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Fourier-transform infrared spectra, mineral composition, and biological potential of lyophilized *Fumaria officinalis* waste extracts

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ABSTRACT:
Plant waste valorisation offers a sustainable approach to extract secondary metabolites with therapeutic, antioxidant, and cosmeceutical potential. *Fumaria officinalis* (fumitory), a traditionally used medicinal plant, contains various biologically active secondary metabolites, but the influence of different extraction methods on its phytochemical yield, mineral composition, and bioactivity remains underexplored. Four extraction techniques, *i.e.* maceration (M), heat-assisted extraction (HAE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE), were applied to obtain fumitory extracts. The extraction yield, mineral composition, phytochemical functional groups (Fourier Transform Infrared analysis - FT-IR spectroscopy), antimicrobial potential, hemolysis inhibition under thermal and hypotonic stress, and sun protection factor (SPF) were assessed. The extraction technique significantly influenced the extraction yield, which ranged from 17.24% (M) to 37.98% (MAE). Potassium was the most abundant macronutrient (271.56–400.37 g/kg), while all micronutrient concentrations were below 1 g/kg. The FT-IR analysis revealed functional groups typical of phenolics, alkaloids, and proteins, confirming the complex chemical structure of the extracts. All of the extracts exhibited antimicrobial activity against *Staphylococcus aureus* and *Enterococcus faecalis*, but not against Gram-negative bacteria or fungi. The UAE and MAE extracts showed superior protection against heat-induced hemolysis (up to 70.4% inhibition at 0.25 mg/mL), and all the extracts demonstrated moderate, dose-dependent protection in hypotonic conditions. The SPF analysis revealed UV-B absorbance across 290–320 nm, with the UAE extract at 100 µg/mL achieving the highest SPF value (1.66 ± 0.01). The study highlights the significantly influence of the extraction method on the physicochemical and biological properties of *F. officinalis* extracts. Ultrasound-assisted extraction and MAE were the most effective in obtaining extracts with enhanced bioactivity. These findings support the potential application of fumitory extracts in natural therapeutics and cosmetic formulations. Future work should focus on isolating specific active constituents and evaluating efficacy for pharmaceutical and cosmeceutical applications in terms of wound-healing or anti-aging activities in novel cell models related to disorders, infections, wounds, burns, or skin ageing.

Keywords: anti-inflammatory potential, antimicrobial activity, fumitory, lyophilisate, sun protection factor

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INTRODUCTION

Fumaria species have been employed in traditional remedies for skin disorders, rheumatism, fever, stomach ache, hypertension, and infertility due to the presence of isoquinoline alkaloids (Erdoğan 2009; Orhan *et al.* 2012; Sharef *et al.* 2020). *Fumaria officinalis* L., also known as common fumitory (earth smoke),

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a species of the Fumariaceae family, is the most prevalent herb of this genus in Western and Central Europe. Fumitory, as an annual herbaceous plant, is characterised by its smooth, slender, and branched stems which vary in height from 10 to 100 cm. The plant bears alternate, finely divided, grey-green leaves and produces both lateral and terminal racemes with purplish-pink flowers, giving it a characteristic smoky appearance (Adham *et al.* 2021).

It is widely recognised for its traditional medicinal applications and richness of biologically active secondary metabolites (Babaeimarzangou *et al.* 2015). *Fumaria officinalis* has been traditionally applied as a remedy for various skin disorders, including eczema, psoriasis, scabies, itches, milk crust, and infections (Hentschel *et al.* 1995). The plant is also used in rheumatism, hypertension, arteriosclerosis, constipation, colicky pains, hepatic, liver, or gallbladder disorders, respiratory, urinary, and cardiac diseases, and allergies (Erdoğan 2009; Neves *et al.* 2009; Pehlivan Karakaş *et al.* 2012). According to the monograph issued by the European Medicines Agency and its Committee on Herbal Medicinal Products, traditional herbal preparations of *F. officinalis* are indicated for use in promoting bile secretion and alleviating symptoms associated with indigestion, including bloating, flatulence, and sluggish digestion (European Medicines Agency 2023). Namely, findings from clinical studies related to *F. officinalis* were considered sufficient to substantiate the traditional use of the plant for its choleretic and digestive benefits (European Medicines Agency 2023). Additionally, pharmacological evidence from studies demonstrating anticholeretic activity, mild antispasmodic effects on smooth muscle, and mild diuretic and laxative properties, further supported its traditional indications for promoting bile flow and relieving dyspeptic symptoms, such as bloating, flatulence, and delayed digestion (Aguiar 2023; European Medicines Agency 2023). In the UK, *F. officinalis* has continued to be used in the modern era as an eyewash for managing conjunctivitis, while in North America, its fresh green leaves are recommended for their general tonic effects, and flowering tops macerated in wine are used for treating digestive disorders (European Medicines Agency 2023). *Fumaria officinalis* has long been available as an herbal medicinal product in France, and currently, fumitory-based preparations are marketed as digestive aids in France and Spain, for biliary tract dyskinesia in Austria, and as herbal tea formulations in Germany (Zhang *et al.* 2020; European Medicines Agency 2023). Additionally, pharmacological research has reported that fumitory shows analgesic, antioxidant, antibacterial, antidiabetic, anticancer, immunity stimulant, and neural activities, as well as beneficial effects in biliary diseases (Neves *et al.* 2009; Al-Snafi 2020).

Fumitory contains alkaloid, carbohydrate, flavonoid, tannin, terpenoid, and saponin components, as well as phytosterols, amino acids, proteins, and steroids. (Evans & Trease 2009; Orhan *et al.* 2012; Al-Snafi 2020). Specifically, previous phytochemical studies on various extracts of *F. officinalis* have identified a broad spectrum of biologically active secondary metabolites. These include flavonoids such as quercetin, its glycosides, and related derivatives; phenolic acids including chlorogenic, *p*-coumaric, ferulic, and caffeoylmalic acids; as well as isoquinoline alkaloids, predominantly of the protopine type (with protopine being the major compound), alongside tetrahydroprotoberine and spirobenzylisoquinoline alkaloids (Ivanov *et al.* 2014; Chlebek *et al.* 2016; Adham *et al.* 2021). In our previous study, the liquid chromatography-mass spectrometry method (performed for fumitory extracts) revealed the presence of caffeoylmalic and chlorogenic acids, rutin, isoquercitrin, quercetin dihexoside, quercetin trihexoside, quercetin pentoside hexoside, quercetin deoxyhexosyl dihexoside, methylquercetin dihexoside, kaempferol deoxyhexosylhexoside, protopine, derivatives of methyl, oxo-, or acetyl protopine, cryptopine, fumariline, as well as fumarophycine (Ahmoda *et al.* 2025).

In addition to the aforementioned studies dealing with the chemical composition of *F. officinalis*, recent studies have investigated the biological potential of *Fumaria* species extracts, including fumitory. Adham *et al.* (2021) showed that fumitory extracts demonstrated cytotoxic activity against two leukaemia cell lines and nine multiple myeloma cell lines. Several research studies have confirmed the antioxidant potential of extracts from various *Fumaria* species (Orhan *et al.* 2012; Ivanov *et al.* 2014; Păltinean *et al.* 2017), as well as diuretic properties (Ivanov *et al.* 2014), and hepatoprotective activity (Orhan *et al.* 2012). *Fumaria* extracts have also exhibited effects on Alzheimer's disease (Chlebek *et al.* 2016) and antimicrobial (Stanojević *et al.* 2018), antidiabetic, antineuropathic, and anti-inflammatory potential (Raafat & El-Zahaby 2020).

