Journal of Chromatography Open 9 (2026) 100275

Contents lists available at ScienceDirect

Journal of Chromatography Open

journal homepage: www.sciencedirect.com/journal/journal-of-chromatography-open

Determination of chemical components, phytochemical (polyphenol) profile, microorganisms and mycotoxins and evaluation of antimicrobial potential of *Pimpinella anisum* (Anise) and *Foeniculum vulgare* (Fennel) against bacteria isolated from cats☆

Marijana Sokolovic [a], Natasa P. Kalogiouri [b], Victoria F. Samanidou [b,*], Camelia Tulcan [c], Camen Dorin [c], Roberta Tripon [c], Snježana Kazazić [d], Fani Krstulović [a], Mirta Balenović [a], Tajana Amšel Zelenika [a], Ana Vulić [e], Claudia Daniela Serban [f], Marija Berendika [a]

[a] Croatian Veterinary Institute, Poultry Centre, Heinzelova 55, Zagreb, Croatia
[b] Laboratory of Analytical Chemistry, School of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece
[c] Faculty of Engineering and Applied Technologies, King Michael I of Romania Life Sciences University, Timisoara, Romania
[d] Ruđer Bošković Institute, Bijenička cesta 54, Zagreb, Croatia
[e] Croatian Veterinary Institute, Laboratory for Analytical Chemistry, Savska cesta 143, Zagreb, Croatia
[f] Doctoral School Engineering of Plant and Animal Resources, University of Life Sciences "King Mihai I" from Timişoara, Aradului Street No. 119, Timisoara, Romania

ARTICLE INFO

Keywords:
Polyphenols
Plants
Pimpinella anisum
Foeniculum vulgare
Antimicrobial activity

ABSTRACT

Nutritional, microbiological and toxicological quality of plants like *Pimpinella anisum* (anise) and *Foeniculum vulgare* Mill (fennel) are important factors in assuring their beneficial characteristics and usage. Besides defining basic nutritional and safety status, understanding the profile of compounds like polyphenols provides insights into their potential phytochemical, antioxidant and antimicrobial effects. The aim of this study was to evaluate chemical composition and polyphenol profile, mycotoxicological and microbiological quality and assess the antimicrobial potential of fennel extracts against bacterial pathogens isolated from cats.

In our study, the proximate chemical compositions of both seeds were in line with previous researches. In fennel and anise seeds, the most abundant group of fatty acids were monosaturated fatty acids (82.15%, 67.16%), followed by polysaturated fatty acids (10.81%, 23.96%) and saturated fatty acids (7.04%, 8.88%). Among individual fatty acids, the most abundant were oleic acid (81.02%, 65.05%), followed by linoleic acid (10.29%, 23.06%), respectively. Polyphenol profiling of plant extracts showed the highest amounts of Hydroxytyrosol (1069.0 mg/kg), Naringenin (420.3 mg/kg) and Epicatechin G (555.4 mg/kg) in fennel, while Naringenin (667.3 mg/kg), Apigenin 360.4 mg/kg), Luteolin (360.4 mg/kg), Coutaric Acid (328.9 mg/kg) and Caftaric acid (317.7 mg/kg) were detected in anise. Microbiological analysis of both plants showed presence of bacteria, fungi, yeasts and tested mycotoxins. Antimicrobial activity of both plants was demonstrated on wild bacterial isolates from cats, with noticed differences for tested extraction solvents. The results of our study show the promising potential of plant extracts for treatment of infections caused by tested pathogenic bacteria.

1. Introduction

Herbs in a medicinal context include any part of a plant that contain pharmacologically active substances and thus might, more or less effectively, aid in healing process (or possess health benefits). The use of various (medicinal) plants and their extracts for prevention, treatment and therapeutic purposes in human and animals is known since ancient times. However, implementation of the modern chemical, microbiological and molecular methodologies provided in depth knowledge on versatility of these immense compounds. Demand for such products is growing rapidly along with a great number of researches

☆ This article is part of a Special issue entitled: 'Dr. Salvatore Fanali 75th birthday' published in Journal of Chromatography Open.

* Corresponding author.
E-mail address: samanidu@chem.auth.gr (V.F. Samanidou).

https://doi.org/10.1016/j.jcoa.2025.100275
Received 4 September 2025; Received in revised form 7 October 2025; Accepted 18 October 2025
Available online 13 November 2025

on beneficial effects of diverse plants confirming their antioxidant, anti-inflammatory, immune-modulating, antimicrobial activity and/or unfavourable properties like allergic symptoms or contamination with bacteria, fungi and mycotoxins [1,2]. Family Apiaceae (Umbelliferae), named after the genus Apium, includes 434 genera and 3780 species of which a great number are aromatic flowering plants while some of them are poisonous species. Among them, species from the genera *Pimipinella* and *Foeniculum* share similar aromatic properties and are commonly used all around the world in the pharmaceutical, food and beverage industries, for therapeutic purposes or as repellents. They share the same characteristics like hollow stem, small umbrella like flower (umbels), specific shape of fruits (or seeds) with oil ducts. The seeds of fennel and aniseed are very similar in appearance and taste [3,4]. They are native in the Mediterranean region and Southwest Asia but can be cultivated all around the world. Their rich flavour, aroma and phytochemical composition are probably responsible their long traditional (medicinal) use for treatment of various disorders and diseases of gastrointestinal, urinary, respiratory, reproductive, immune and neural systems, problems with skin, degenerative joint diseases like osteoarthritis, pain, and cancer. Emerging interest of various research groups is focused on characterization of their secondary metabolites (e.g. polyphenols) as well as evaluation and testing of essential oils and their valuable fatty acids (e.g. oleic acid, etc.) [5].

