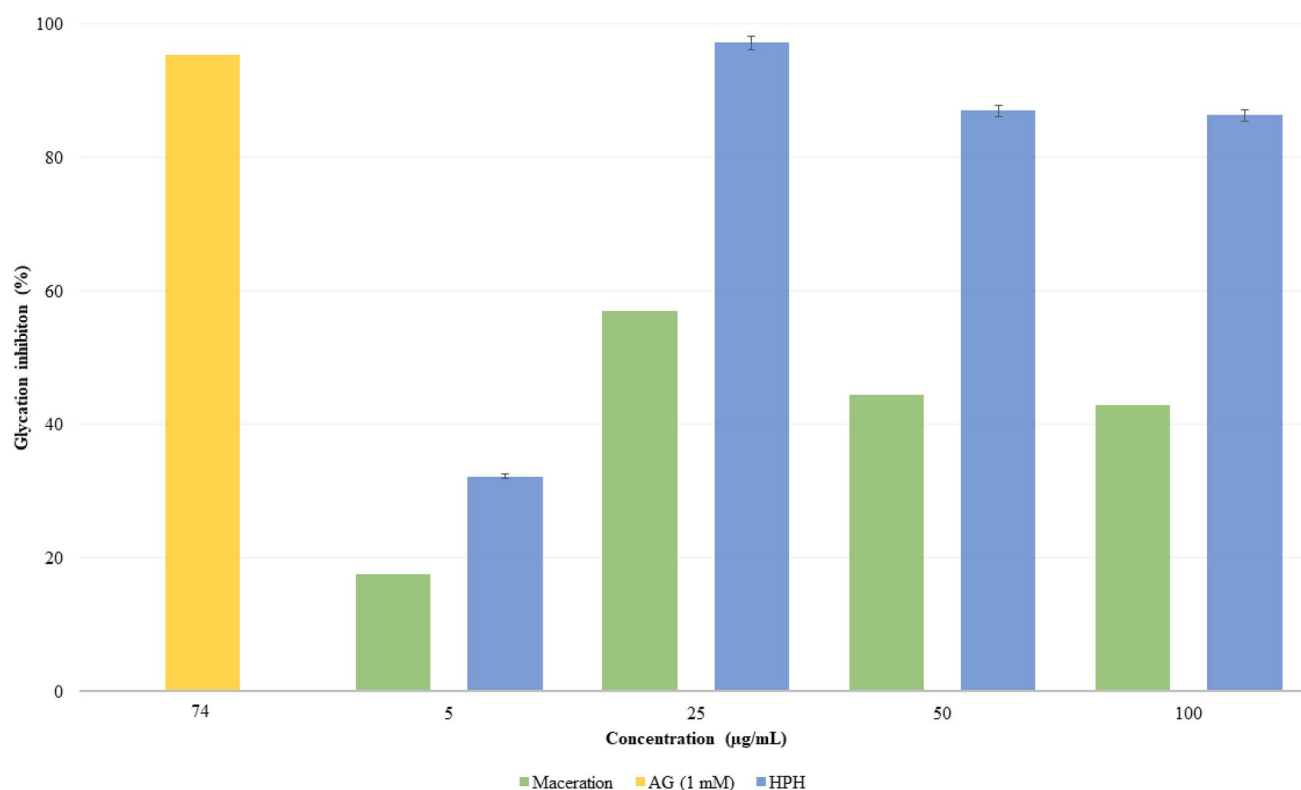


Fig. 3 The results of the antiglycation activity test

Energy Consumption and CO₂ Emissions

The energy consumption and carbon emission patterns for each technique are illustrated in Table 4. HPH resulted in a remarkable reduction in energy consumption and carbon emissions compared to maceration. As presented in Table 4, the energy consumption was 0.22 kWh/kg extract for HPH and 38.4 kWh/kg extract for maceration for isolating sesquiterpene lactones from IHRE. Accordingly, HPH reduced the energy consumption 174.5-fold when compared with maceration (conventional extraction). The same trend was reflected in the carbon footprint pattern as well. Energy consumption and carbon emissions are key factors to consider when determining industrial profitability. It is vital to lower these factors to reduce environmental pollution and production costs. According to previous studies, HPH

technology has energy requirements ranging from 0.02 to 147 kWh/kg sample (Grossmann et al., 2018; Günerken et al., 2015; Zhang et al., 2021), depending on the sample structure and processing aim. The value of our current study (0.22 kWh/kg extract) was in line with the literature. Otherwise, Valdovinos-García et al. (2022) reported a consumption of CO₂ of 11 kg CO₂/kg sample for oil production from microalgae, whereas in our present work, this value was 0.18 kg CO₂/kg extract for extraction of SL by HPH.

In conclusion, HPH, as a green extraction procedure, enables greater functional and economic uses of SL by considerably reducing carbon emissions and energy costs while improving yields (Table 3).

These findings demonstrated that the HPH enabled higher AL and IAL yields and lower energy consumption and carbon emissions, considering other extraction techniques.

Table 4 Comparison of HPH and maceration in terms of AL and IAL yield, energy consumption, and carbon emissions

Extraction techniques	AL yield (mg/g)	IAL yield (mg/g)	Energy consumption (KWh/kg extract)	CO ₂ emission (kg CO ₂ /kg extract)
Maceration	20.0 ± 0.2 ^B	18.9 ± 0.7 ^B	38.4	30.72
HPH	38.2 ± 0.7 ^A	34.4 ± 0.2 ^A	0.22	0.18

Values are mean ± SD. Different letters indicate significant differences between groups ($p < 0.05$)

Conclusions

This study evaluated the extraction of sesquiterpene lactones from *I. helenium* roots with high-pressure homogenization. The results unequivocally revealed that high-pressure homogenization significantly increases the efficiency of SL extraction, thus enhancing the antioxidant, antiglycation, and antimicrobial activities of the extracts (an approximately 90% improvement for all parameters). SEM results showed that the HPH technique damages the microstructure of *I. helenium* root cells, which is considered to increase the permeability of the extraction solution in *I. helenium* root cells, enhance the solubility of the intracellular components, and shorten the extraction period. This study also demonstrated that HPH is a good alternative to conventional techniques regarding energy consumption and carbon emissions at the pilot scale. Thus, HPH can be considered a feasible and sustainable technology to extract sesquiterpene lactones from *I. helenium* root extracts at the industrial level. Consequently, the HPH treatment offers advantages, including shorter extraction times, lower energy consumption and carbon emissions, and higher extraction yields of sesquiterpene lactones. The results and advantages of this study suggest that the HPH technique might be used on an industrial scale as a future potential alternative to traditional extraction techniques.

The following supporting information: Fig. S1: LC–MS/MS chromatogram for AL and IAL and their structural formulas; Fig. S2: The preliminary trial results of maceration.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11947-024-03361-9>.

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Data Availability The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

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