Various branches of industry generate considerable plant waste, resulting in environmental pollution. Nevertheless, such waste may serve as a valuable source of biologically active secondary metabolites, including terpenes, polyphenols, peptides, minerals, and phytosterols (Lizárraga-Velázquez *et al.* 2020). Hence, an increasing number of research studies have been directed towards addressing the recovery of biologically active secondary metabolites from valuable herbal waste by employing green extraction processes and GRAS (Generally Recognized As Safe) extraction mediums, as more efficient and sustainable technologies (Kumar *et al.* 2017; Fierascu *et al.* 2019; Mrkonjić *et al.* 2024). To the best of our knowledge, no studies have been carried out on the chemical composition and biological potential of lyophilized *F. officinalis* dust extracts obtained using traditional and modern extraction techniques and their comparison. In addition, the anti-inflammatory activity, shown as erythrocyte membrane stabilisation, and sun protection capacity of *F. officinalis* extracts have not been documented either. Therefore, the goals of the present research were: (1) the development of fumitory extracts from plant waste by using traditional (maceration) and novel extraction protocols (heating, ultrasonic, and microwave extractions) and GRAS solvent; (2) the examination of the extraction yield, presence of functional groups (Fourier Transform Infrared analysis, FT-IR) and mineral composition of the extracts; and (3) the investigation of the biological potential related to dermal application, including antimicrobial, anti-inflammatory, and sun protection activities.

MATERIAL AND METHODS

Plant material. *Fumaria officinalis* (air-dried aerial parts) in the form of waste (dust), from the Institute for Medicinal Plants Research "Dr Josif Pančić" (Serbia), was used as the plant material. The identification of the wild-growing plant (collected in September 2023 in the flowering stage, in the village of Žitkovac, the municipality of Aleksinac, Nišava District, Serbia, N 43.5095°, E 21.6949°) was carried out by the Quality Control Sector at the Institute for Medicinal Plants Research "Dr Josif Pančić". A voucher specimen with the same serial number was kept at the Institute. The plant material of *F. officinalis* consisted of herbal dust generated through intensive mechanical grinding using an industrial mill (UMČ 30, Biljotehnika, Pančevo, Serbia). This material originated from a heterogeneous mixture of plant parts (stems, leaves, and flowers) processed within the production sector of the Institute for Medicinal Plant Research "Dr Josif Pančić", with a final particle size not exceeding 0.3 mm. Following particle size separation using standard pharmaceutical sieves, the resulting fine powder was classified as processing residue or herbal dust. In accordance with national regulations governing the quality standards for tea, herbal tea, and related products in the Republic of Serbia, such material is not permitted for inclusion in commercial tea preparations intended for sale or distribution (Anonymous 2012). A detailed analytical qualification and quan-

tification of the biologically active secondary metabolites in the *F. officinalis* extracts and the chromatogram figures were published in our previous study (Ahmoda *et al.* 2025).

Preparation of the plant extracts. *Fumaria officinalis* herb extracts were prepared employing 4 extraction procedures: maceration, heat extraction (HAE), ultrasonic extraction (UAE), and microwave extraction (MAE) (Ahmoda *et al.* 2025). Namely, the maceration process was performed at 25°C in a shaker (IKA, Germany) for 60 min using a 50% water-ethanol mixture (Fisher Science, UK) at a 30:1 mL/g solvent-to-solid ratio. HAE was conducted at 80°C (30 min) using the same extraction solvent, ratio, and incubator shaker as in the maceration process. UAE was realized in an ultrasonic bath device (ARGO LAB, Italy) at 25–27°C for 15 min using the same parameters. An Erlenmeyer flask containing the plant material and extraction medium was cooled using ice during the process. In all 3 extraction procedures, the flasks were covered. MAE was performed at 100°C in a Monowave 300 reactor (Anton Paar, Austria) for 120 s in a closed vial using the same parameters as in the above-described protocols. After extraction, all the samples were filtered separately using fine-pore filter paper (0.45 μm).

Determination of the extraction yield. The yield of the extraction from *F. officinalis* was calculated using the following equation (Adam *et al.* 2019):

$$\text{Yield of extraction (\%)} = 100 - \frac{(a-b)\times 100}{m} \quad (1),$$

a - weight of the dish containing liquid extract after drying at 105°C for 3 h in a drying oven (Memmert GmbH, Germany), *b* - weight of the empty dish, and *m* - weight of the herbal matrix necessary to prepare 2 mL of the extract (the volume of the extracts used for the analysis). The extraction yield is expressed as a percentage (%).

Extract lyophilisation. Subsequently, the obtained fumitory waste extracts were prepared for the lyophilisation process. Namely, ethanol was evaporated at 50°C and 50 mbar for 40 min. The extracts were frozen at -80°C for 1 h and freeze-dried in an Alpha 2–4 LSCplus device from Christ (Germany) at 0.011 mbar for 24 h.

FT-IR analysis. The freeze-dried *F. officinalis* samples were analysed using a FT-IR spectrophotometer. The spectra were obtained by the ATR technique in a Nicolet IS35 FTIR-ATR spectrometer (Thermo Fisher Scientific, Sweden) in the absorption range between 500 and 4000 cm^{-1} (Budiman *et al.* 2024).

Mineral composition analysis. Fumitory extracts for mineral composition analysis were prepared by acid-wet digestion (Jaćimović *et al.* 2022). Each lyophilised sample weighing 0.1 g was then placed in a glass jar and heated for 20 min at 80°C using 15 mL of HNO_3 (65%). Following cooling, the solutions were filtered through 0.45 μm filter paper, moved to a 25 mL volumetric flask, and diluted with deionised water to the appropriate level. A Thermo Fisher Scientific model 7400 dual ICP-OES spectrometer (Sweden) was used to quantify the elements. A mono-element Hg standard solution (Mercury solution, 1000 ppm) and a multi-element standard solution (Multi stock: Certipur ICP multi-element standard solution 1000 ppm) were used to generate the analytical standards for the instrument calibration. Multielement stock standards were serially diluted to create the working standard solution. Each experiment was run in duplicate at 25°C. A blank solution was made using the same technique as the samples. The results are presented as mean value ± RSE (relative standard error) (g/kg).

Determination of the antimicrobial potential. The antimicrobial effect of the freeze-dried *F. officinalis* extracts was examined by employing the disc diffusion method (Borotová *et al.* 2021). The research protocol was approved (0203-07-013/006/2025) by the ethical committee of the Institute for the Application of Nuclear Energy INEP, University of Belgrade, and informed consent was obtained. All the microorganisms used (bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Enterococcus faecalis*, and one fungus: *Candida albicans*) were obtained from patients' samples from the laboratory of the Institute for the Application of Nuclear Energy INEP, University of Belgrade. The cultivation of microorganisms was carried out aerobically on blood agar for 24 h (bacteria) and using Sabouraud agar (fungus, 48 h) at 37°C. The inoculum was spread on Mueller-Hinton agar (for bacteria; Promedia, Serbia) or Sabouraud agar (for fungus; Promedia, Serbia). The *Fumaria officinalis* extracts (175 mg/mL) were transferred into wells of agar for incubation (24 h, 37°C). Antimicrobial capacity was assessed based on the inhibition zone around the applied extract (< 0.5 cm is very weak inhibition, > 0.5 cm is weak inhibition, > 1 cm is medium inhibition, while > 1.5 cm is very strong inhibition). Antibiotics commonly used for antibiograms (amoxicillin, ciprofloxacin, trimethoprim, fosfomycin, a mixture of amoxicillin and clavulanic acid, cephalexin, ceftriaxone, gentamicin, amikacin, and nitrofurantoin) and antimycotic fluconazole were used as the positive controls.

Erythrocyte membrane stabilisation assay. The erythrocyte membrane stabilisation assay (Ranasinghe *et al.* 2012) was performed to examine the *in vitro* anti-inflammatory capacity of 4 fumitory extracts. The research protocol was approved (0203-07-013/005/2025) by the ethical committee of the Institute for the Application of Nuclear Energy INEP, University of Belgrade, and informed consent was obtained. Blood was obtained from the biochemical laboratory of the Institute for the Application of Nuclear Energy INEP, University of Belgrade. Sodium oxalate (Sigma Aldrich, Germany) was used to prevent clotting, and the blood was stored at 4°C for 24 h. The blood was centrifuged for 5 min at 770×g (Thermo Scientific, USA). A sterile isotonic saline solution was employed for washing, and centrifugation was repeated. The process was performed in triplicate, and the packed blood cells were mixed using PBS (pH 7.4, 10 mM, Sigma Aldrich, Germany) to obtain a 40% v/v suspension.