Anise (*Pimpinella anisum* L) and fennel *(Foeniculum vulgare* Mill) are examples of widely studied plants due to their specific composition. That is to say, besides their nutritional quality, mineral and vitamin contents, these plants contain compounds with promising antioxidant and antimicrobial effects. For example, essential oils of anise and fennel have shown antibacterial activity against bacteria from the genera *Actinomyces*, *Bacillus*, *Escherichia*, *Enterococcus*, *Klebsiella*, *Lactobacillus*, *Listeria*, *Pseudomonas*, *Salmonella*, *Staphylococcus*, *Streptococcus*, against certain fungal species and parasites [6–11]. Both, aniseeds and fennel extracts showed high oxidant activity by use of various antioxidant tests [12,13].

Polyphenols are plant-derived compounds of various chemical structures and biological activity. Classified by their chemical structure, biological function, and origin, or as phenolic acids / alcohols or in different groups in respect to the strength of their phenolic ring, they represent and enormous group of compounds. Their production (and presence in the plants) also depends upon various factors including biological functions in plants, environment (biotic and abiotic stress), etc. [14,15]. The most known are: flavonoids, lignans, stilbenes, phenolic acids, non-phenolic metabolites and other polyphenols. Due to their distribution in plants in various forms that are considered important for their biological potential, analysis of plants in respect to their profile is an important step in further research and testing of beneficial effects of plants [16]. Polyphenols (e.g. flavonoids, phenolic acids, coumarin, tannins, phenolics, etc.) are found in all of plants of fennel in various quantities while a total phenolic content (TPC) in methanolic extract is around 1017.3 g GAE/100 g DW. In aniseeds, detected is a great number of polyphenols (e.g. anisol, anisalcohol, areluteolin, guajacol, isoorientin, isovitinexin, quercetin 3-O glucuronide and vanillin) also in diverse concentrations, while TPC of methanolic extracts of aniseeds is usually in a range from 17.4 mg to 46.2 mg GAE/g [17–19]. However, their (phyto)chemical composition is influenced by various factors including genetic background, food availability and diverse environmental conditions. Therefore, determination of moisture, ash, fibre, fat, protein, sugar and starch provides insights in plant nutritional composition and enables comparisons among plants of different origin and other plants in general.

An important aspect of plant use is assessment of their safety, i.e. determination of various contaminants. Namely, during their lifetime, plants can be contaminated with pesticides, heavy metals, chemicals, toxins or by microbial contaminants. Among these, contamination with fungi and mycotoxins can often occur, irrespective of the growing and production phase, transport or storage phases. For majority of plants (mainly plants used as food and feed) pretty scattered data from studies in different laboratory settings can be found in scientific literature. Certain adverse effects of plants might be due to their specific components (allergic reaction) or due to the presence of contaminating microorganisms and/or their metabolites [1,2,20]. Therefore, microbiological testing and determination of bacteria, fungi and their toxins are of high importance. Summing all said above, if adequately analysed and evaluated, fennel and aniseeds are promising candidates in fighting the growing worldwide threat of antimicrobial resistance. In a case of companion animals, the trend of owner and animal interactions besides numerous benefits, also brings a higher risk of transmission of potential zoonotic and antimicrobial resistant pathogens. In cats, the spread of diseases can occur through bites, scratches, urine, faeces, and contact [21–23]. Since a number of antibiotics are currently not adequately effective, a lot of efforts are made in a search of alternative treatments of such infections. Determination of antimicrobial activity of plant-derived compounds against pathogens has been focus of recent research efforts and mainly includes effects of their essential oils on selected pathogens [24,25]. Since the antimicrobial activity of plants' extracts depends upon their chemical and polyphenol profile, the aim of this study was to evaluate chemical and microbiological quality and safety of *Pimpinella anisum* and *Foeniculum vulgare* and to assess their antimicrobial potential against wild resistant *Staphylococcus* strains isolated from cats.

2. Experimental

2.1. Chemicals, reagents and solvents

If not otherwise stated, all chemicals used in this study were of pro analysis grade. Specific chemicals and reagents are mentioned later in the text.

