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Multicriteria comparison of green and conventional phenolic extraction technologies applied to *Ficus carica* L. leaves

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ABSTRACT

Phenolic compounds are key bioactive constituents with antioxidant and antimicrobial properties relevant to food applications. This study provides a multicriteria comparison of conventional solvent extraction and green-assisted technologies for the recovery of phenolics from *Ficus carica* L. agro-industrial residues. Conventional solid-liquid extraction using solvents of different polarity was compared with ultrasound-assisted extraction and homogenisation-ultrasound-assisted extraction. Extracts were characterised in terms of phenolic profile, antioxidant capacity, and antimicrobial activity, while process sustainability was evaluated through life cycle and life cycle cost analyses. The highest phenolic recovery was achieved using 40% ethanol-water under HUAE (36.5 mg_{GAE}/g_{DW}; 28.6 mg_{QE}/g_{DW}), with antioxidant activity strongly correlated to phenolic content ($R^2 > 0.85$), while extracts exhibited the strongest antimicrobial activity against *Listeria monocytogenes*. Life cycle assessment revealed a reduction of up to 90% in environmental impact for ultrasound-assisted extraction, rendering an efficient and relatively low-cost alternative for food-grade phenolic recovery.

1. Introduction

Phenolic compounds have garnered immense scientific and industrial interest due to their demonstrated efficacy as antioxidants, anti-inflammatory agents, antimicrobials, and potential modulators of chronic disease pathways (Sun et al., 2024). Their incorporation into nutraceutical, cosmeceutical, and pharmaceutical formulations has driven a need for efficient, scalable, and sustainable recovery methods from plant matrices. Among these matrices, the agro-industrial residues of fig (*Ficus carica* L.) represent an underutilised source of high-value phenolics (e.g., chlorogenic acid, rutin, quercetin glycosides), offering a promising feedstock for circular bioeconomy initiatives (Xyderou-Malefaki et al., 2025).

The cultivation is concentrated in the Mediterranean basin, where annual global production surpasses one million tonnes, with Turkey, Egypt, and Algeria collectively contributing more than half of this yield (Food and Agriculture Organization of the United Nations, 2025). Industrial processing of figs for fresh, dried, and processed products generates substantial quantities of by-products, primarily leaves and peels, which are frequently discarded, landfilled, or incinerated. Such practices not only exacerbate greenhouse gas emissions and environmental pollution (Teruel-Andreu et al., 2021) but also result in the loss of bioactive compounds found in these by-products. Ayuso et al. (2022) have identified fig leaves (FL) and peels as major side streams, rich in phenolic acids (e.g., gallic, chlorogenic), flavonoids (e.g., rutin, quercetin), flavanols (catechin, epicatechin), and anthocyanidins (cyanidin derivatives), which collectively contribute to the leaves' traditional use in treating gastrointestinal, urinary, respiratory, and dermal conditions (Abdel-Rahman et al., 2021; Liu et al., 2024).

The efficient extraction of these compounds hinges on the choice of solvent and processing technology. Conventional solid-liquid extraction (SLE) methods typically employ organic solvents of varying polarity, such as methanol (MeOH), ethanol-water mixtures (EtOH–W), water (W), or ethyl acetate (EtAc) to maximise solute solubility and selectivity.

Abbreviations: SLE, solid-liquid extraction; UAE, ultrasound-assisted extraction; HUAE, homogenisation-ultrasound-assisted extraction; TPC, total phenolic content; TFC, total flavonoid content; AC, antioxidant capacity; MIC, minimum inhibitory concentration; EtOH, ethanol-water; MeOH, methanol; W, distilled water; EtAc, ethyl acetate; EtOH:W40%, mixture of 40% of ethanol in water; Rt, retention time; FL, Fig leaves; n.a., no antimicrobial activity observed.

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However, these approaches are often associated with high solvent consumption, lengthy processing times, and significant energy requirements, raising concerns over operational costs, worker safety, and environmental sustainability (Boateng, 2023; Picot-Allain et al., 2021). In response, green extraction technologies such as ultrasound-assisted extraction (UAE) and homogenisation-ultrasound-assisted extraction (HUAE) have emerged. UAE applies high-frequency waves to induce cyclic compression in the solvent, generating localised pressure differentials and microstreaming that disrupt plant cell walls and facilitate solute release (Tang et al., 2024). HUAE augments this effect by integrating high-shear mechanical homogenisation, reducing particle size and increasing surface area with sonication, thus this process can synergistically enhance mass-transfer kinetics and reduce extraction times up to 80%, compared with conventional ones (Tang et al., 2024). Preliminary studies have demonstrated that these methods can achieve total phenolic contents (TPC) comparable to those of SLE, while reducing solvent volumes by up to 60% and energy inputs by 30–45% (Ștefănescu et al., 2024).

The actual environmental and economic benefits of green extractions compared to conventional ones remain largely context-dependent and underexamined. Life cycle assessment (LCA) provides a standardised framework to quantify cradle-to-gate impacts-including global warming potential (kg CO_2-eq), enabling a holistic comparison of process alternatives. While LCA studies have evaluated tannin, essential oil, and general polyphenol extractions (Baquero et al., 2025; Ding et al., 2017; Vauchel et al., 2018), a critical research gap remains concerning the LCA-integrated appraisal of phenolic recovery from FL residues.

The present study addresses this gap by conducting a side-by-side assessment of SLE, UAE (bath mode), and HUAE for the extraction of phenolics from FL. The working hypothesis is that green extraction can achieve a superior balance between chemical efficiency (phenolic yield and profile), biological functionality (antioxidant and antimicrobial activity), and environmental sustainability compared with conventional SLE. Three solvents (pure methanol, distilled water, and a 40% (v/v) ethanol-water solution) were selected to span a range of polarities and be food-grade. A 40% (v/v) ethanol-water mixture was selected as an adequate compromise between extraction efficacy and environmental sustainability, as has been experimentally shown to efficiently extract medium-polar phenolic compounds. Extracts were evaluated for TPC, antioxidant capacity (DPPH and ABTS assays), and antimicrobial efficacy against representative Gram-positive and Gram-negative strains. By integrating extraction performance with environmental impact metrics, this work aims to delineate the optimal extraction process for valorising fig agro-industrial by-products, thereby advancing sustainable bioactive recovery.

2. Materials and methods

2.1. Chemicals and reagents

The reference standards (protocatechuic acid, catechin, chlorogenic acid, epicatechin, apigenin-xylosyl-glucoside, apigenin-malonyl-glucoside, luteolin-glucoside, kaempferol-xylosyl-rhamnoside, apigenin-rutinoside, quercetin-rutinoside, apigenin-glucoside, ellagic acid, kaempferol-rutinoside, quercetin-glucoside) were purchased from Sigma-Aldrich (Steinheim, Germany). Chemical reagents required for extraction, as well as chemical and biological characterisation, were also sourced from the same supplier. Culture media for antimicrobial activity assays (PDA and TSA) were obtained from HiMedia (India) and Sigma-Aldrich (Steinheim, Germany). All High-Performance Liquid Chromatography (HPLC) standards for phenolic compound identification and the solvents were chromatographic grade (>99%). Acetonitrile, HPLC-gradient, was provided by Merck (Germany), and water was purified with a Direct-Q UV system by Millipore (USA).

Table 1
Summary of extraction conditions (temperature, time, and equipment) applied for solid–liquid extraction (SLE), ultrasound-assisted extraction (UAE), and homogenizer-assisted ultrasound extraction (HUAE).