Heat-induced hemolysis. 10 µL of red blood cell (RBC) suspension was added to 1 mL of the isotonic saline solution with fumitory extract (25, 50, and 100 µg/mL) and mixed gently. A pure isotonic solution (without extract) was mixed with the RBC suspension (negative control). The same volume of isotonic solution containing diclofenac (75 µg/mL, prepared from a 25 mg/mL ampoule of diclofenac, Galenika, Serbia) was also mixed with the RBC suspension (positive control). The samples were incubated (54°C, 20 min) in a water bath (Thermo Scientific™ TSGP10PMO5, Thermo Fisher Scientific, United States of America). The other tubes were preserved at a temperature of 4°C for 20 min in ice. All the samples were centrifuged for 5 min at 12300×g. The absorbance reading of the supernatant was determined spectrophotometrically (560 nm) in triplicate in a UV-1800 Shimadzu (Japan). The inhibition of erythrocyte lysis was determined according to the following equation:

$$\% \text{ of the lysis inhibition} = 100 \times \left(1 - \frac{OD_2 - OD_1}{OD_3 - OD_1} \right) \quad (2),$$

OD_1 = unheated sample, OD_2 = heated sample, and OD_3 = heated control.

Hypotonicity-induced hemolysis. 10 µL of RBC suspension was added to 1 mL of the hypotonic saline solution with fumitory extract (25, 50, and 100 µg/mL), and

mixed gently in centrifuge tubes. The same volume of pure hypotonic solution (without extract) was mixed with the RBC suspension to serve as the negative control, while the same volume of hypotonic solution with diclofenac (75 µg/mL) was mixed with the RBC suspension for the positive control. The samples were incubated (25°C, 10 min). The tubes were centrifuged for 5 min at 12300×g. The absorbance reading of the supernatant was determined spectrophotometrically (560 nm). The erythrocyte lysis inhibition was determined using the following equation:

$$\% \text{ of the lysis inhibition} = 100 \times \left(1 - \frac{OD_2 - OD_1}{OD_3 - OD_1} \right) \quad (3),$$

where OD_1 = sample with isotonic solution, OD_2 = sample with hypotonic solution, and OD_3 = control with hypotonic solution.

Determination of the photoprotective activity of the extracts. The photoprotective potential of the fumitory extracts was tested using the previously described *in vitro* protocol (Oliveira *et al.* 2021). The sun protection factor, *i.e.* SPF value, was determined according to the Mansur equation, and the absorbance readings from 290 nm to 320 nm to express the photoprotective capacity of the extracts. Namely, 3 extract concentrations (25, 50, and 100 µg/mL) were used in the assay. The absorbance reading of each sample was performed in triplicate against the blank. The SPF value was determined using the following equation:

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad (4)$$

CF - correction factor (10), EE (λ) - erythemal effect spectrum, I (λ) - solar intensity spectrum, and Abs (λ) - absorbance of the sample at each wavelength. The values of EE×I are constants (Sayre *et al.* 1979).

Statistical analysis. One-way ANOVA and Duncan's post hoc test (STATISTICA 7.0) were employed to determine the statistically significant differences among the data from the tests used to analyse the extraction yield, mineral composition, antimicrobial, and hemolysis inhibition activity, and photoprotective potential ($P < 0.05$). To assess the normality of the samples, histograms were used, while for homogeneity of variances, Levene's test was employed ($P > 0.05$). The results in the tables and graph are presented as mean±standard deviation.

RESULTS

Extraction yield. The extraction yield of the fumitory waste extracts prepared using 4 extraction processes are summarised in Table 1.

The extraction technique significantly influenced the variable and varied widely from 17.24 to 37.98% (microwaves > heat and ultrasounds > maceration). The extract formulated using microwaves showed the highest extraction yield (37.98 ± 0.87%), while significantly lower and similar extraction yields were determined in the samples from HAE and UAE (21.74 ± 0.75% and 21.99 ± 0.87%, respectively). As expected, maceration produced the lowest extraction yield, 17.24 ± 0.75%.

Mineral composition of the fumitory extracts. The mineral composition of the lyophilised fumitory extracts is presented in Table 2.

The most dominant element in all the extracts was potassium (271.56–400.37 g/kg), followed by significantly lower amounts of magnesium (16.23–30.54 g/kg), sodium (5.88–22.47 g/kg), barium (6.50–9.28 g/kg), and calcium (1.20–5.40 g/kg) (Table 2). The concentration of all the microelements was lower than 1 g/kg.

Table 1. The extraction yield of the fumitory waste extracts prepared by maceration (M), heat extraction (HAE), ultrasonic extraction (UAE), and microwave extraction (MAE).

Extraction method	Extraction yield (%) (mean±SD)
M	17.24^{c}±0.75*
HAE	21.74^{b}±0.75
UAE	21.99^{b}±0.87
MAE	37.98^{a}±0.87

*Values with different letters show statistically significant differences ($P < 0.05$); SD - standard deviation.

Table 2. The concentration of the detected elements in the lyophilised *Fumaria officinalis* extracts prepared using maceration (M), heat extraction (HAE), ultrasonic extraction (UAE), and microwave extraction (MAE).

Elements	Extraction method			
	M	HAE	UAE	MAE
	mean value±RSE* (g/kg)			
Na	22.47^{a}±2.25**	17.27^{b}±1.73	8.56^{c}±0.85	5.88^{d}±0.59
K	348.31^{b}±3.48	399.40^{a}±31.94	271.56^{c}±27.15	400.37^{a}±40.31
Mg	28.45^{a}±2.84	30.54^{a}±3.05	25.03^{a}±2.50	16.23^{b}±1.62
Ca	5.40^{a}±0.54	4.14^{b}±0.41	4.20^{b}±0.42	1.20^{c}±0.12
Ba	7.68^{b}±0.46	6.50^{c}±0.39	6.99^{c}±0.42	9.28^{a}±0.56
Mn	0.021^{a}±0.001	0.019^{a}±0.002	0.022^{a}±0.002	0.008^{b}±0.000
Cr	0.001^{c}±0.000	0.005^{b}±0.000	0.001^{b}±0.000	0.016^{a}±0.001
Fe	0.097^{c}±0.006	0.091^{c}±0.005	0.140^{b}±0.008	0.299^{a}±0.018
Ni	0.004^{c}±0.001	0.009^{b}±0.000	0.003^{c}±0.000	0.016^{a}±0.001
B	0.136^{c}±0.008	0.498^{a}±0.030	0.051^{a}±0.003	0.226^{b}±0.015
Cu	0.018^{c}±0.001	0.023^{b}±0.002	0.017^{c}±0.001	0.028^{a}±0.002
Zn	0.123^{a}±0.007	0.103^{b}±0.006	0.099^{b}±0.006	0.099^{b}±0.005
P	0.881^{a}±0.053	0.526^{b}±0.032	0.780^{a}±0.047	0.198^{c}±0.014
S	7.28^{a}±0.44	0.075^{c}±0.004	6.89^{a}±0.41	4.29^{b}±0.26

*RSE - relative standard error; **The same letter between the columns (differences between various tested extraction procedures for the same element) represents the absence of statistically significant differences ($P > 0.05$).

Boron (0.051–0.498 g/kg), iron (0.091–0.299 g/kg), and zinc (0.099–0.123 g/kg) had the highest measured values among the micronutrients. The content of sulphur varied widely in the extracts prepared using different extraction methods, ranging from 0.075 g/kg (HAE) to 7.28 g/kg (M), whereas the concentration of phosphorus was 0.198–0.881 g/kg. Chrome and nickel exhibited the lowest concentration in all the tested extracts.