2.2. Plant materials

The dried plant products (seeds) of *Pimpiella anisum* and *Foeniculum vulgare* were purchased from the local store (Šuban d.o.o., Strmec Samoborski, Croatia) in a scope of the NextGenerationEU project NPOO15: Comprehensive Research on the Impact of Plant Extracts against resistant *Staphylococcus* (CRISP). Upon acquisition, the plant samples were homogenized and grinded (ZM200 RETSCH, Germany), in the sterile conditions, so that the laboratory sample passed completely through a sieve with 1 mm apertures. For further analysis, samples were weighed on the Laboratory Analytical Balance (Ohaus DV215CD, USA). All samples were analysed upon their collection so that all procedures were done in such a way that deterioration and change in composition were minimized.

2.3. Proximate analysis and mineral content of samples

Determination of chemical composition of plants was carried out in the Feed Analysis Laboratory, Branch of Poultry Centre of Croatian Veterinary Institute. **Chemicals:** Acetanilide standard, acetone, ammonium heptamolybdate solution, ammonium monovadate solution, boric acid, calcium carbonate, copper (II) sulphate pentahydrate (catalyst), diatomaceous earth, ethanol, filter aid (Celite 545), hydrochloric acid, light petroleum, minerals' standards, nitric acid, potassium hydroxide, potassium sulphate, saccharose, sodium hydroxide, sodium sulphate, tryptophan standard, water (grade 3 according to ISO 3696) and zinc acetate solution. **Procedures:** The Proximate Analysis and Mineral Content were determined with validated International Organisation for Standardisation (ISO) methods. Content of moisture, crude ash, crude fibre, total fat, starch and crude protein in plant samples were determined with ISO 6496:1999, ISO 5984:2022, ISO 6865:2000, ISO 6492:1999, ISO 6493:2000 and ISO 5983-1:2005/Cor 1:2008/ISO 5983-2:2009, respectively. Determinations of **moisture, other volatile**

matter and **ash** content were made with gravimetric methods using microwave furnace (Pyro Ashing System-MLS 1200 Milestone, USA), electric muffle-furnace (Nabertherm, Germany) and drying oven (Ele International, USA). The moisture and other volatile matter content were determined as a mass fraction of substances that is lost during drying of the sample at 103 °C ± 2 °C in a specific time period to achieve constant mass. The ash content was calculated from weight difference of a test portion after it was carbonized and incinerated at 550 °C ± 20 °C and cooled. Determination of **fibre** content was made with automatic system Fibretherm (Gerhardt, Germany) according to the manufacturer's Instructions and adapted to the principle of the method specified in the aforementioned ISO standard. For **fat analysis,** we have applied the Soxhlet method with a hydrolysis step prior to extraction using automated system Soxtherm (Gerhardt, Germany) according to the manufacturer's protocol adapted to corresponding standard method. **Total protein** content was determined according to the Kjeldahl method using block digestion on Kjeldatherm (Gerhardt, Germany) and steam distillation on Vapodest 500 (Gerhardt, Germany). The method follows the Instruction of the manufacturer that are adapted to corresponding ISO standard and includes digestion of the sample with sulphuric acid, distillation of the digestion solution and titration of the distillate. The protein content was calculated from the measured nitrogen content using conventional factor. **Sugar** content was determined with the validated colorimetric method according to protocols developed by Nelson and Somogyi [26,27]. The method is based on estimation of reducing sugar (glucose) and their measurement at specified wavelength was made using spectrophotometer Lambda 25 (Perkin Elmer, USA). The **starch** content was determined after a test portion is decomposed with diluted hydrochloric acid. The solubilized starch is gelatinized and hydrolysed. The content of starch was measured by determination of the total optical rotation of the clarified solution using Polarimeter IP20 (Zeiss, Germany). All analyses were made in duplicate and the results were expressed as an average percentage ratio in dry feed of samples with corresponding standard deviation and standard error. Determination of mineral contents were made after the samples were ashed in microwave oven (Milestone, USA) and electric muffle furnace (Nabertherm, Germany) at specified temperature. The content of phosphorus was measured with spectrophotometer Lambda (Perkin Elmer, USA) according to ISO 6491:1998, while the other minerals (Ca, Cu, Fe, Na, K, Mg, Mn and Zn) were determined according to the ISO 6869:2000 using atomic absorption spectrophotometer PinAAcle 800 (Perkin Elmer, USA) using appropriate standards for preparation of calibration curve. All analyses were made in duplicate and the results were expressed as mg/kg ration in dry feed samples with corresponding standard deviation and standard error.

2.4. Determination of fatty acid profile of samples

Fatty Acid Profile of Samples (extracted fat during Proximate Analysis) was carried out in the Laboratory for Analytical Chemistry of Croatian Veterinary Institute. **Chemicals:** Standard solution of fatty acid methyl esters (FAME) was prepared by dissolving 100 mg of standard SupelcoTM 37 Component FAME Mix (Pennsylvania, USA) in 10 mL of hexane. **Procedure**: Extracted fat was used for fatty acid methyl esters preparation according to ISO 12966-4:2015 with certain modifications. Briefly, the extracted fat was dissolved in isooctane, and methanolic transesterification was performed. Afterwards, saturated sodium chloride solution was added. After the separation of layers, the upper isooctane layer was transferred into another tube and anhydrous sodium hydrogen sulphate was added. The aliquot of each sample was filtered through a PTFE filter 0.2 μm pore size into vials. Methyl esters of fatty acids were analysed by GC-FID (gas chromatography with flame ionization detector) method using the Agilent 7890 A Gas Chromatograph (Agilent Technologies, USA) equipped with a DB-23 capillary column (60 m length, internal capillary diameter 0.25 mm, and stationary phase thickness 0.2 μm (Agilent Technologies, USA). The carrier gas was helium. A split/splitless injector was used to inject one microlitre of each sample in split ratio of 1:50. FAME identification was performed by comparing the sample FAME retention time to standard solution mixture FAME. The results were expressed as the percentage of a given fatty acid in the total fatty acid share.