Extraction method	Temperature (°C)	Time (min)	Agitation/Equipment
SLE	25	30	Stirrer (350 rpm, C-MAG HS4)
UAE	37	15	Ultrasonic bath (*Elmasonic E15H*)
HUAE	37	15	Ultrasonic homogenizer (*Sonopuls HD220*, 20 kHz, 180 W, probe tip area 1.13 cm^2)

2.1.1. Plant material

FL (*Ficus carica* L. leaves) was harvested in September 2023 from a local agricultural site in Kissamos Province, Chania, located on the island of Crete, Greece (geographical coordinates: 35.455605° N, 23.616234° E). The leaves were sun-dried outdoors in the shade for 15 days under a protective tulle. Once dried, the leaves were stored in shielded plastic bags for further processing. Using a grinder (Optimum 9200A 2nd Gen Blender, 2611W), the dried leaves were finely ground and passed through a 40-mesh sieve to ensure uniform particle size. The powders were stored in sealed bags under dry, dark, and ambient conditions until further extraction procedures.

2.2. Extraction methods

All extraction procedures (Table 1) were performed using a solid-to-solvent ratio of 1:20 (*w/v*) (Xyderou-Malefaki et al., 2025), dispersing FL powder in one of four solvents: water, 40% ethanol in water (EtOH: W40%), methanol (MeOH), or ethyl acetate (EtAc) (Gil-Martín et al., 2022). EtOH: W40% was selected based on previous studies showing that hydroalcoholic mixtures optimise solvent polarity and enhance the extraction of both polar and moderately polar phenolic compounds (Ștefănescu et al., 2024), while also representing a food-grade and more cost-effective solvent system suitable for potential industrial applications. After extraction, all samples were centrifuged at 10,000 rpm for 10 min, and the supernatants were filtered using filter paper (Whatman No. 1) and stored at −18 °C. Before analysis, all extracts were concentrated 20-fold using a rotary evaporator (Buchi Rota vapor R124, Switzerland) at 40 °C, 18 mbar for 30 min to avoid phenolic degradation and eluted in the appropriate solvent to a final dry matter concentration of 2 g/L. After that, 1 mL of each concentrated extract was diluted with 4 mL of the corresponding solvent to obtain a final volume of 5 mL, filtered through a 0.45 μm Chromafil nylon filter, and used for analytical protocols and other analyses. The remaining 1 mL was used for biological protocols.

2.2.1. Solid-liquid extraction (SLE)

This method was adapted from Ammar et al. (2015). The extraction was performed at 25 °C with continuous magnetic stirring at 350 rpm for 30 min, selected as the optimal compromise between extraction efficacy and environmental sustainability (Costa et al., 2014; Xyderou-Malefaki et al., 2025).

2.2.2. Ultrasound-assisted extraction (UAE)

Based on Ștefănescu et al. (2024), extraction was conducted in a 100 mL Erlenmeyer flask using an ultrasonic bath (Elmasonic E15H, Elma, Singen, Germany) at 37 °C for 15 min.

2.2.3. Homogenisation-ultrasound-assisted extraction (HUAE)

A Bandelin Sonopuls HD220 ultrasonic horn (Germany) was used, operating at 20 kHz and 180 W, equipped with a titanium probe (1.13cm^2 active surface) (Ozsefil & Ziylan-Yavas, 2023; Yang et al., 2021).

Table 2
Experimental design and abbreviations of *Ficus carica* leaf (FL) extracts obtained using different extraction methods (UAE, HUAE, and SLE) and solvents.

Number	Extract Name	Extraction Method	Solvent
1.	FLUAEEtOH: W40%	UAE	40% EtOH in Distilled water
2.	FLUAEEtAc	UAE	Ethyl Acetate
3.	FLUAEW	UAE	Distilled water
4.	FLHUAEEtOH: W40%	HUAE	40% ethanol in Distilled water
5.	FLHUAEEtAc	HUAE	Ethyl acetate
6.	FLHUAEW	HUAE	Distilled water
7.	FLSLEEtOH: W40%	SLE	40% EtOH in Distilled water
8.	FLSLEEtAc	SLE	Ethyl acetate
9.	FLHUAEMeOH	HUAE	Methanol
10.	FLUAEMeOH	UAE	Methanol
11.	FLSLEMeOH	SLE	Methanol
12.	FLSLEW	SLE	Distilled water

2.3. Solvent selection and experimental design

The choice of solvents was based on polarity and environmental considerations. W was selected as a green solvent, MeOH and EtAc as conventional solvents, and EtOH: W 40% as a broad-spectrum mixture (Rieckmann et al., 2024; Ștefănescu et al., 2024). The combinations of extraction method and solvent used are summarised in Table 2, along with their abbreviations for clarity in subsequent sections.

2.4. HPLC-DAD-ESI-MS analysis

Phenolic compound analysis was conducted using an Agilent HP-1200 high-performance liquid chromatography (HPLC) system (Agilent Technologies, USA), equipped with a vacuum degasser, quaternary solvent delivery pump, autosampler, diode array detector (DAD), and a 6110 single-quadrupole mass spectrometer with an atmospheric pressure ionisation electrospray (API-ESI) interface. Phenolic compounds were detected in positive ionisation mode (ESI^+), with fragmentor voltages ranging from 50 to 100 V. Separation was performed on a Kinetex XB-C18 column (5 μm; 4.5 × 150 mm) from Phenomenex (USA). The mobile phase consisted of (A) water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. A multistep linear gradient was applied, starting with 5% B for 2 min, increasing to 90% B over 20 min, keeping it at 90% B for 4 min, and then returning to 5% B over 6 min. The total run time was 30 min, with a flow rate of 0.5 mL/min and an oven temperature of 25 ± 0.5 °C (Ștefănescu et al., 2024). Mass spectrometric detection was performed in Scan mode for positively charged ions. The experimental conditions included a gas temperature of 350 °C, a nitrogen flow rate of 7 L/min, a nebuliser pressure of 35 psi, a capillary voltage of 3000 V, a fragmentor voltage of 100 V, and an *m/z* range of 120–1500. Chromatograms were recorded at 280 nm and 340 nm wavelengths, with data acquisition managed using ChemStation software (Agilent Technologies, CA, USA), version Rev. B.02.01-SR2. Phenolic compound identification and quantification were based on retention times, UV–Vis absorbance spectra, and mass spectral data, compared with three reference standards. Flavanols were quantified using a calibration curve of catechin (10–200 μg/mL), expressed as catechin equivalents ($mg_{catechin}/g_{DW}$) ($y = 15.224x - 130.24$, $R^2 = 0.9985$). Hydroxycinnamic acids were quantified based on a chlorogenic acid calibration curve (10–50 μg/mL), expressed as chlorogenic acid equivalents ($mg_{chlorogenic\ acid}/g_{DW}$) ($y = 22.585x - 36.728$, $R^2 = 0.9937$). Flavonol quantification was performed using a quercetin calibration curve (10–200 μg/mL), expressed as quercetin equivalents ($mg_{quercetin}/g_{DW}$) ($y = 87.392\times + 78.795$, $R^2 = 0.9951$). The flavone content was calculated using a five-point calibration curve of luteolin ($y = 68.857x - 25.113$, $R^2 = 0.9972$) in the linearity range of 1–100 μg/mL (Tables S1 and S2).

2.5. Extraction yield

After the extraction was completed, the herbal residue was separated from the liquid extract as described and the recovered liquid extract was measured in a volumetric cylinder. The solid content of the liquid extracts was determined gravimetrically. Pre-weighed vials containing known volumes of the extracts were either placed directly in a drying oven at 100 °C overnight (for aqueous extracts) or left to evaporate in a fume hood overnight (for organic solvent-based extracts, MeOH or EthAc) to allow for solvent removal under safe conditions. Subsequently, all vials were transferred to a drying oven at 100 °C for an additional 1 h to ensure complete drying, then cooled in a desiccator and reweighed using an analytical balance. The solid content was expressed as g/L ± standard deviation (SD).