FT-IR spectra of the fumitory extracts. The FT-IR spectra of the lyophilised *F. officinalis* extracts obtained using various extraction techniques are shown in Fig. 1. The spectra show overlapped bands, related to the functional groups present in complex structures of polyphenols, flavonoids, tannins, alkaloids, and proteins.

Broad bands, observed at 3235–3271 cm^{-1} in all the spectra, relate to O-H stretching vibrations of hydroxyl, phenolic, and carboxyl groups present in the secondary metabolites of the extracts (Cakić *et al.* 2018; Li *et al.* 2021). These

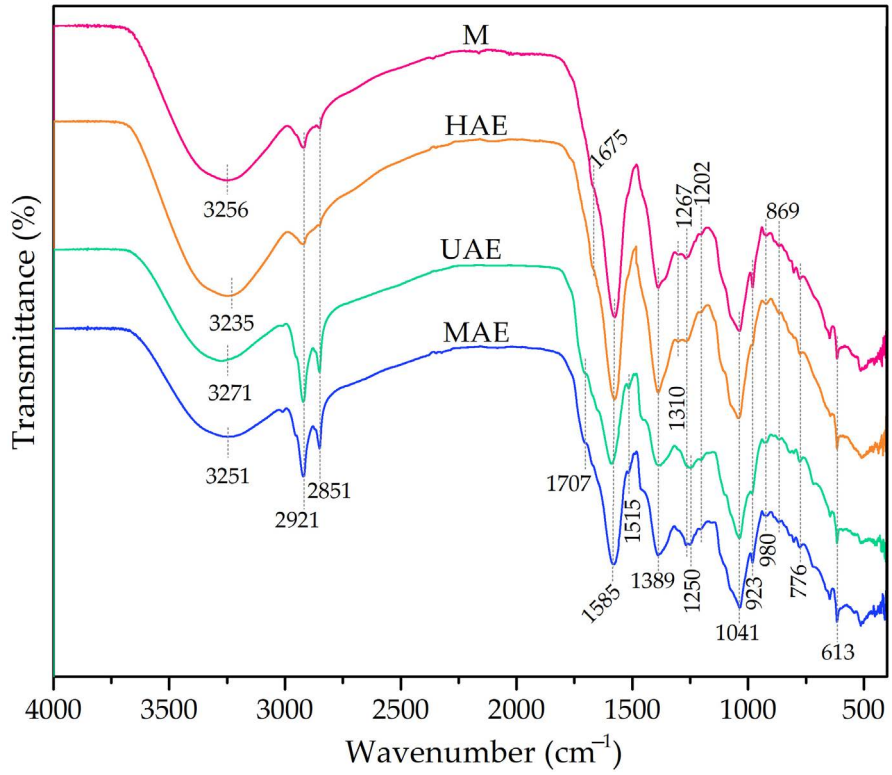

Fig. 1. Fourier Transform Infrared (FT-IR) spectra of the freeze-dried *Fumaria officinalis* extracts obtained by maceration (M), heat extraction (HAE), ultrasonic extraction (UAE), and microwave extraction (MAE).

bands overlap with N-H stretching vibrations of amino groups (Cakić *et al.* 2018) present in the detected proteins and alkaloids. Changes in peak overlap intensity and band broadening indicate the intermolecular hydrogen bonding between phenolic compounds and structures containing nitrogen. Furthermore, 2 vibration peaks at 2921 cm^{-1} and 2851 cm^{-1} are related to the C-H stretching vibration of aliphatic methylene and methyl groups, while the bending vibrations of these groups are observed in the 1380–1310 cm^{-1} region (Cakić *et al.* 2018; Li *et al.* 2021). In the UAE and MAE spectra, a low intensity band at 1707 cm^{-1} is attributed to the stretching vibrations of the carboxyl group in phenolic acids (Li *et al.* 2021). Analogously, in the spectra of M and HAE, the small observable shoulder of the peak at 1675 cm^{-1} indicates that the phenolic acid content is similar to that of the extracts prepared using ultrasound and microwave techniques. These findings are in accordance with the secondary metabolites previously detected in *F. officinalis* extracts (Ahmoda *et al.* 2025). Intense and sharp bands noticed at 1585 cm^{-1} and 1515 cm^{-1} may be related to the C=C stretching vibration of aromatic and/or vinyl moiety in the structures of secondary metabolites. Also, the band at 1585 cm^{-1} may be attributed to N-H bending and C-N stretching vibrations (referred to as amide II bands), *i.e.* related to functional groups of structures such as proteins, fumariline, cryptopine, and protopine (Shurvell 2006). Weak bands at 1267 cm^{-1} and 1250 cm^{-1} indicate C–N stretching of the aromatic amino skeletal structures of alkaloids and peptide groups in proteins (Shurvell 2006; Cakić *et al.* 2018). The bands in the 1202–980 cm^{-1} wavelength region are characteristic of the absorption of C-O, C-O-C, and C-O-H groups and their accompanying asymmetric and symmetric vibrations (Shurvell 2006; Li *et al.* 2021). The bands in the 923–613 cm^{-1} region originate from the C–H *out-of-*

plane deformational vibrations of the aromatic and vinyl parts within the structures (Shurvell 2006; Cakić *et al.* 2018). In this region, 2 modes at 869 cm^{-1} and 776 cm^{-1} correspond to N–H wagging vibrations of molecules (Shurvell 2006). These results indicate the complex structure of the *F. officinalis* extracts.

Antimicrobial effects of the fumitory extracts. The data showing the antimicrobial capacity (expressed as the zone of inhibition) towards two Gram-positive bacteria (*E. faecalis* and *S. aureus*), four Gram-negative bacteria (*E. coli*, *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa*), and one fungus (*C. albicans*) are presented in Table 3.

Escherichia coli was resistant to amoxicillin, ciprofloxacin, trimethoprim, and fosfomycin, exhibited intermediate sensitivity to a combination of amoxicillin and clavulanic acid, and was sensitive to cephalexin, ceftriaxone, gentamicin, amikacin, and nitrofurantoin. Two Gram-positive bacteria (*S. aureus* and *E. feacalis*) were sensitive to all the used antibiotics, while *P. mirabilis*, *K. pneumoniae*, and *P. aeruginosa* demonstrated intermediate sensitivity to amoxicillin, ciprofloxacin, trimethoprim, fosfomycin, a combination of amoxicillin and clavulanic acid, and were sensitive to cephalexin, ceftriaxone, gentamicin, amikacin, and nitrofurantoin. *Candida albicans* was sensitive to the antimycotic fluconazole. The fumitory extracts achieved antibacterial potential against *S. aureus* and *E. faecalis*. The inhibition zones ranged from 13.1 to 14.0 mm for *S. aureus*, while the inhibition activity against *E. faecalis* was in the range from 11.2 to 12.2 mm (Table 3). No significant differences were observed in the inhibitory potential among the various fumitory extracts on the growth of either of the tested Gram-positive bacteria. However, the Gram-negative bacteria and the fungus were resistant to the tested fumitory extracts (Table 3).

Erythrocyte membrane stabilisation potential of the fumitory extracts. The anti-inflammatory potential of the lyophilised fumitory extracts was assessed using the erythrocyte membrane stabilisation method, applying both heat-induced and hypotonic solution-induced hemolysis models. The data are presented in Table 4.