2.5. Determination of polyphenol profile of samples

The dried plant samples were ground in a simple mixer, homogenized, and five grams of each plant was transferred in the plastic Falcon Tube, and refrigerated at −20 °C with the closed lid. After 24 h of refrigerating, the lids of falcon tubes were taken away, the tubes were covered with the parafilm and three holes were made with a needle. The tubes were transferred in the Lyophilizer Alpha 1-4 (Christ, Germany) for 24 h. When the process of lyophilization was completed, the tubes with the samples were closed, refrigerated and transported to the Laboratory of Chemistry of Aristotle University of Thessaloniki for further analysis. From each sample, 20 mg of lyophilized material was accurately weighed and transferred into Eppendorf-type microtube. Subsequently, 2 mL of extraction solvent (methanol/water, 80:20 v/v) was added and the samples were vortexed for 1 min. Then, the samples were centrifuged at 8.000 rpm for 10 min. The aliquots were collected and filtered through 0.22 microlitre nylon syringe filters with pipette (CAPP Aero EcoPipette Single, EU) prior to the injection in the chromatographic system. **Chemicals and standards**: LC-MS grade methanol, water, and formic acid 98%–100% were provided by Merck (Darmstadt, Germany). Apigenin 98%, catechin 98%, epicatechin 98%, hesperidin 98%, isorhamnetin 98%, luteolin 98%, myricetin 98%, naringenin 98%, caffeic acid 98%, protocatechuic acid 98%, quercetin 98%, rutin 98%, syringaldehyde 98%, taxifolin 98%, ferulic acid 98%, resveratrol 98%, syringic acid 98%, p-coumaric acid 98%, ellagic acid 98%, gallic acid 98%, coutaric acid 98%, hydroxytyrosol 98%, epicatechin gallate 98%, and caftaric acid 98% were purchased from Sigma-Aldrich (Steinheim, Germany). Stock standard solutions of all the aforementioned standard compounds were prepared in LC-MS grade methanol at 1000 mg/L and were afterwards stored in dark brown glass bottles at −20 °C. **Instruments:** Analytical balance SCALTEC SBA33, accuracy ± 0.0001 g (Gujarat, India), Small vortex mixer, Glass-col (Terre Haute, USA), Mini centrifuge 2507/15 Nahita (Auxilab S.L., Spain), Adjustable micropipette 20–200 microlitre (CAPP Aero EcoPipette Single, EU), Adjustable micropipette 10–1000 microlitre (MinipipetVar, Servoprax GmbH, Wesel, Germany), 2 mL conical microcentrifuge tubes (Eppendorf type), 1 mL microsyringe, Braun inject-F (Bad Arolsen, Germany).

LC-QTOF-MS/MS analysis: Analyses were performed using an Agilent 6530 Quadrupole Time-of-Flight (Q-TOF) Liquid Chromatography/Mass Spectrometry (LC/MS) system (Agilent Technologies, Santa Clara, CA, USA), equipped with an electrospray ionization (ESI) source operating in negative ion mode. The chromatographic separation was achieved using an Agilent 1290 Infinity II UHPLC system coupled to the Q-TOF mass spectrometer. Chromatographic separation was carried out on an XBridge BEH C18 column (2.1 × 100 mm, 2.5 micrometer particle size; Waters Corp., Milford, MA, USA), maintained at 40 °C. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in methanol (solvent B), delivered at a flow rate of 0.2–0.4 mL/min according to the gradient elution program specified in Table 1.

Data acquisition and processing were performed using Agilent MassHunter Workstation software. For the confirmation of the presence of the target analytes, the mass accuracies of the precursor ion and the qualifiers, the retention times (Rt), and the MS/MS spectra of the real samples and standard solutions were compared.

Table 1
Gradient elution program for LC-QTOF-MS/MS analysis of Polyphenols.

Time (min)	A [%]	B [%]	Flow [mL/min]
0	99	1	0.2
4	61	39	0.2
12	5	95	0.4
15	5	95	0.4
16	99	1	0.2
20	99	1	0.2

Mobile Phases: A: 0.1% Formic acid in Water, B: 0.1% Formic acid in Methanol. **The MS detector** was operated according to the settings listed in Table 2.

Table 2
Tne MS detector settings for LC-QTOF-MS/MS analysis of Polyphenols.