The extraction yield (%) was calculated as the mass of total solids obtained from 100 g of dried plant material, according to Eq. (1).

$$\text{Yield of extraction (\%)} = \frac{100 \times \text{total solids (g)}}{\text{corresponding dry herbal material}} \quad (1)$$

The moisture content of the herbal material was expressed as g/100 g ± SD. In glass beakers, triplicate samples of 1 g of material were weighed on an analytical balance and then placed at 105 °C for 1 h (Dimopoulou & Kontogiorgos, 2020). They were then placed in a desiccator until they reached room temperature and were weighed.

2.6. Total phenolic content

TPC was evaluated using a modified Folin-Ciocalteu assay in a 96-well microplate. A 20 μL aliquot of each sample was added to the wells, followed by 100 μL of Folin-Ciocalteu reagent (1:10, v/v). The plate was then incubated at room temperature in the dark for 3 min. After incubation, 80 μL of sodium carbonate solution (7.5% *v/w*) was added to each well, and the mixture was further incubated for 30 min. A solvent blank was prepared to account for background interference, and its absorbance was measured along with the samples at 760 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA). A standard calibration curve was made using gallic acid 20s, ranging from 0.01 to 1.00 mg/mL. The total phenolic content (TPC) was calculated and expressed as milligrams of gallic acid equivalents (mg_{GAE}/g_{DW}) per gram of dry weight (Zhang et al., 2006).

2.7. Total flavonoid content (TFC)

TFC was determined using the aluminium chloride colourimetric method, as per Shraim et al. (2021), with slight modifications. The extracts were first diluted with 720 μL of distilled water in a 6-well plate, followed by the addition of 90 μL of 5% $NaNO_2$ (*w/v*). After 5 min incubation, 90 μL of 10% ethanolic $AlCl_3$ (w/v) was added, and the mixture was incubated for another 5 min. Subsequently, 600 μL of 1 N NaOH was added to complete the reaction. Absorbance was measured at 510 nm using quercetin as the reference standard. Each measurement was performed in triplicate. Absorbance was measured at 510 nm using a multi-mode plate reader (BioTek, Winooski, VT, USA), and the total flavonoid content was expressed as milligrams quercetin equivalents (QE) per gram dry weight (DW) (mg_{QE}/g_{DW}).

2.8. In vitro antioxidant activity

For antioxidant capacity assays, extracts obtained with ethyl acetate, were evaporated to dryness and subsequently re-dissolved in methanol prior to analysis to ensure compatibility with the aqueous reaction medium, following commonly applied antioxidant assay protocols (Christodoulou et al., 2022).

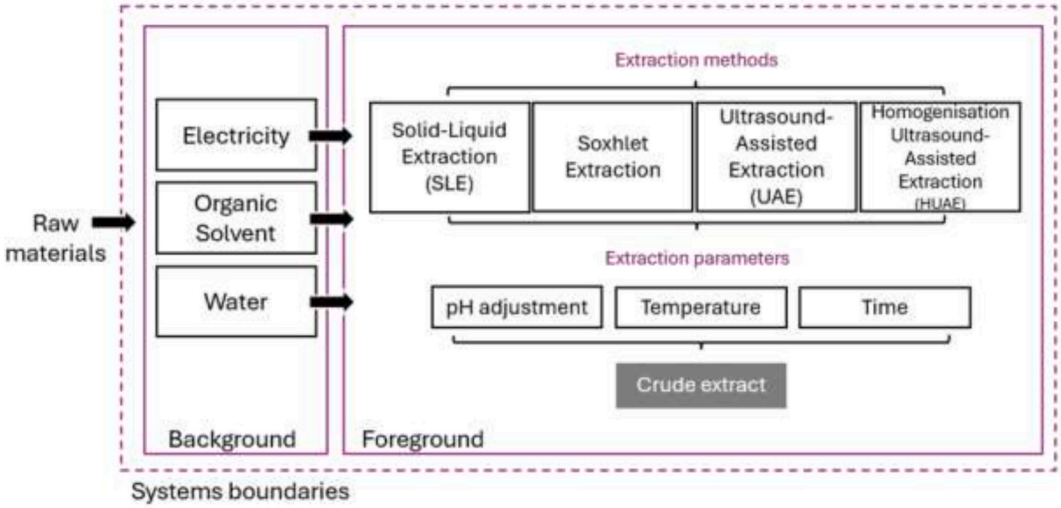

Fig. 1. Life cycle assessment (LCA) framework applied to evaluate selected extraction methods of *Ficus carica* leaf by-products. The scheme shows the systems' boundaries as well as the background and the foreground system.

2.8.1. DPPH assay

The antioxidant capacity of the extracts was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay. To determine the antioxidant capacity, triplicate samples were prepared by mixing 35 μL of the extracted sample with 250 μL of a methanol-based DPPH solution (Sharma & Bhat, 2009) in a 96-well microplate. The mixture was then incubated in the dark at room temperature for 30 min. Absorbance was measured at 515 nm using a multi-mode plate reader (BioTek, Winooski, VT, USA), and a calibration curve was generated using a Trolox standard. Results were expressed as micromolar Trolox equivalent per gram dry weight of extract ($\mu M_{TE}/g_{DW}$).

2.8.2. CUPRAC assay

The total antioxidant capacity of the extracts was also assessed using the CUPRAC (copper reducing antioxidant capacity) assay, as described in a previously published method (Apak et al., 2004). In a 96-well microplate, 25 μL of each sample, solvent (blank), or Trolox standard was pipetted in triplicate. Subsequently, 175 μL of the CUPRAC reagent solution, prepared at a 1:1:0.5 ratio of ammonium acetate buffer (1 M, pH 6.8–7), $CuCl_2$ solution (10 mM), and neocuproine solution (7.5 mM), was added. The plate was then incubated in the dark for 30 min. After incubation, absorbance was measured at 450 nm using a microplate reader (BioTek Instruments, Winooski, VT, USA). A calibration curve was constructed using Trolox concentrations ranging from 25 to 700 μM. The results were expressed as micromolar Trolox equivalent per gram dry weight of extract ($\mu M_{TE}/g_{DW}$).

2.9. Antimicrobial activity

2.9.1. Microbial strains

The following eight standard microbial and fungal strains, obtained from the Food Biotechnology Laboratory at UASVM Cluj-Napoca, Romania, were tested: *Staphylococcus aureus* subsp. *aureus* ATCC 29213, *Candida parapsilosis* ATCC 22019, *Candida albicans* ATCC 20367, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Listeria monocytogenes* DSMZ 115292, *Aspergillus niger* ATCC 6275, and *Aspergillus brasiliensis* ATCC 16404. The strains were cultivated in test tubes containing 9 mL of specific media, as indicated by the supplier: tryptic soy broth (TSB), tryptic soy yeast extract (TSYM), and potato dextrose agar (PDA). Incubation conditions were *C. parapsilosis, E. coli, P. aeruginosa, S. aureus* in TSB, and *L. monocytogenes* in TSYM at 37 °C for 24 h. *C. albicans*, *A. niger,* and *A. brasiliensis* in PDB at 30 °C for 24–48 h. A loopful of each inoculum was transferred to an agar growth medium, with plates incubated for 24 h at either 37 °C or 30 °C, depending on the strain. Bacterial morphology was examined using optical microscopy (Nikon ECLIPSE Ci-L, Tokyo, Japan) to ensure accurate strain identification and confirm microbial response (Martău et al., 2021).

2.9.2. Disc diffusion protocol

The disk diffusion method was implemented as described in the standardised antimicrobial susceptibility test from Hudzicki, (2009), and it was used to evaluate the effectiveness of antibiotics against bacterial isolates. The agar plates were inoculated with a standardised bacterial suspension (10^5 for bacteria and 10^6 CFU/mL for yeasts), ensuring even distribution across the surface using a Drigalski spatula. The 9 mm paper disks were then placed onto the agar, and 10 μL from the test extract was added, allowing the extract, the antibiotic, or the solvent to diffuse outward. The plate containing the fungi was incubated at 30 °C for 36 h, while the plate containing the bacteria was incubated at 37 °C for 24 h. Following the incubation period, the diameter of inhibition zones was measured using a digital caliper (Lumytools). For positive controls, Gentamicin (0.4 mg/mL in saline) and Ketoconazole (1 mg/mL in DMSO) were used (Ștefănescu et al., 2024), and for the negative control was the solvent.