The data in Table 4 show that at all the tested cncentrations, the lyophilised fumitory extracts (0.05–0.25 mg/mL) protected the human red blood cell membranes against heat-induced hemolysis in a concentration-dependent manner. Namely, the percentage of heat-induced lysis inhibitions varied, with higher concentrations exhibiting stronger effects (from 57.2 to 70.4% at 0.10 and 0.25 mg/mL, respectively). Further, a significant difference was noted in the capacity to inhibit heat-induced erythrocyte lysis among the samples obtained by different extraction techniques, particularly at higher concentrations (0.10 and 0.25 mg/mL). The UAE and MAE extracts showed the highest inhibition potential in heat-induced lysis, followed by the HAE sample at higher concentrations, while at 0.05 mg/mL, no statistically significant differences were observed among the samples (from 39.9 to 42.0%). In the hypotonic-induced lysis, all the fumitory samples showed dose-dependent activity, as well as a significantly lower inhibitory capacity in comparison to the heat-induced hemolysis at all the tested concentrations (Table 4). The highest level of hypotonic lysis inhibition was achieved in the presence of the HAE, UAE, and MAE samples at the highest dose (63.1–65.0%). At lower concentrations, the trend of prevention of hypotonic-induced lysis was as follows: UAE and MAE ≥ HAE ≥ maceration (at 0.05 mg/mL) and UAE ≥ UAE ≥ HAE > maceration (at 0.10 mg/mL). However, the inhibitory effects on heat- and hypotonic-induced hemolysis in the presence of the fumitory waste extracts were significantly lower compared to the measured value of 0.075 mg/mL for the nonsteroidal anti-inflammatory drug diclofenac.

***In vitro* sun protection factor of the fumitory extracts.** All the tested samples showed absorption across the entire UV range (UV-A, UV-B, and UV-C). The

wavelengths and respective absorbance values of 4 fumitory extracts at concentrations of 25, 50, and 100 μg/mL are shown in Supplementary Table S1, while the sun protection factors at the same extract concentrations are presented in Fig. 2.

Biologically active secondary metabolites from herbal materials, particularly antioxidants (flavonoids and other phenolic components), have been investigated as sunscreen candidates due to their UV-absorbing characteristics (Sutar & Chaudhari 2012). In addition to their photoprotective effects, their potent antioxidant activity also provides a platform for exploiting various combinations of plant extracts as potential ingredients in sunscreen preparations. The SPF of the fumitory extracts was determined using Mansur's equation in the UV-B region. This region possessed the highest incidence of radiation over periods of longer exposure. The absorbance spectrum of the plant extracts indicates that the extract components can absorb UV irradiation from 200 nm to 400 nm. A decrease in the SPF values was observed alongside a decrease in concentration (Fig. 2). The highest SPF value was recorded for the UAE fumitory extract at 100 μg/mL (1.66 ± 0.01), followed by HAE and M (1.25 ± 0.02 and 1.16 ± 0.01, respectively), while MAE had the lowest values at the same concentration (0.98 ± 0.01). The same trend was noted for the other 2 tested concentrations (25 and 50 μg/mL): UAE (0.40–0.82) > M (0.30–0.60) and HAE (0.29–0.62) > MAE (0.26–0.50).

DISCUSSION

The obtained percentages of the extraction yield are significantly higher than the results of *F. officinalis* ethanol extracts investigated in the studies carried out by Pehlivan Karakaş *et al.* (2012) and Wasu & Muley (2009) (8–11%). Nevertheless, Mohajerani *et al.* (2019) reported that *F. vaillantii* Loisel. ethanol extract possessed a high extraction yield (20.3%), similar to the results of the presented study. The difference between extraction yields can be explained by the chemical composition, which differs from one species to another, the nature and composition of the soil, the plant material, *i.e.* the plant part, the nature of the extraction medium, and employed extraction procedures (Sofiane & Seridi 2021). The observed differences in the extraction yield between the fumitory samples prepared using 4 different extraction procedures were expected due to the presence/absence of mechanical or thermal effects during the process. Microwaves provide a higher yield of extraction for a short time because of heat and mass transfer (Gil-Martín *et al.* 2022). Elevated temperatures in heat extraction can increase the extraction yield due to the destruction of plant cells. Rakotondramasy-Rabesiaka *et al.* (2008) also showed that extraction parameters, including temperature, water-ethanol ratio, pH, mixing, the particle size of the plant material, etc. significantly influenced the extraction yield of protopine from *F. officinalis*. In the case of *F. indica*, the highest extraction yield was achieved by using 100% methanol as an extraction medium (14.4%), while a reduction in the methanol content caused a significant decrease in the mentioned parameter (8.5%) (Bharat *et al.* 2024). Dutta *et al.* (2020) reported that the extraction yield of *F. officinalis* leaf extracts prepared using organic solvents varied widely, ranging from 1.7% to 14.8%.

The content of elements in the soil is influenced by numerous factors which can impact their mobility and accumulation by plants, including soil reaction and the content of organic components (Bošković *et al.* 2018). Additionally, other factors can affect their mobility and harmful effects, including humidity, calcium carbonate, hydrated oxides of iron and aluminium content, exchange capacity of cations, redox potential, as well as groundwater levels. Thus, the analysis of the mineral composition of plants is a very important stage in the investigation of their quality. The most dominant abundant element in the fumitory dust extracts was potassium, followed by magnesium, sodium, barium, and calcium, which is

Table 3. The antimicrobial activity of the lyophilised fumitory waste extracts obtained by maceration (M), heat extraction (HAE), ultrasonic extraction (UAE), and microwave extraction (MAE), expressed as the inhibition zone, measured using the disk diffusion method.

Microorganism	M	HAE	UAE	MAE	antibiotics/ fluconazole**
	zone of inhibition (mm)				
Staphylococcus aureus	13.4^{a}±0.9*	14.0^{a}±0.7	13.9^{a}±0.2	13.1^{a}±0.8	S
Enterococcus faecalis	11.2^{a}±0.7	11.5^{a}±0.6	12.2^{a}±0.4	11.7^{a}±0.9	S
Proteus mirabilis	no inhibition	no inhibition	no inhibition	no inhibition	I/S
Klebsiella pneumoniae	no inhibition	no inhibition	no inhibition	no inhibition	I/S
Escherichia coli	no inhibition	no inhibition	no inhibition	no inhibition	R/I/S
Pseudomonas aeruginosa	no inhibition	no inhibition	no inhibition	no inhibition	I/S
Candida albicans	no inhibition	no inhibition	no inhibition	no inhibition	S

*Values with the same letter showed no statistically significant differences ($P > 0.05$); S, sensitive to the used positive control; I, intermediate sensitive to the used positive control; R, resistant to the used positive control.
**Antibiotics (amoxicillin, ciprofloxacin, trimethoprim, fosfomycin, amoxicillin and clavulanic acid, cephalexin, ceftriaxone, gentamicin, amikacin, and nitrofurantoin) - positive control for bacteria; fluconazole - positive control for fungus.

Table 4. The effect of the lyophilised fumitory waste extracts obtained by maceration (M), heat extraction (HAE), ultrasonic extraction (UAE), and microwave extraction (MAE), on the red blood cell membrane in the models of heat- and hypotonic-induced hemolysis.

Extraction method	Concentration (mg/mL)	Inhibition of heat-induced hemolysis (%)	Inhibition of hypotonic-induced hemolysis (%)
M	0.05	39.9^{g}±1.5*	35.0^{g}±1.0
	0.10	57.2^{f}±1.7	48.2^{e}±1.2
	0.25	65.4^{d}±1.3	55.8^{cd}±1.9
HAE	0.05	41.5^{g}±1.3	37.4^{fg}±1.6
	0.10	59.5^{ef}±0.9	53.8^{d}±0.4
	0.25	67.8^{cd}±1.2	63.1^{b}±1.2
UAE	0.05	42.0^{g}±0.8	38.1^{f}±1.5
	0.10	60.2^{e}±0.9	56.1^{c}±0.9
	0.25	69.3^{bc}±1.6^{-2}	65.0^{b}±1.1
MAE	0.05	41.7^{g}±1.0	38.7^{f}±0.7
	0.10	61.1^{e}±1.1	55.9^{cd}±1.2
	0.25	70.4^{b}±1.0	64.3^{b}±1.3
Diclofenac (control)	0.075	88.5^{a}±1.0	86.9^{a}±1.2

*Different letters (a-g) in each column represent the presence of statistically significant differences ($P < 0.05$).