Parameter	Value
Gas temperature [°C]	200
Drying gas flow [L/min]	10
Nebulizer pressure [psi]	40
Sheath gas flow [L/min]	12
Sheath gas temperature [°C]	250
Ion source	ESI (negative mode)
Capillary voltage (V)	3500
Fragmentor voltage	180
Acquisition Mode	Full scan and MS/MS
Mass range	m/z 100–1000

2.6. Mycotoxin analysis of samples

All procedures were carried out in the Feed Analysis Laboratory, Branch of Poultry Centre of Croatian Veterinary Institute. **Chemicals**: deionized water (Milli-Q Integral 5, USA), Methanol and Mycotoxin standards were purchased from Sigma Aldrich, USA. **Procedure:** Mycotoxicological profile of seven mycotoxins was determined using validated enzyme-linked immunosorbent assays (ELISA) in a quantitative form as Agraquant® test kits T-2/HT-2 ELISA test, Zearalenone Plus ELISA test, Total Aflatoxin ELISA test, Ochratoxin ELISA test, Deoxynivalenol Plus ELISA test and Fumonisin ELISA test (RomerLabs®, USA). All procedures were made according to the manufacturer's instructions. Diacetoxyscirpenol was analysed by Thin Layer Chromatography Method as described earlier [28]. All samples that were positive with ELISA tests were confirmed using validated High-Performance Liquid Chromatography methods [28,29].

2.7. Microbiological analysis of samples

Isolation, counting and microbiological identification of bacteria and fungi were carried out in the Feed Analysis Laboratory, Branch of Poultry Centre of Croatian Veterinary Institute. Isolated microorganisms were confirmed by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS, Bruker, Germany) at Ruđer Bošković Institute. All samples were tested for the presence of bacteria and fungi using standard ISO methods (ISO 4833-2:2013/ISO 4833-2:2013/Amd 1:2022, ISO 6579-1:2017, ISO 6579-1:2017/Amd 1:2020, ISO 15213-1:2023/ISO 15213-2:2023, ISO 16654:2001/ISO 16654:2001/Amd 1:2017/ISO 16654:2001/Amd 2:2023, ISO 6888-1:2021/ISO 6888-1:2021/Amd:2023 and ISO 21527-2:2008). Isolated bacteria were identified according to colony appearance on selective agar media, microscopic examination of Gram stains and standard biochemical tests. Fungal species were confirmed by morphological macroscopic and microscopic characteristics using relevant fungal keys [30].

2.8. Antimicrobial potential of plant extracts

All procedures were carried out in the Feed Analysis Laboratory, Branch of Poultry Centre of Croatian Veterinary Institute. Antimicrobial activity of plant extracts was evaluated using extracts of plants prepared with water and different organic solvents.

2.8.1. Plant extracts preparation and TPC content

The Antimicrobial activity of plant extracts was evaluated using extracts of plants prepared with water and different organic solvents (methanol, water/ethanol (50/50, v/v) and DMSO). In short, extracts of plants with deionized water, DMSO, methanol, water/ethanol (50/50, v/v) were prepared by sonicating of 2.0 g of homogenized sample with 40 ml of selected solvent for 30 min in ultrasonic bath, centrifuging at 5.000 rpm/10 min and filtering through Whatman filter papers. Samples were stored at cold temperature (2–8 °C) until further analysis. **Total phenolic content (TPC):** Folin-Ciocalteu Colorimetric (F-C) Assay was performed according to Singleton et al. [31] to evaluate extraction efficacy of the tested solvents. All extraction and dilution procedures were protected from light. For F-C Assay, 1 ml of sample, blank and reference standard (Gallic acid, GAE) was added to 60 ml of de-ionized water in a 100-ml volumetric flask. After adding 5.0 ml of Folin Ciocalteu Reagent, the mixture was vortexed for one minute, added was 15 ml of 20% sodium carbonate solution and the volume was adjusted to 100.0 ml. The samples were kept at 25 °C for 120 min for development of the blue colour. The results were measured with UV–VIS spectrophotometer SPECTROstar NANO (BMG Labtech, Germany) at 750 nm in a one cm quartz cuvette. All the samples were analysed in triplicates and the concentration of total phenolic in samples were calculated from the calibration curve and expressed as gallic acid equivalents (mg of GAE per g of sample). Calibration curve was prepared with gallic acid standard solutions in the range 50–500 mg/L. The linear regression analysis was used to determine the slope, y-intercept and the R-squared value (that was accepted if greater than 0.990).