2.9.3. Determination of the minimum inhibitory concentration (MIC)

Multiple colonies of each strain were then suspended in 9 mL of sterile saline solution (8.5 g/L NaCl) and adjusted to match the turbidity of the McFarland 0.5 standard (10^8 CFU/mL). The microbial suspensions were adjusted to final concentrations of 10^5 CFU/mL for bacterial strains and 10^6 CFU/mL for fungal strains and subsequently inoculated into individual wells of the microtiter plates. The spore suspension was prepared with a 0.1% Tween 80 solution and sterile glass beads to dislodge the spores from the surface of the agar plates. A 1 mL sample from the concentrated spore suspension was collected from the agar and transferred into a 9 mL sterile saline solution. The concentration of spores in the suspension was then quantified using a Thoma counting chamber. The spore suspension was adjusted to a final concentration of 1×10^6 spores/mL for inoculation.

A resazurin microtiter plate-based antibacterial assay was used to determine the *minimum inhibitory concentration* (MIC). First, 100 μL of a sterile culture medium was added to the wells of a 96-well microplate. Then, 100 μL of each extract (at a concentration of 71.43 mg/mL) was introduced into the first well, followed by a series of 2-fold dilutions across the row. Afterwards, 10 μL of inoculum was added to all wells. For controls, gentamicin (0.4 mg/mL in saline) or ketoconazole (1 mg/mL in

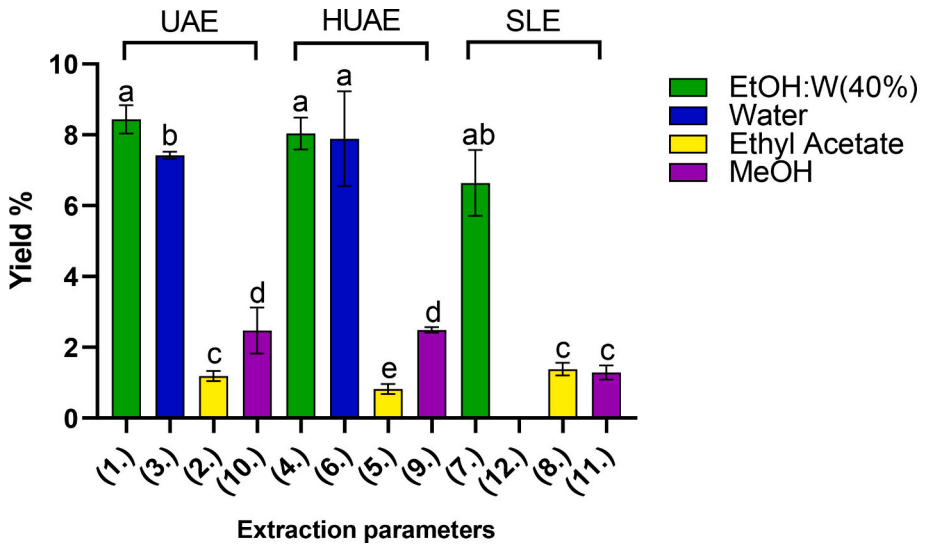

Fig. 2. Yield (%) of FL extracts obtained using different extraction parameters. Notes: Numbers (1.-12.) correspond to specific extraction conditions combining method and solvent, as detailed in the methodology section. Extraction methods are indicated in abbreviations and brackets (UAE, SLE, HUAE), while different colors represent the solvent used (blue for W, purple for MeOH, yellow for EtAc, green for EtOH: W40%). Values are presented as mean ± SD ($n = 3$). Different letters indicate statistically significant differences between treatments ($p < 0.05$), according to one-way ANOVA followed by Tukey's post hoc test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

DMSO) served as positive controls (C^+), while the extraction solvent (EtOH: W40%) was used as the negative control (C^-). The microplates were incubated for 20–22 h at 37 °C or 30 °C. After incubation, 20 μL of sterile resazurin solution (0.2 mg/mL) was added to each well, followed by a further 2-h incubation at the same temperature. The lowest concentration at which the blue colour remained unchanged indicated complete bacterial growth inhibition, representing the MIC (Karuncharoenpanich et al., 2025). All experiments were conducted in duplicate ($n = 2$).

2.10. LCA and LCC

A life cycle assessment (LCA) was conducted to evaluate the environmental and economic performance of different extraction techniques for obtaining phenolic extracts from the FL waste. The methodological approach follows the LCA framework outlined in ISO14040/44:2006 (ISO, 2006), which comprises four key stages: (1) goal and scope definition, establishing the study's objective and setting the spatial and temporal boundaries of the extraction system; (2) life cycle inventory (LCI), compiling an inclusive list of all inputs and outputs associated with the extraction process; (3) life cycle impact assessment (LCIA), quantifying the environmental impact indicators based on the inventory flows and relevant characterisation factors; and (4) interpretation of results, which draws broader conclusions regarding the sustainability of each extraction method. The impact indicators employed to evaluate the environmental impact of different extraction methods from FL in this study are illustrated in Table S3 (Rodríguez-Meizoso et al., 2012).

Different extraction techniques, SLE, UAE, and HUAE, were assessed with a focus on phenolic compounds extracted from the FL waste. The analysis was performed using SimaPro 9.2 academic license, the latest ecoinvent database, and the environmental footprint 3.0 methodology.

The system boundaries, as illustrated in Fig. 1, include all the processes considered in the extraction system for obtaining the crude extracts from raw materials. This system is divided into background and foreground components, where background processes include the provision of essential inputs such as electricity, organic solvents, and water. Foreground processes represent the core operational stages where extraction occurs, encompassing the extraction methods selected for this work: SLE, UAE, and HUAE. These methods operate under specific extraction parameters (pH adjustment, temperature, and time) to influence the yield and quality of the crude extract. The functional unit is defined as 1 mg_{GAE} of phenolic compounds, providing a common basis for comparing the different techniques.

Alongside the environmental assessment, a cost analysis is conducted, considering the price of solvents, energy, and other operational inputs. The Life Cycle Cost (LCC) approach analysis is defined by the international standards published in 2008 (ISO15686-5). However, since there are no shared methodologies to perform the analysis (Gluch & Baumann, 2004), we have adopted an ad hoc method for this paper. The cost of the different extraction methods was considered to perform the LCC, focusing on the extraction material and cost of energy, without considering the equipment cost. By integrating environmental and economic dimensions, the study aims to identify the most sustainable and cost-effective extraction method, thereby guiding stakeholders in optimising waste valorisation for phenolic compound recovery.

2.11. Statistical analysis

The results from each experiment, conducted in triplicate ($n = 3$) or quadruplicate ($n = 4$), were reported as mean values ± standard deviation (SD). Statistical analyses were carried out using GraphPad Prism version 8.0.1 (GraphPad Software Inc., San Diego, CA, USA). One-way analysis of variance (ANOVA) was applied, followed by Tukey's post hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Yield of the extractions

The results indicate that the best solvent for extracting phenolic compounds and maximising yield was EtOH: W(40%) ($p < 0.05$), followed by water (W), methanol (MeOH), and ethyl acetate (EtAc) (Fig. 2). The highest extraction yield was obtained using EtOH: W(40%), reaching 8.7 ± 0.3%, followed by W (7.8 ± 0.4%), MeOH (2.5 ± 0.2%), and EtAc (1.2 ± 0.1%). The combination of ethanol and water facilitates the solubilisation of both hydrophilic and moderately lipophilic phenolics, thereby enhancing extraction efficiency (Kaur & Kaur, 2014; Keddar et al., 2020). Li et al. (2006) used aqueous ethanol to extract phenolics from citrus peels and reported a high extraction efficiency.