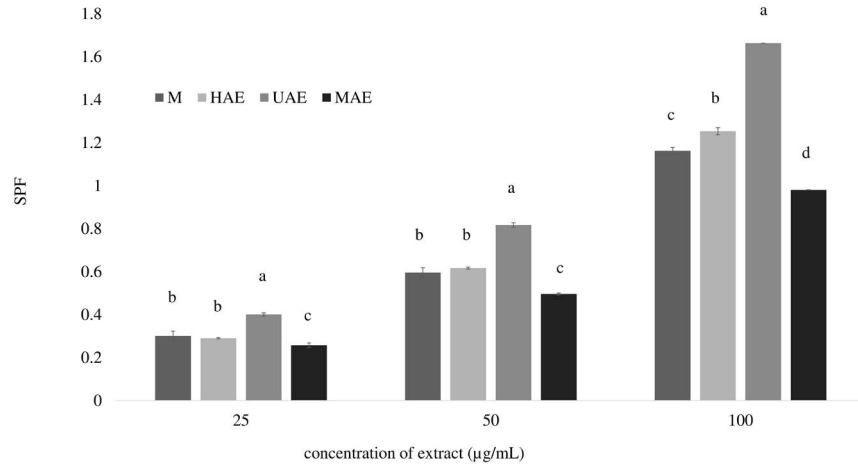

Fig. 2. Sun protection factor (SPF) of the lyophilised fumitory waste extracts obtained by maceration (M), heat extraction (HAE), ultrasonic extraction (UAE), and microwave extraction (MAE), at different concentrations; the same letter above the bars (for each used concentration separately) represents the absence of statistically significant differences ($P > 0.05$).

in line with the literature data (Bošković *et al.* 2018). Although the extraction procedure significantly affected the concentration of all the elements, no trend was observed. For example, the highest levels of some macroelements, including sodium and calcium, were measured in the fumitory macerates, while the highest potassium content was found in the HAE and MAE extracts. Conversely, the lowest concentration of magnesium and the highest concentration of barium were obtained in the MAE extract. In addition, the highest sulphur, phosphorus, and manganese contents were recorded in the macerate and UAE extract, whereas chrome, iron, nickel, and copper reached their highest concentrations in the MAE extract. The HAE extract yielded the sample with the highest boron content, whereas M contained the highest level of zinc. The content of all the microelements was very low, but consistent with the levels reported in the literature related to medicinal plants (Zafar *et al.* 2010; Lavrinenko *et al.* 2024). A significantly higher content of zinc in comparison to copper in the fumitory dust extracts is due to the antagonistic interaction between the mentioned elements, reflected in the adoption of one reducing the acceptance of another (Arias *et al.* 2006). According to the literature, zinc plays an essential role in wound healing and as an antioxidant, while manganese also shows antioxidant potential, which are both essential for the potential use of fumitory dust extracts in skin disorders (Zafar *et al.* 2010). Conversely, minimal amounts of nickel in all the tested extracts (significantly lower than those reported in the literature data) are favourable due to nickel's significant carcinogenic health risks (Lavrinenko *et al.* 2024). The presence of sulphur in the fumitory extracts is also beneficial due to its antibacterial activity against the bacteria *Propionibacterium acnes* which causes acne, as well as its antifungal potential, and skin relaxation and exfoliant effects. Such properties underscore the role of the obtained extracts in the treatment of different skin conditions, including acne vulgaris, seborrheic dermatitis rosacea, dandruff, etc. (Gupta & Nicol 2004). A study dealing with the mineral composition of various *Fumaria* species showed that the parts of the plants significantly differed in terms of the concentration of micro- and macroelements (Marichkova & K'ostarova 1985). Namely, the roots contained a higher level of sodium, copper, iron, and zinc, whereas the aerial parts contained higher quantities of potassium and calcium. In the tested *F. officinalis* lyophilised extracts, the calcium content was positively correlated with the magnesium and

zinc levels, while the iron level was negatively correlated with sodium concentration. Janda *et al.* (2021) reported that the mineral content in infusions of *Papaver rhoeas* L. (a species from the same family as fumitory, Papaveraceae) depended on the temperatures used, A similar trend was observed in the extracts developed in the present study, where the extraction conditions significantly influenced the aforementioned variable. For example, in the cited study, the sodium concentration was significantly higher in the samples obtained at room temperature than in those prepared using elevated temperatures (70–90°C). As can be seen from the results of the lyophilised fumitory extracts, the sodium level was significantly higher after maceration (room temperature) compared to the HAE and MAE extracts (80°C and 100°C, respectively), which aligns with the aforementioned literature data. Saleh Mohammed *et al.* (2023) also showed that the most dominant elements in various *Papaver* species (the same plant family as the *Fumaria* species) were potassium, sodium, magnesium, and calcium, as in the case of the fumitory extracts.

According to the literature, *S. aureus*, *E. coli*, and *C. albicans* are the most common causes of skin infections (Del Giudice 2020; Marques & Fernandes Abbade 2020; Hu *et al.* 2021). In addition, infections of the skin or soft tissues by *P. aeruginosa* range from superficial discolorations to serious and life-threatening conditions because of its invasive and toxigenic characteristics (Nagoba *et al.* 2017). *Enterococcus faecalis*, as one of the most frequently isolated bacteria from wounds, such as diabetic ulcers, surgical sites, and burns, was also selected for the antibacterial analysis of the extracts (Chong *et al.* 2017). *Klebsiella pneumoniae* is also known to cause wound infections, with data from the literature linking skin and soft tissue infections in aquaculture workers to this bacterium (Xie *et al.* 2024). *Proteus mirabilis* is found in skin abscesses, open wounds, or surgical sites, particularly in humans with compromised immune systems, diabetes, or prolonged antibiotic use which disrupts the normal flora of the skin (Mistry *et al.* 2010). Therefore, *K. pneumoniae* and *P. mirabilis* were also selected evaluating the antimicrobial capacity of the prepared extracts. Previous studies have reported the significant bactericidal activity of fumitory against Gram-positive microorganisms, such as *Staphylococcus* species and *Bacillus anthracis* (Babaeimarzangou *et al.* 2015). However, in a disc diffusion method, water and ethanol extracts of *F. officinalis* did not demonstrate antibacterial capacity against *E. coli*, *P. aeruginosa*, *P. vulgaris*, *K. pneumoniae*, and *S. aureus* (Pehlivan Karakaş *et al.* 2012). Several studies have also shown the resistance of Gram-negative bacteria to fumitory extracts, *i.e. E. coli* (Erdogrul *et al.* 2002; Stanojević *et al.* 2018), *P. mirabilis,* and *K. pneumoniae* (Sengul *et al.* 2009) which is consistent with the data obtained in this study. Nevertheless, the study conducted by Stanojević *et al.* (2018) showed antibacterial potential against *P. aeruginosa*, *P. vulgaris*, and *K. pneumoniae*, but the absence of any antibacterial effect on *S. aureus*, which contrasts with the data obtained in the present study. The reason for these differences may stem from the various origins of plant material and the bacteria used, as well as the extract preparation and assay specificity. Cakić *et al.* (2018) reported the strong antimicrobial potential of silver nanoparticles with *F. officinalis* extract against *S. aureus*, *B. cereus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *P. vulgaris*, and *C. albicans* due to the synergistic effect of silver and fumitory secondary metabolites. Sengul *et al.* (2009) demonstrated that the methanolic extract obtained from the aerial parts of *F. officinalis* exhibited moderate antimicrobial activity against the aforementioned spectrum of microbs. Conversely, in the same study, the aqueous extract showed little to no inhibitory effect on the tested microorganisms. In a separate study, Erdogrul (2002) found that ethyl acetate, methanol, chloroform, and acetone extracts of *F. officinalis* exhibited no antimicrobial activity against the panel of 12 microorganisms evaluated. *Fumaria indica* extracts showed varying antibacterial activity against *S. aureus*, *B. subtilis*, and *E. coli* depending on the extraction medium used (Riaz *et al.* 2019). Regarding