2.8.2. Antimicrobial potential

Evaluation was made according to the Procedure described earlier [32]. In short, all bacterial strains were cultured on Brain Heart Infusion liquid medium (Thermo Scientific™, USA) at 37 °C ± 1 °C for six hours. The strains were cultured on the Mueller-Hinton agar plates (Thermo Scientific™, USA) at concentration of 10^6 cells/mL. On each agar plate with bacteria, filter paper discs (6 mm diameter) inoculated with 50 µl of each extract was placed. After 24 ±1 h of incubation at 37 °C ± 1 °C, the zones were measured. The controls were the pure solvents and commercial antibiotic discs: cefoxitin, chloramphenicol, gentamicin and vancomycin that were previously tested along with other antimicrobial discs to evaluate antimicrobial resistance of standard and isolated strains (data are provided as Supplementary File S1 and S2). The tests were performed on standard bacterial strains *S. aureus* ATCC 6538, 29213, 256923 and *E. coli* ATCC 25922. Multi-drug Resistant Wild Strains were isolated from swabs originated from cats, sampled in a scope of the NextGenerationEU project NPOO15: Comprehensive Research on the Impact of Plant Extracts against resistant *Staphylococcus* (CRISP). Isolation was made according to the protocol for detection of *Staphylococcus* spp. and MRSA *Staphylococcus* [33,34]. The procedure included: examination of swabs within 72 h of sampling (maintained at 4–8 °C), their incubation in Mueller-Hinton broth (Thermo Scientific™, USA) containing 6,5% NaCl, incubation at 35-37 °C in thermostat (Memmert, Germany) for 16–24 h, subsequent inoculation of selective MRSA agar (CHROMagar™ MRSA) for 16–24 h. All presumptive colonies were sub-cultured on Manitol Salt Agar (Thermo Scientific™, USA), Baird Parker Agar (Thermo Scientific™, USA), Columbia Agar (Thermo Scientific™, USA) and Columbia Blood Agar (Thermo Scientific™, USA). Each strain was confirmed by characteristic growth on agar plates, gram stain, catalase, oxidase and qualitative slide agglutination test

Table 3
Results of the Proximate analysis and mineral content of *Pimpinella anisum* (Anise) seeds.

Pimpinella anisum	MEAN	SD	SE
Proximate Analysis			
Moisture (%)	8.54	0.08	0.05
Crude Ash (%)	5.90	0.06	0.04
Total Fat (%)	27.90	0.29	0.21
Crude Fibre (%)	30.61	0.09	0.07
Crude Protein (%)	18.40	0.10	0.07
Starch (%)	1.43	0.41	0.29
Sugar content (%)	4.39	0.05	0.04
Macro-minerals (mg/kg)			
Ca	6,650.00	70.71	50.00
K	20,300.00	141.42	100.00
Mg	2,720.00	0.00	0.00
Na	324.00	84.85	60.00
P	4,190.00	42.43	30.00
Trace Minerals (mg/kg)			
Cu	17	0.00	0.00
Fe	187.00	1.41	1.00
Mn	26.00	0.00	0.00
Zn	71.00	7.07	5.00

SD — standard deviation, SE — standard error.

(Staphylase™ Test, Thermo Scientific™, USA). All positive strains were additionally confirmed by MALDI-TOF MS. Antimicrobial resistance of strains was determined by disk diffusion method specified in CLSI M100 standard [35], oxacillin and cefoxitin test and confirmation of methicillin resistance by a multiplex polymerase chain reaction protocol for detection of *mec*A, *mec*C, *spa* and PVL genes [36] analysed using Thermocycler SimpliAmp (Thermo Fisher Scientific, USA), gel electrophoresis unit PowerPro 300 (Cleaver Scientific, UK) and visualized with GelDoc GO Imaging System (BioRad, USA). All strains selected for testing were resistant to three or more antibiotic and *S. aureus* strains were *mecC* positive with PCR method.

3. Results

3.1. Proximate analysis and mineral content of plants

Chemical composition of *Pimpinella anisum* and *Foeniculum vulgare* seeds are presented in Tables 3. and 4.

3.2. Fatty Acid Methyl Esters (FAME) analysis

The results were expressed as the percentage (%) of a given fatty acid in the total fatty acid share. The Results are presented in Table 5.

3.3. Polyphenols

Lyophilization allows water removal without the application of high temperatures, thereby minimizing the degradation of thermolabile bioactive compounds. It ensures long-term stability of natural products and is an internationally accepted preparation method for analytical purposes. In our study, *Foeniculum vulgare*, fructus contained the highest amounts of Hydroxytyrosol (1069.0 mg/kg), Naringenin (420.3 mg/kg), Epicatechin G (555.4 mg/kg) and Rutin (70.9 mg/kg) followed with lower amounts of Syringic Acid (27.4 mg/kg), *p*-coumaric acid (26.2 mg/kg), Protocatehuic acid (16.9 mg/kg) and caffeic acid (9.4 mg/kg). *Pimpinella anisum* seeds extracts were the most abundant in Naringenin (667.3 mg/kg), Apigenin (360.4 mg/kg), Luteolin (360.4 mg/kg), Coutaric Acid (328.9 mg/kg), Caftaric (317.7 mg/kg), Hydroxytyrosol (268.7 mg/kg), Epicatechin G (154.0 mg/kg), Protocatechuic (123.2 mg/kg), *p*-coumaric acid (83.0 mg/kg) and Rutin (76.3 mg/kg), followed with the lower concentrations of Cafeic (32.8 mg/kg), Syringic acid (19.1 mg/kg), Quercetin (13.6 mg/kg), and Hesperidin (5.6 mg/kg) (Table 6.)