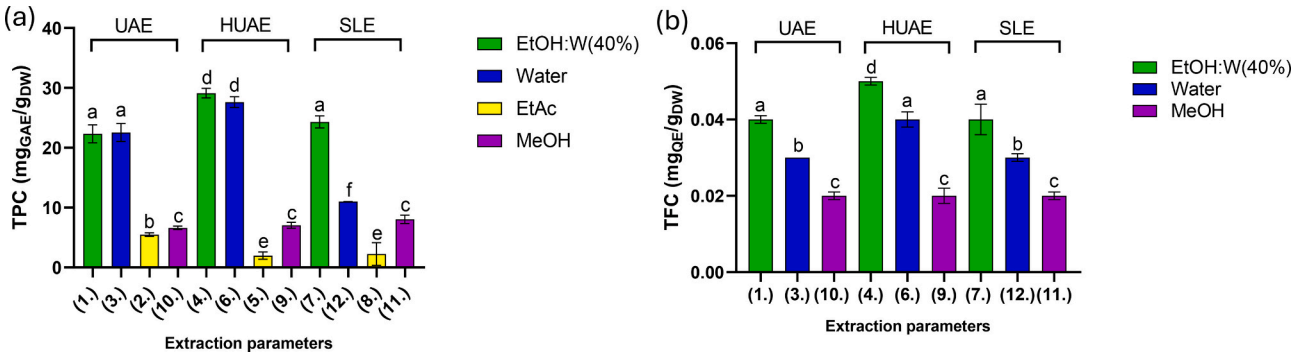

Fig. 3. (a) Total phenolic content (TPC) of FL extracts obtained using different extraction parameters. Values are expressed as mg_{GAE}/g_{DW} (mean ± SD, $n = 3$) and (b) Total flavonoid content (TFC) of FL extracts obtained under different extraction parameters. Values are expressed as mg_{QE}/g_{DW} (mean ± SD, $n = 3$). Extraction methods are grouped, with each condition coded from (1.) to (12.) according to specific combinations of method and solvents described in the methodology section. Solvents are represented by colour: EtOH:W 40% (green), Water (blue), EtAc (yellow), and MeOH (purple). Different letters indicate statistically significant differences between treatments ($p < 0.05$), according to one-way ANOVA followed by Tukey's post hoc test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

They found that using ~70% ethanol (v/v) yielded significantly higher total phenolic recovery compared to pure water or pure ethanol. Therefore, the enhanced performance of the ethanolic mixture is due to its ability to extract both hydrophilic and moderately lipophilic phenolics (Plaskova & Mlcek, 2023).

3.2. TPC and TFC of the extractions

Among the tested extraction methods, HUAE extract 4, using EtOH: W 40% and W, yielded the highest total phenolic content (TPC) (Fig. 3), with values of 36.5 ± 1.2 mg_{GAE}/g_{DW} and 35.8 ± 1.1 mg_{GAE}/g_{DW}, respectively. These were followed by the UAE extract (1.) FLUAEEtOH: W40% (28.7 ± 0.9 mg_{GAE}/g_{DW}) and extract (7.) with EtOH: W40% (27.9 ± 1.0 mg_{GAE}/g_{DW}). Lower TPC values were recorded for extracts obtained using EtAc or methanol, such as UAE EtAc (sample (2.), 9.3 ± 0.7 mg_{GAE}/g_{DW}), HUAE EtAc (sample (5.), 7.1 ± 0.6 mg_{GAE}/g_{DW}), and SLE-EtAc (sample (8.), 5.3 ± 0.40 mg_{GAE}/g_{DW}). Both in HUAE and UAE, there was no significant difference between EtOH: W40% and W extracts.

HUAE extract 4 yielded higher TPCs than previously achieved. The findings that EtOH: W40% was more efficient than water for flavonoid extraction are in line with previous studies. Akhtar et al. (2019) compared UAE with maceration techniques across 18 fig cultivars. The TPC values obtained via UAE ranged from 12.94 ± 0.23 to 23.04 ± 0.31 mg_{GAE}/g_{DW}, which were higher than those obtained through maceration (10.70 ± 0.20 to 21.18 ± 0.68 mg_{GAE}/g_{DW}). Merzić et al. (2021) observed TPC values of 12.8 mg_{GAE} in fig leaf. Also, although MeOH and EtAc are polar solvents, their lower TPC results (UAE–MeOH, 11.8 ± 0.5 mg_{GAE}/g_{DW}) are influenced by matrix-solvent interactions (Wakeel et al., 2019). A similar result has been reported by Gómez-Mejía et al. (2021) in leaf extractions from *Artocarpus heterophyllus*, another *Moraceae* species, where EtOH: W40% outperformed EtAc and MeOH in phenolic yield.

EtOH: W40% emerged as the most effective solvent for extracting phenolic compounds (samples (1.), (4.), and (7.), yielding significantly higher TPC values compared to MeOH, W, and EtAc ($p < 0.05$). Among the extraction techniques, HUAE provided the highest polyphenol yield and antioxidant capacity, followed by SLE and UAE. The efficiency of ultrasound-assisted methods, HUAE and UAE, highlights their effectiveness in disrupting plant cell walls and enhancing the release of bioactive compounds (Zhang et al., 2021).

The TFC (Fig. 3) of FL extracts varied significantly across the different extraction conditions, ranging from 3.86 ± 0.20 mg_{QE}/g_{DW} to 28.60 ± 0.56 mg_{QE}/g_{DW}. The highest TFC was observed in extract (7.), obtained by HUAE using EtOH: W40%, while the lowest TFC was recorded in extract (8.), extracted with water using the same method. Among the three techniques, HUAE generally resulted in superior flavonoid recovery, particularly when paired with EtOH: W40%. This synergistic effect enhanced cell disruption and promoted the release of both free and bound flavonoids, as previously reported for *Ficus carica* and other plant matrices (Zhang et al., 2021; Ahmed & Hassan, 2023).

Comparison with the TPC data shows that samples with high

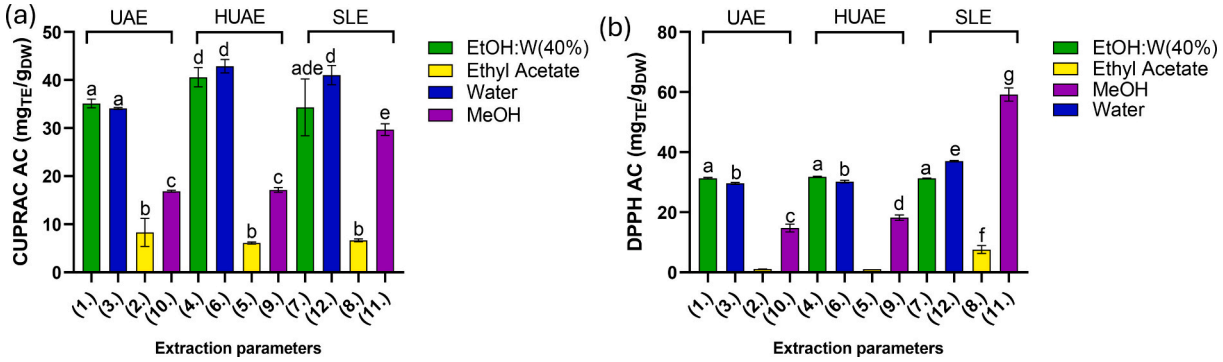

Fig. 4. (a) CUPRAC antioxidant capacity (CUPRAC AC) and (b) DPPH antioxidant activity (DPPH AC) of FL extracts obtained under different extraction parameters. Values are expressedas mg_{TE}/g_{DW} (mean ± SD, $n = 3$). Extraction methods are grouped (UAE, HUAE, and SLE, witheach treatment coded from (1.) to (12.), as defined in the methodology. Solvents are representedby different colors: EtOH: W40% (green), EtAc (yellow), W (blue), and MeOH (purple). Different letters indicate statistically significant differences between treatments ($p < 0.05$), based on one-way ANOVA followed by Tukey's post hoc test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