the antifungal activity of fumitory extracts, Bagheri *et al.* (2015) and Sengul *et al.* (2009) reported that the methanol fumitory extracts exhibited no significant effect on *C. albicans*, while Stanojević *et al.* (2018) reported the significant antifungal potential of the fumitory extracts against the same yeast. In contrast, two other studies have shown the antifungal potential of fumitory extracts against filamentous fungi, including *Aspergillus niger*, *A. flavus*, *Ganoderma lucidum*, *Alternaria alternata*, and *Cladosporium herbarum* (Sengul *et al.* 2009; Riaz *et al.* 2019). According to a recently published study (Ahmoda *et al.* 2025), caffeoylmalic acid, quercetin dihexoside, quercetin pentoside hexoside, rutin, and methylquercetin dihexoside were the most abundant components in the fumitory extracts. These extracts also contained alkaloids of the protopine type (protopine, oxo-, methyl, and/or acetyl protopine derivatives, and cryptopine), and of the spirobenzylisoquinoline type (fumariline and fumarophycine). Alkaloids, as the secondary metabolites of numerous plants, have demonstrated antimicrobial activity, influencing fungal biological functions at very low concentrations, particularly *via* efflux pump inhibition. For example, the minimal inhibitory concentration of fumariline and protopine (alkaloids also present in fumitory waste extracts, Ahmoda *et al.* 2025) amounted to 4–8 μg/mL for *C. albicans*, and 32–64 μg/mL for *E. coli*, *P. mirabilis*, *P. aeruginosa*, *K. pneumoniae*, as well as *S. aureus* (Bagheri *et al.* 2015). In addition, flavonoids are also known to be potent antibacterial agents. Specifically, kaempferol and quercetin (also present in *F. officinalis* waste extracts) displayed satisfactory *E. coli* DNA gyrase inhibition and membrane interaction effects (Shamsudin *et al.* 2022), while quercetin can also inhibit the formation of *P. aeruginosa* biofilm (Huang *et al.* 2022). However, in the case of the fumitory dust extracts, the antibacterial components were only effective against *S. aureus* and *E. faecalis*. The absence of any antibacterial effects on *E. coli*, *P. mirabilis*, and *K. pneumoniae* may be explained by an insufficient amount of quercetin in the selected fumitory extracts. Namely, the concentration of quercetin (determined in our previous study, Ahmoda *et al.* 2025) in the dilution of the fumitory extract used in the disc diffusion method was in the range of 0.131 to 0.177 μg/μL, while Shamsudin *et al.* (2022) reported that the minimal inhibitory concentration of quercetin was > 4 μg/μL for *E. coli* and *K. pneumoniae* and 0.5 μg/μL for *P. mirabilis*. The concentration of kaempferol varied from 0.124 to 0.177 μg/μL in the fumitory dust extracts, whereas in the literature, the minimal inhibitory concentration for the *E. coli* membrane interaction effect was reported as 0.025 μg/μL (Wu *et al.* 2013). Nevertheless, the tested extracts did not show any antibacterial activity toward the mentioned bacteria, probably due to the diversity and adaptations of *E. coli* pathotypes (Siniagina *et al.* 2021). Tannins, also present in fumitory extracts as strong binding agents for proteins and other membrane compounds, may affect microbes by disrupting and destabilising the cell membrane and consequently increasing membrane permeability (Villanueva *et al.* 2023). Gram-positive bacteria possess a thick cell wall, whereas Gram-negative species have a relatively thin cell wall as well as an additional outer membrane, which contains numerous pores and appendages, making them more resistant to antibacterial agents, which is also the case in the tested fumitory extracts. For example, Villanueva *et al.* (2023) reported that in order to achieve the inhibition of Gram-negative bacteria tannins should be derivatised with enough positive charge-introducing ammonium functional groups.

Kaempferol and rutin (also detected in fumitory waste extracts, Ahmoda *et al.* 2025) are flavonoids which possess anti-inflammatory capacity (Ullah *et al.* 2020). Among the four tested fumitory extracts, the MAE extract produced the highest rutin yield, but the lowest kaempferol content (Ahmoda *et al.* 2025), while the other extracts contained similar amounts of these flavonoids, which may explain the absence of any significant differences in the anti-inflammatory potential of the extracts at 0.5 g/mL in the heat-induced erythrocyte lysis process. Additionally, flavonoids such as kaempferol are potent inhibitors of cyclooxygen-

ase-2 (Abubakar *et al.* 2019). Biologically active secondary metabolites in plant extracts, including polyphenols and alkaloids, can protect lysosomal membranes through the activation of phospholipases, showing anti-inflammatory potential in terms of membrane stabilisation (Truong *et al.* 2019). The most potent activity of the MAE and UAE samples at higher concentrations in the inhibition of heat-induced hemolysis may lie in the higher total alkaloid content in the samples. Alkaloids are an important class of secondary metabolites with anti-inflammatory effects due to the increase in cytokine expression, and the inhibition of lipid mediators, enzymes, and histamine related to inflammatory reactions (Souza *et al.* 2020). Islam *et al.* (2023) indicated that plant extracts containing alkaloids, flavonoids, and tannins exhibit a promising *in vitro* membrane stabilising function, with a significant capacity to prevent RBC hemolysis in a concentration-dependent manner. In hypotonic-induced lysis of red blood cells, the fumitory dust extracts showed stabilising effects on the RBC membrane, but were lower in comparison to heat-induced hemolysis. This inhibition of hypotonic lysis of erythrocytes may be attributed to the prevention of the release of active inflammatory mediators and lytic enzymes (Yesmin *et al.* 2020). The investigation related to protopine also showed its anti-inflammatory properties (Vrba *et al.* 2011). The anti-inflammatory activity of plant extracts can be due to the flavonoids, as shown in the study of Radhakrishnan & Selvarajan (2021). Studies applying the RBC membrane stabilisation assay to *Fumaria* species have not been found in the available literature. However, a great deal of research supports anti-inflammatory effects using other well-validated models for *Fumaria* extracts. Raafat *et al.* (2020) demonstrated that the anti-inflammatory effects of *F. officinalis* extracts (using a different method) alleviate both carrageenan-induced acute inflammation and chronic hind paw edema. Serum analysis revealed decreased concentrations of the pro-inflammatory cytokines TNF-alpha and IL-6, alongside an increase in the anti-inflammatory cytokine IL-10. The alkaloid fumitory extract demonstrated notable anti-inflammatory activity, as evidenced by its inhibition of bovine serum albumin denaturation *in vitro* and a reduction of carrageenan-induced paw edema *in vivo* (Yahiaoui *et al.* 2022). The total alkaloid extract from *F. capreolata* significantly alleviated both macroscopic and microscopic indicators of intestinal inflammation and also modulated the expression of inflammatory mediators by reducing pro-inflammatory and increasing anti-inflammatory markers, while promoting the expression of proteins associated with intestinal barrier integrity (Bribi *et al.* 2020). In addition, various fractions of *F. indica* showed very low hemolytic activities, *i.e.* less toxicity (Riaz *et al.* 2019).

According to the literature, the acute and subacute toxicity of alkaloids from the aerial parts of *F. officinalis* determined in an *in vivo* study showed that the median lethal dose was estimated at 1341.11 mg/kg, suggesting its designation as a plant with limited toxic potential (Yahiaoui *et al.* 2022). Acute toxicity testing of the ethanolic extract derived from *F. officinalis* leaves also indicated a median lethal dose cut-off value of 2000 mg/kg body weight (Sharma *et al.* 2015). The cytotoxic potential of various *F. officinalis* extracts was assessed through the brine shrimp lethality bioassay. Among the tested extracts, the n-hexane fraction exhibited notable cytotoxicity, with a median lethal dose of 901.24 µg/mL against *Artemia salina* larvae (Al-Snafi 2020). Additionally, in our previous study (Ahmoda *et al.* 2025), the absence of keratinocyte cytotoxicity of the *F. officinalis* extracts in concentrations of 25, 50, and 100 µg/mL was proven in the keratinocyte cell (HaCaT) model.