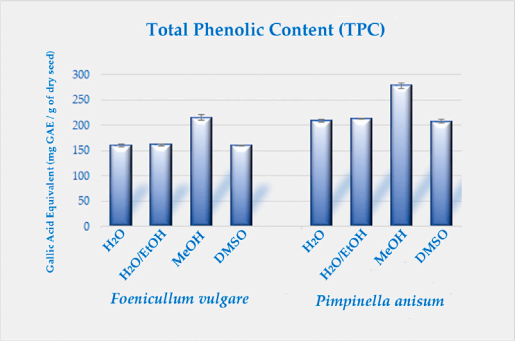

Fig. 1. Total Phenolic Content (TPC) of different extracts of *Pimpinella Anisum* and *Foeniculum vulgare* used for determination of antimicrobial activity against tested pathogens, expressed as gallic acid equivalents (mg of GAE per g of sample) (n = 3).

3.4. Mycotoxins in samples of Pimpinella anisum (Anise) and Foeniculum vulgare (Fennel) seeds

The Results of this study showed presence of potentially pathogenic fungi from the genera *Aspergillus, Fusarium, Penicillium, Alternaria* and Yeasts in both plant samples and all samples contained all tested mycotoxins in diverse range of concentrations (Table 7.). The mycotoxicological profiles of tested plants were in accordance with current legislation for ochratoxin, the only mycotoxin for which there is a Commission Regulation (EU) that propose maximum tolerable levels (MTL) for Herbs (EU 2023/915). For other tested mycotoxins: aflatoxin, diacetoxyscirpenol, deoxynivalenol, fumonisin, zearalenone and T-2/HT-2 toxin there are no guidance levels nor proposed tolerable limits for this kind of samples.

3.5. Microbiological evaluation of samples of seeds of Pimpinella anisum (Anise) and Foeniculum vulgare Mill (Fennel)

The results of microbiological evaluation of samples are presented in Table 8. Determined numbers of total bacteria and moulds indicated that the samples are microbiologically safe and in line with protocols for microbiological quality assessment. Determination of total bacterial count, presence of potentially pathogenic bacteria and fungi did not result with detection of pathogens, but certain number of bacteria and fungi were confirmed. This highlights the importance of evaluation of their safety before further use. This is especially important since the low numbers of fungi from the genera *Aspergillus, Fusarium, Penicillium* and *Alternaria* as well as yeasts were detected in both samples. In line with this are the findings of tested mycotoxins.

3.6. Antimicrobial potential of plant extracts

The total phenolic content (TPC) of plants *Pimpinella anisum* and *Foeniculum vulgare* extracts in water, methanol, water/ethanol and DMSO, calculated as gallic acid equivalents (mg of GAE per g of sample) are presented in Fig. 1. The highest TPC was achieved with methanolic extracts, followed by ethanol/water extracts, while water and DMSO showed almost similar TPC content for both plants.

Antimicrobial activity of plant extracts: Antimicrobial activity of plants extracts prepared with water and different organic solvents (methanol, ethanol and DMSO) showed different activities against tested bacterial strains (Table 9.).

Table 4
Results of the proximate analysis and mineral content of *Foeniculum vulgare* Mill (Fennel) seeds.

Foeniculum vulgare	MEAN	SD	SE
Proximate Analysis			
Moisture (%)	9.19	0.06	0.04
Crude Ash (%)	7.83	0.21	0.15
Total Fat (%)	13.75	0.34	0.24
Crude Fibre (%)	25.11	0.13	0.09
Crude Protein (%)	16.67	0.11	0.07
Starch (%)	ND	–	–
Sugar content (%)	4.80	0.11	0.08
Macro-minerals (mg/kg)			
Ca	10,920.00	169.71	120.00
K	22,400.00	1,131.37	800.00
Mg	4,120.00	0.00	0.00
Na	1,173.50	75.66	53.50
P	5,035.00	35.36	25.00
Trace Minerals (mg/kg)			
Cu	14.00	0.00	0.00
Fe	106.00	2.83	2.00
Mn	48.00	0.00	0.00
Zn	49.00	4.24	3.00

SD — standard deviation, SE — standard error, ND — not detected.

4. Discussion

Besides research on their efficacy, natural products need to be evaluated for their quality and safety. The chemical analysis of plants that includes separation and quantification of major components, included in proximate analysis, is the first step in evaluation of quality and safety of plants. As stated before, chemical and phytochemical composition of plants depends upon various factors from genetic background, the part of the plant, nutrient availability and diverse environmental conditions. The place where and when certain plant grows is an important factor for chemical composition as well as for production of biomolecules [37]. Additional information on the essential inorganic nutrients like minerals (major or macro-minerals and trace minerals) provide valuable information of their faculties. Furthermore, total fat content provides information about the amount of lipid or fat in the analysed sample. Evaluation of the fatty acid profile of ether extracts encompasses greater knowledge on their (biochemical) origin, biological role and potential (beneficial) functions and is necessary tool to assure their (therapeutic) use [38].