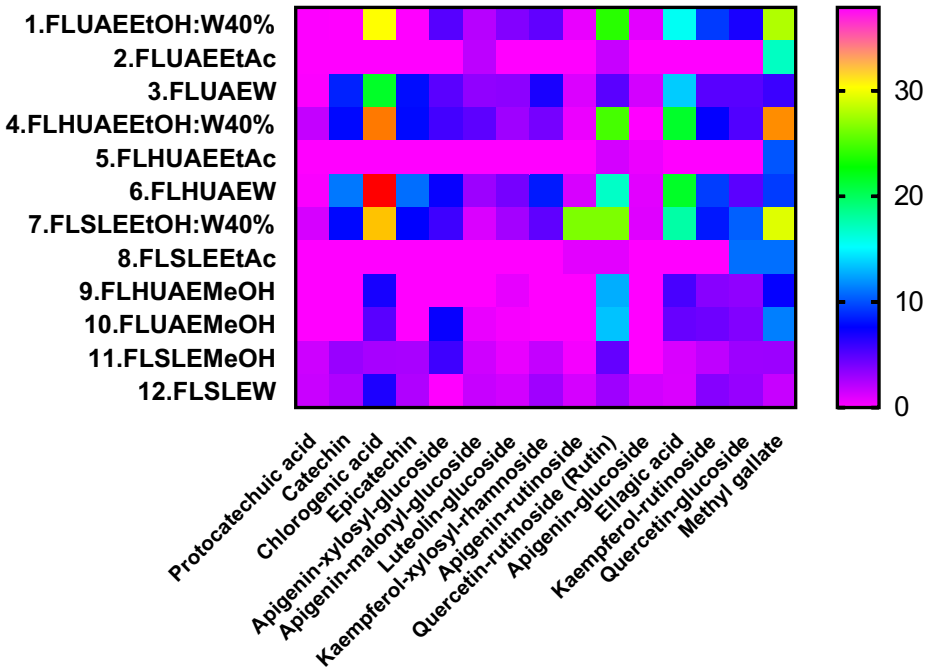

Fig. 5. Heat map of phenolic profiles in *Ficus carica* leaf extracts obtained under twelve extraction conditions. Colour difference indicates the abundance of each compound (scaled 0–40 within columns), with red representing the highest levels and pink the lowest. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

flavonoid content also tended to exhibit elevated phenolic levels, as seen in extract (7.) (TFC: 28.60 mg_{QE}/g_{DW}; TPC: 247.19 mg_{QE}/g_{DW}) and extract (4.) (TFC: 25.75; TPC: 240.81 mg_{QE}/g_{DW}). Conversely, extracts obtained using MeOH and Water consistently showed reduced TFC and TPC, highlighting the inefficiency of those polar solvents. EtAc showed no flavonoid content, which agrees with the very low TPC.

3.3. Antioxidant activity of the extracts

Among all extraction conditions, the HUAE extract obtained with EtOH: W40% showed the highest CUPRAC value (47.2 ± 1.5 mg_{TE}/g_{DW}), followed by MeOH (44.8 ± 2.0 mg_{TE}/g_{DW}), while the lowest antioxidant capacity was recorded for the ethyl acetate extract obtained by SLE (9.5 ± 0.8 mg_{TE}/g_{DW}). A strong positive correlation ($R^2 = 0.85$, $p < 0.01$) was observed between TPC and antioxidant capacity (Fig. 4), suggesting that polyphenol-rich extracts have higher radical scavenging potential. This correlation supports the hypothesis that phenolic compounds are the primary contributors to the antioxidant properties of the extracts. Also, a strong correlation was observed between AC and TFC ($R^2 = 0.95$, $p < 0.01$).

The results of the DPPH radical scavenging activity and CUPRAC assay showed a pattern like TPC. Regression analysis revealed a significant correlation between DPPH and TPC ($R^2 = 0.89$, $p < 0.01$), highlighting the importance of polyphenols as antioxidants. Comparably, olive leaf hydroalcoholic extracts, for instance, exhibit CUPRAC values between 40 and 55 mg_{TE}/g_{DW}, strongly correlated with TPC ($R^2 > 0.80$) (Benavente-Garcıa et al., 2000). Grapevine leaf extracts obtained with ethanol–water mixtures similarly show high reducing capacities (45 mg_{TE}/g_{DW}) (Norchai, 2022), while moringa leaf extracts range between 35 and 50 mg_{TE}/g_{DW} depending on the solvent and method (Siddhuraju & Becker, 2003).

3.4. Identification and quantification of phenolic compounds through HPLC-MS

The phenolic contents were expressed in μg/g extract. Among the extracts, extraction (7.) exhibited the highest TPC at 182.34 $\mu g_{GA}/g_{DW}$, followed closely by extraction (4.) (161.92 $\mu g_{GA}/g_{DW}$) and extraction (6.) (146.44 $\mu g_{GA}/g_{DW}$). In contrast, the lowest phenolic yields were recorded in extraction (5.) (11.88 $\mu g_{GA}/g_{DW}$), (2.) (20.55 $\mu g_{GA}/g_{DW}$), and (8.) (23.35 $\mu g_{GA}/g_{DW}$) in EtAc, indicating significant variation in phenolic recovery depending on the extraction method and solvent employed.

Across all samples (Fig. 5, Table S4), the most abundant compounds were chlorogenic acid, quercetin-rutinoside (rutin), methyl gallate, and ellagic acid. Chlorogenic acid reached its highest concentration in extract (6.) (37.87 $\mu g_{GA}/g_{DW}$), rutin peaked in extract (7.) (26.43 $\mu g_{GA}/g_{DW}$), methyl gallate was most abundant in extract (4.) (33.66 $\mu g_{GA}/g_{DW}$), and ellagic acid was particularly enriched in extract (6.) (21.54 $\mu g_{GA}/g_{DW}$). These results suggest that certain extraction methods promoted selective recovery of hydroxycinnamic acids and flavonol glycosides. In comparison with the literature, Akhtar et al. (2019) reported the presence of rutin, chlorogenic acid, and catechin in UAE-derived FL extracts, with rutin being the predominant compound. Zhang et al. (2021) identified quercetin, rutin, and kaempferol derivatives in UAE extracts of *Ficus carica*, achieving concentrations up to 60.07 mg/g for total flavonoids. The variability in TPC among samples is attributed to the synergistic effects of the extraction method and solvent polarity. Extract (7.), with the highest phenolic yield, benefitted from homogenisation-ultrasound synergy, enhancing cell disruption and solubilisation of polyphenols. Flavonoid glycosides such as apigenin-rutinoside, kaempferol-xylosyl-rhamnoside, and quercetin-glucoside were frequently detected across high-yielding extracts (Waizumi et al., 2024). The prominence of glycosylated forms may be attributed to the natural abundance of these derivatives in *Ficus carica* leaves (Masiala et al., 2024) or sugar-assisted stabilisation during extraction (Mojzer et al., 2016). Glycosylation enhances compound solubility in aqueous-organic systems and protect phenolics from degradation, as noted in similar plant matrices (Li et al., 2017).

The trend also supports the hypothesis that combining ethanol and ultrasound enhances the solubilisation and diffusion of flavonoid glycosides such as rutin, apigenin-glucoside, and kaempferol derivatives, all of which were major compounds identified in the HPLC-MS analysis. The lower TFC in water-based extracts may also be due to degradation or

incomplete release of flavonoids bound to the cell wall matrix (Petchsomrit et al., 2022).