Khazaeli & Mehrabani (2008) reported that sun protection factor of plant extracts is attributed to their high content of flavonoids and other phenolic compounds. In support of this, Mishra *et al.* (2012) emphasised that phenolics, particularly flavonoids, possess strong antioxidant and photoprotective activities. However, although the fumitory extracts possess polyphenols, the SPF values of the developed lyophilised extracts were significantly low. Therefore, in the devel-

opment of topical sunscreen formulations, it is necessary to screen for the specific flavonoids and other polyphenols in the selected plant species which are the carriers of the sun protection potential of the plant extracts. Due to the presence of polyphenolic molecules, including flavonoids, plant extracts can protect from UV irradiation and act as sun-protecting agents by inhibiting the production of free radicals as the main cause of skin-related disorders (Abidi *et al.* 2018). Flavonoid components have been widely used in cosmetics, skincare, and anti-wrinkle skin formulations (Ullah *et al.* 2020). The highest SPF was measured in the UAE sample, followed by the HAE and M extracts, while the lowest value was measured for the MAE sample, which can be explained by their chemical profiles. Namely, our previous study (Ahmoda *et al.* 2025) showed that the contents of chlorogenic and caffeoylmalic acids, methylquercetin dihexoside, kaempferol deoxyhexosylhexoside, and methylquercetin deoxyhexosylhexoside are higher in the M, HAE, and UAE extracts than the MAE sample. According to the literature, kaempferol is characterised by absorption at wavelengths relatively higher than other flavonoids, thus enabling the extracts containing kaempferol to provide a broad spectrum of protection in the UVA and UVB regions, along with high levels of antioxidant potential on the skin. Similarly, quercetin is characterised by absorption in both the UVA and UVB regions (Filho *et al.* 2016). Further, Evans-Johnson *et al.* (2013) demonstrated that a mixture of flavonoids containing kaempferol and quercetin, among others, significantly reduced dermal fibroblast apoptosis and keratinocyte proliferation induced by UVA radiation. Literature reports indicate that chlorogenic acid serves as a potent UV filter (Choquenet *et al.* 2009; Baldisserotto *et al.* 2018). Extracts rich in polyphenols, including chlorogenic and caffeic acids, can protect the skin from oxidative damage. Furthermore, these secondary metabolites have sunscreen capacity due to their chromophore groups, *i.e.* the conjugated single and double bonds which absorb UVA and UVB rays and consequently decrease their intensity on the skin (Zulfaidah *et al.* 2023). However, the fumitory dust extracts provided a low SPF value at all the tested concentrations since an SPF of 6 is generally considered the minimum degree necessary for UVB protection.

CONCLUSIONS

The study demonstrates that extraction techniques exert a significant influence on the phytochemical composition and biological activity of lyophilised *F. officinalis* waste extracts. Among the tested methods, microwave- and ultrasound-assisted extraction proved the most effective in maximising yield and enhancing membrane-stabilising activity. The presence of essential macro- and microelements, alongside complex secondary metabolites identified through FT-IR analysis, confirms the chemical richness of these extracts. The tested fumitory extracts exhibited moderate and comparable antibacterial activity against Gram-positive *S. aureus* and *E. faecalis*, while showing no efficacy against the Gram-negative bacteria or fungi. The ability of fumitory extracts to inhibit erythrocyte hemolysis under heat and hypotonic stress suggests promising anti-inflammatory (membrane stabilisation) properties. Moreover, while the SPF values were modest, the UV-absorbing potential, particularly of the UAE extracts, supports the further exploration of *F. officinalis* as a complementary ingredient in natural photoprotective formulations. Given the limitations of the present study, including the reliance on *in vitro* assays, the lack of *in vivo* validation, and the need for compound-specific testing, future perspectives will include the examination of the biological potential of the developed extracts, as well as their individual compounds in animal models. Future work should also focus on optimising formulation strategies, scaling up extraction techniques, and validating bioactivity in clinical settings in order to harness the full therapeutic and cosmeceutical potential of this traditionally valued plant.

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COMPREHENSIVE PLANT BIOLOGY

REZIME

FT-IR spektri, mineralni sastav i biološka aktivnost liofilizovanih ekstrakata otpada *Fumaria officinalis*

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Eksploatacija biljnog otpada u cilju ekstrakcije sekundarnih metabolita sa terapeutskim, antioksidativnim i kozmetičkim potencijalom, podržava principe održivog razvoja. *Fumaria officinalis* (dimnjača), tradicionalno korišćena lekovita biljna vrsta, sadrži različite biološki aktivne sekundarne metabolite, ali uticaj različitih metoda ekstrakcije na prinos ekstrakcije, mineralni sastav i bioaktivnost ove biljke još uvek nije dovoljno istražen. Četiri ekstrakcione tehnike, tj. maceracija (M), ekstrakcija na povišenoj temperaturi (HAE), ekstrakcija ultrazvučnim talasima (UAE) i ekstrakcija mikrotalasima (MAE), primenjene su za dobijanje ekstrakata dimnjače. Određeni su prinos ekstrakcije, mineralni sastav, karakteristične funkcionalne grupe (tehnika infracrvene spektroskopije sa Furijeovom transformacijom, FT-IR), antimikrobni potencijal, inhibicija hemolize izazvane termalnim i hipotonim stresom, kao i faktor zaštite od sunca (SPF). Tehnika ekstrakcije značajno je uticala na prinos ekstrakcije, koji je varirao od 17,24% (M) do 37,98% (MAE). Kalijum je bio najzastupljeniji makronutrijent (271,56–400,37 g/kg), dok su koncentracije svih mikronutrijenata bile ispod 1 g/kg. FT-IR analiza je pokazala prisustvo funkcionalnih grupa karakterističnih za fenole, alkaloide i proteine, potvrđujući složen hemijski sastav ekstrakata. Svi ekstrakti su pokazali antimikrobnu aktivnost prema bakterijama *Staphylococcus aureus* i *Enterococcus faecalis*, ali ne i protiv testiranih Gram-negativnih bakterija ili gljiva. UAE i MAE ekstrakti su pokazali visok nivo zaštite eritrocita od hemolize izazvane termalnim stresom (do 70,4% inhibicije pri koncentraciji od 0,25 mg/mL), dok su svi ekstrakti ispoljili umerenu i dozno-zavisnu zaštitu eritrocita u hipotoničnim uslovima. SPF analiza je pokazala UV-B apsorpciju u opsegu od 290–320 nm, pri čemu je UAE ekstrakt u koncentraciji od 100 μg/mL postigao najvišu SPF vrednost (1,66 ± 0,01). Studija pokazuje da metoda ekstrakcije značajno utiče na fizičko-hemijska i biološka svojstva *F. officinalis* ekstrakata. UAE i MAE su se pokazale kao najefikasnije metode za dobijanje ekstrakta sa najvećim biološkim potencijalom. Ovi rezultati podržavaju potencijalnu primenu ekstrakata dimnjače u prirodnim terapeutskim i kozmetičkim formulacijama. Buduća istraživanja treba da se fokusiraju na izolaciju specifičnih aktivnih jedinjenja i procenu njihove efikasnosti u farmaceutskim i kozmetičkim formulacijama, posebno u kontekstu zarastanja rana i delovanja protiv starenja kože, na novim ćelijskim modelima koji simuliraju oboljenja, infekcije, rane, opekotine i starenje kože.

Ključne reči: antiinflamatorni potencijal, antimikrobna aktivnost, dimnjača, liofilizat, faktor zaštite od sunca