3.4.1. Disc diffusion

The antimicrobial activity of different FL extracts varied (Fig. S3; Table S5) depending on the extraction method and the target microorganism. *L. monocytogenes* was the most susceptible, with inhibition observed in extracts from (4.) FLHUAEEtOH: W40%, (7.) FLSLEEtOH: W40%, (8.) FLSLEEtAc, and (10.) FLUAEMeOH. No inhibition was observed with the solvent control (DMSO, ETOH: W40%, EtAc, MeOH, W), which was used as solvent to solubilise the dry extracts. This suggests that ethanol-water and methanol were effective solvents.

In contrast, *S. aureus* showed more selective inhibition but very low, with activity mainly observed in (7.) FLSLEEtOH: W40%, (8.) FLSLEEtAc, (4.) HUAEEtOH: W40%, and (10.) FLUAEMeOH, but at lower levels than *L. monocytogenes*, indicating potential resistance mechanisms. *C. albicans* was the least affected, with inhibition seen only for sample (8.), suggesting that the antimicrobial compounds present are ineffective against fungi. In Farhan et al., 2022, leaves had a very weak effect vs *S. aureus* (1.3 mm zone) using ethanol extract. Shafique et al., 2021, found in leaves (methanol) good inhibition of *S. aureus* (Jeong et al., 2009). This pattern is consistent with the lierature, where phenolic-rich plant extracts generally exhibit stronger activity against Gram-positive bacteria than against fungi, as the latter often require higher concentrations or more targeted compounds for effective inhibition. The lower MIC values observed for the Gram-positive bacterium *S. aureus* compared with the fungal strain *C. albicans* indicate higher susceptibility of Gram-positive bacteria, likely due to their simpler peptidoglycan cell wall facilitating phenolic penetration. In contrast, the more complex fungal cell wall and ergosterol-rich membranes, along with detoxification and efflux mechanisms, limit intracellular accumulation of bioactive phenolics such as rutin, quercetin, and chlorogenic acid, which disrupt membranes, induce oxidative stress, and interfere with metabolic enzymes (Maria Daglia, 2012; Davidova et al., 2024; Simonetti et al., 2020).

Notably, ultrasound-assisted extracts (1.) FLUAEEtOH: W40% and (2.) FLUAEEtAc showed little to no inhibition, indicating that ultrasound extraction may not be an efficient method for recovering antimicrobial compounds. The more potent activity of HUAE extracts implies that homogenisation, breaking cell walls, enhances the solubility or release of bioactive compounds. Additionally, the activity of EtAc extracts suggests the presence of semi-polar antimicrobial compounds. Overall, EtOH: W40% and MeOH extracts were the most effective against *C. albicans* and *L. monocytogenes*. Consistently, *L. monocytogenes* was inhibited by plant extracts at MICs ~78–625 μg/mL. against Gram-positive food-borne pathogens (Nostro et al., 2016).

3.4.2. Minimum inhibitory concentration (MIC) of fig leaf extracts

The antimicrobial activity (Table S6) of selected FL extracts was evaluated against six microbial strains using the minimum inhibitory concentration (MIC) method ((Djais et al., 2019)). *Listeria monocytogenes* exhibited the highest sensitivity to FL extracts. The lowest MIC value (4.72 mg/L) was recorded for the UAEEtOH: W40% extract, followed by (7.) FLSLEEtOH: W40% (14.84 mg/L) and HUAE EtOH: W40% (71.88 mg/L). No inhibition was observed for this strain with water extracts.

This strong inhibition can be due to the high content of bioactive polyphenols, particularly chlorogenic acid, methyl gallate, and rutin, which were previously quantified in the UAE and SLE extracts. Chlorogenic acid, a hydroxycinnamic acid, can induce oxidative stress through ROS generation and metal ion chelation, impairing bacterial metabolism and membrane stability (Wang et al., 2022). Rutin, a glycosylated flavonoid, and its aglycone quercetin can intercalate into bacterial membranes, altering their potential and disrupting cell homeostasis (Al-Shabib et al., 2017). Methyl gallate, with its trihydroxylated ring, is also known to increase membrane permeability and inhibit bacterial biofilms (Liang et al., 2023).

The improved efficacy of HUAE over UAE is due to the enhanced cell disruption and micelle formation, which facilitates the extraction of mid-polar compounds like chlorogenic acid and rutin more efficiently than homogenisation (Chemat et al., 2017). Furthermore, the HUAE prevents thermal degradation and facilitates faster solvent penetration, resulting in higher radation and supports faster solvent penetration, leading to greater phenolic yield (Siddiqui et al., 2025). These findings align with previous studies. For example, Mahmoudi et al. (2016) reported MIC values of 6.25–12.5 mg/L against *L. monocytogenes* using methanolic fig leaf extracts, supporting our results. Moreover, a study by Aslan (2024) highlighted the sensitivity of Gram-positive bacteria to fig phenolics due to their lack of outer membranes, allowing for easier polyphenol access and accumulation in the cytoplasmic space.

No antimicrobial activity was detected against *Escherichia coli* or *Pseudomonas aeruginosa* in any of the tested extracts. This resistance is likely due to the outer membrane structure of Gram-negative bacteria, which acts as a permeability barrier to phenolics and other bioactive compounds (Li et al., 2017). Enzymatic degradation (e.g., β-lactamases and polyphenol oxidases) further contributes to their resistance (Kim et al., 2017). Despite high TPC values, polyphenol-rich extracts fail to penetrate the outer lipid-rich membrane or are inactivated by detoxifying enzymes (Veiko et al., 2023). These findings are consistent with Olufemi and Olusegun (2013), who observed minimal inhibition of Gram-negative strains by FL methanolic and aqueous extracts.

Among yeasts, *C. albicans* was inhibited by all polyphenolic extracts to varying extents. The (1.) FLUAEEtOH: W40% extract again showed the lowest MIC (37.75 mg/L), followed by (7.) FLSLEEtOH: W40% (118.75 mg/L), (4.) HUAEEtOH: W40% (143.75 mg/L), and HUAEW (70.5 mg/L). No inhibition was recorded for water extracts via SLE.

C. parapsilosis was only inhibited by (4.) HUAEEtOH: W40% with an MIC of 37.75 mg/L. The antifungal activity is linked to the synergistic effects of flavonoids and hydroxybenzoic acids. Flavonoids like rutin and kaempferol derivatives have been shown to inhibit ergosterol biosynthesis, disrupt fungal cell membranes, and interfere with hyphal formation (Rodriguez Vaquero et al., 2011). Chlorogenic acid and methyl gallate may also induce ROS accumulation and DNA fragmentation in yeasts, contributing to antifungal effects (Álvarez-Martínez et al., 2020). The relatively low MIC of UAEEtOH: W40% supports its extraction efficiency for antifungal phenolics.

No detectable antifungal activity was recorded against either filamentous fungal strain, *A. niger* and *A. brasiliensis*. This may be due to their robust spore-forming capacity, cell wall rigidity, or lower permeability to polar phenolics. Additionally, fungal melanin cell walls may act as a natural barrier against ROS or chelating agents (Li et al., 2017). No significant correlation was observed for *E. coli* or *S. aureus*, implying that phenolic quantity alone is not sufficient; their chemical structure, polarity, and microbial resistance traits also play crucial roles.

Among the tested microorganisms, *L. monocytogenes*, *C. albicans*, and *C. parapsilosis* showed varying susceptibility, while no inhibitory activity was observed for *E. coli*, *S.s aureus*, *P. aeruginosa*, *A. niger*, and *A. brasiliensis* across any extract. *C. albicans* was inhibited by four extracts, with UAEEtOH: W40% again showing the lowest MIC at 37.75 mg/L. The (4.) HUAEEtOH: W40% extract had a MIC of 143.75 mg/L, SLEEtOH: W40% 118.75 mg/L, and HUAE water 70.5 mg/L. The high antifungal performance of the UAE extract may be attributed to improved solubilisation and diffusion of slightly polar polyphenols in the EtOH: W system, as supported by Do et al. (2014), who demonstrated that hydroethanolic solvents efficiently extract phenolics with antifungal activity.

Our results align with Aslan (2024), who reported the strongest inhibition of *C. albicans* among the fungi tested with SLE. The lack of activity in water-only SLE extracts indicates that solvent polarity significantly affects antifungal compound recovery. MeOH and EtOH: W mixtures are particularly effective in solubilising chlorogenic acid, rutin, and caffeoylquinic acids (Ignat et al., 2011), compounds with known antifungal potential. *C. parapsilosis* was inhibited only by the (4.)

Table 3
Condensed comparison of extraction groups based on solvent and technique, summarising overall performance across yield, phenolic content, antioxidant activity, antimicrobial effects, sustainability, and cost. Extracts obtained with 40% ethanol, particularly HUAE, showed the strongest overall performance.

Extraction technique	Solvent	Yield	TPC	TFC	Antioxidant	Antimicrobial	Sustainability (LCA)	Cost (LCC)
HUAE	EtOH:Water 40%	Highest	Highest	High	Highest	Moderate	Moderate	Moderate
SLE	EtOH:Water 40%	High	High	Highest	High	Strong vs Gram+	Moderate	Low
UAE	EtOH:Water 40%	Moderate	High	Good	High	Best MIC values	Higher	Low
Water Extractions (All methods)	Water	Good Yield	Moderate	Low	Low–Moderate	No MIC effect	Best	Lowest
EtAc Extractions (All methods)	Ethyl Acetate	Low	Low	Zero	Very Low	Weak	High	High

HUAEEtOH: W40% extract, with a MIC of 37.75 mg/L. No activity was detected with the other extraction methods or solvents. This selective inhibition suggests that only the (4.) HUAEEtOH: W40% combination was effective in extracting antifungal bioactive in sufficient concentrations. Similar selectivity for *C. parapsilosis* has been reported in studies using polyphenol-rich extracts from fig and grape leaves (Barros et al., 2014). No inhibition was observed against *S. aureus*, *E. coli*, *P. aeruginosa*, *A. niger*, or *A. brasiliensis* with any extract or solvent tested. This suggests a microorganism-specific spectrum of activity. The lack of *S. aureus* inhibition (MIC >143.75 mg/L) contradicts some earlier reports (Mahmoudi et al., 2016), possibly due to strain variability or low diffusion rates of polyphenols through its thick peptidoglycan layer, which can impede bioactive uptake (Kim et al., 2021). Similarly, Gram-negative bacteria such as *E. coli* and *P. aeruginosa* are known to resist polyphenol-based antimicrobials due to the barrier presented by their outer membrane (Bradley, 2014). Solvent controls included EtOH: W40%, MeOH, and W. None of these solvents alone exhibited inhibitory effects, confirming that the observed antimicrobial activity was solely due to the bioactive compounds in the extracts. The c extract consistently exhibited the lowest MIC values for both *L. monocytogenes* (4.72 mg/L) and *C. albicans* (37.75 mg/L), indicating that UAE enhanced the recovery of antimicrobial compounds more effectively than HUAE or SLE. This can be attributed to cavitation effects and better penetration of solvent into plant matrices (Chemat et al., 2017). Additionally, EtOH: W40% mixtures are superior to pure water in solubilising moderate polar compounds like chlorogenic acid and rutin, both of which show broad antimicrobial activity (Ignat et al., 2011; Trombino et al., 2019).

3.5. LCA

The Life Cycle Assessment (LCA) and environmental impact analysis of the three extraction methods, SLE, UAE, and HUAE, was conducted using the life cycle inventory (LCI) detailed in Table S7. The comparison reveals notable differences in resource use and energy consumption among the methods (Fig. S4).

SLE shows high environmental impact (Table S8), especially in EtAc-based extractions (0.0057 mPt/g_{leaves} and 11.5306 mPt/mg_{GAE}), making it the least sustainable option. HUAE has the lowest environmental impact when water is used as a solvent. Among different UAE/HUAE solvent choices, EtOH: W40% and MeOH extractions have slightly higher impacts than purely water-based UAE. The environmental impact was assessed using two functional units: (a) one gram of dry leaves and (b) one milligram of Gallic Acid Equivalents (mg_{GAE}) to compare the dry leaf matter corresponding to the phenolic compounds expressed as mg_{GAE}.

Fig. S4 shows the environmental impact characterisation of the 10 extraction scenarios for FL extracts, based on midpoint indicators from Life Cycle Assessment (LCA) categories. Each bar aggregates contributions to categories such as Climate Change (GWP), Ozone Depletion (ODP), Ionising Radiation (IR), Human Toxicity (HTPC, HTPNC), Eutrophication (FAETP, MEP, TEP), and Resource Use (ADP, MD). Extraction 6. HUAEW exhibited the highest total impact, primarily driven by its contributions to climate change, human toxicity, water use, and resource use. This is due to the high electricity consumption and the low yield (0.124 g extract per 4 g of leaves). Also, extraction 1. UAEEtOH: W40% had a high impact (5.3 impact points), particularly due to water use and climate change. Extraction 10. UAEMeOH had the third-highest impact of 4.5 impact points, especially influenced by climate change and human toxicity categories. All the other scenarios showed substantially lower environmental impact. UAE-W reduced environmental impact by 82% per gram of leaves and more than 98% mg_{GAE} in the least sustainable extraction, assuming that lower = better and that the efficiency score of the best option = 100%.

3.5.1. LCC considerations

The purchase costs per unit of raw materials, utilities, and chemicals that were used during the different extraction techniques. SLE is the cheapest method in terms of price per gram of leaves, but it has extremely high environmental costs. The UAE and HUAE, combined with water, are the most cost-effective methods (Table 3), as they combine low environmental impact with low costs. HUAE and UAE with ethyl acetate or methanol are among the most expensive methods (Tables S9 and S10).

4. Conclusion

This study shows that both solvent composition and extraction technique critically influence the phenolic profile, antioxidant capacity, and antimicrobial activity of FL extracts. Among the tested conditions, EtOH: W 40% extracts consistently outperformed W extracts, confirming the importance of intermediate solvent polarity for efficient polyphenol recovery. HUAE emerged as the most effective method overall, delivering the TPC, the strongest antioxidant activity, and the broadest antimicrobial effects.

15 key antioxidant compounds were identified, supporting the strong positive correlations observed between TPC and antioxidant assays and underscoring the central role of polyphenols in radical scavenging activity. A moderate correlation between TPC and antimicrobial activity suggests that phenolics contribute to microbial inhibition, although other bioactive compounds and synergistic interactions are also likely involved. The EtOH: W 40% extracts showed notable antimicrobial activity against *L. monocytogenes* and *Candida* spp., whereas no inhibition was observed against *S. aureus*, *E. coli*, *P. aeruginosa*, or the tested filamentous fungi, highlighting species-specific susceptibility.

From a sustainability perspective, W UAE represents the most environmentally friendly option, albeit with reduced bioactivity. SLE offers cost advantages but requires further optimisation to improve efficiency. Overall, HUAE with EtOH: W 40% is recommended when maximising phenolic yield, antioxidant capacity, and antimicrobial efficacy is the primary objective. While these findings indicate promising in vitro potential for food preservation and antifungal applications, further studies addressing extract stability, safety, bioavailability, and performance in complex food matrices are necessary before practical application.

CRediT authorship contribution statement

Angeliki Xyderou-Malefaki: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Gheorghe Adrian Martău:** Writing – review & editing, Supervision, Resources, Methodology. **Lavinia Florina Călinoiu:** Writing – review & editing,

Validation, Supervision, Project administration, Conceptualization. **Floricuta Ranga:** Validation, Methodology, Formal analysis, Data curation. **Dan Cristian Vodnar:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Maria Dimopoulou:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Athanasios Angelis-Dimakis:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the author(s) used ChatGPT and Grammarly to enhance the vocabulary, coherence of the text, and individual icons included in the graphical abstract. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2026.149512.

Data availability

Data will be made available on request.

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