In general, **Aniseeds** have moisture content around 9%–13%, crude ash around 10%, crude fibre from 12%–25%, crude protein around 18%, starch around 5%, carbohydrates 55%, soluble sugars 5%, 8%–11% of fixed oil (of which 56% is petroselinic acid) and essential oils from 2%–7% [39]. **Fennel seeds** have moisture 6.3–8.8%, crude ash 8.2–13.4%, crude fat 14.9%, crude fibre 15.7–28.4%, crude protein 9.5–15.8%, carbohydrates 36.6–50.1%, soluble sugars around 1.1%, 1.2 g Ca, 19 mg Fe, 1.0 g K, 385 mg Mg, 88 mg Na, 487 mg P, 28 mg Zn, 135 IU of vitamin A, 6 mg of niacin, 0.41 mg of thiamine, 0.35 mg of riboflavin, 10–14.4% fixed (mainly petroselenic (70%–80%), oleic (around 25%), linoleic (around 15%) and palmitic (around 5%) acids) and essential oil (containing anethole, estragol, fenchone, limonene and other monoterpenes) [40,41]. In our study, in *Foenicullum vulgare,* the most abundant group of fatty acids (FA) were monosaturated fatty acids (MUFA) (82.15%) followed by polysaturated fatty acids (PUFA) (10.81%) while saturated fatty acids (SFA) (7.04%) were very low. If we observe individual fatty acids, it can be noticed that the most abundant fatty acid was oleic (MUFA, C18:1n9c, 81.02%), followed by essential linoleic acid (PUFA, C18:2n6c, 10.29%) and palmitic acid (SFA, C16:0, 4.44%), while there was a very low content of stearic acid (SFA, C18:0, 1.78%), cis-vaccenic acid (MUFA, C18:1n7, 0.42%) and essential alpha-linolenic acid (PUFA, C18:3n3 (ALA), 0.36%). In *Pimpinella anisum*, the pattern on FAs group was similar to *Foeniculum vulgare* with MUFAs being the most abundant (67.16%) group, followed by PUFA (23.96%) and SFA (8.88%). The most abundant FA in *Pimpinella anisum* was also oleic acid (MUFA, C18:1n9c, 65.05%), followed by essential linoleic acid (PUFA, C18:2n6c, 23.06%), palmitic acid (SFA, C16:0, 3.79%), lauric acid (SFA, C12:0, 2.94%), stearic acid (SFA, C18:0, 1.38%), cis-vaccenic acid (MUFA, C18:1n7, 1.41%), and in less than 1% were butyric acid (SFA, C4:0, 0.46%), essential alpha-linolenic acid (PUFA, C18:3n3, ALA, 0.45%), linolenic acid with a trans double bond (PUFA, C18:2n6t, 0.39%) and cis-palmitoleic acid (MUFA, C16:1n7c, 0.35%). Knowledge of chemical composition is the starting point in evaluation of plants for further research and use since it directly relates to nutritional and therapeutic value and this has been confirmed in our study. FAs acids are known for their strong antimicrobial properties and although the mechanism of action is not well described, their application (often in combination with other compounds) was effective against different pathogenic bacteria. For example, among SFA, lauric acid was effective against gram-positive bacteria including wild isolates of *S. aureus*, while their activity against gram-negative bacteria was less pronounced. Similar results were observed for palmitic and stearic acid [42–45]. Acids in MUFA and PUFA groups like docosahexanoic acid and eicosapentaenoic acid also exhibited antibacterial effects against gram positive bacteria [45,46]. In a study made by Butt et al. [47], microemulsions of FAs palmitoleic and alfa-linolenic acid showed to be effective against *S. aureus.* For oleic acid, that was the most abundant in our study, an interesting research results indicated that synthetized liposomal oleic acid was able to restore sensitivity of certain bacteria against known classes of antibiotics [48]. Finding the novel drugs in the world of FAs is not easy since different bacteria react differently against different antibiotic. In addition, the benefit of FAs is probably also in their observed synergistic effects with other compounds, including already used antibacterial agents. However, presence of these FAs in our study in respect to above mentioned studies show their potential synergistic effects with other chemical compounds in anise and fennel in reaching the antimicrobial activity against multi-drug resistant bacteria.

Polyphenols have also been investigated for their antibacterial properties and are considered as a promising natural alternatives to currently used antimicrobials in practice. In our study, *Pimpinella anisum* seeds extracts were the most abundant in Naringenin (667.3 mg/kg), Apigenin (360.4 mg/kg), Luteolin (360.4 mg/kg), Coutaric Acid (328.9 mg/kg), Caftaric (317.7 mg/kg), Hydroxytyrosol (268.7 mg/kg), Epicatechin G. (154.0 mg/kg), Protocatechuic (123.2 mg/kg), *p*-coumaric acid (83.0 mg/kg) and Rutin (76.3 mg/kg), followed with the lower concentrations of Cafeic (32.8 mg/kg), Syringic acid (19.1 mg/kg), Quercetin (13.6 mg/kg), and Hesperidin

Table 5
Fatty acid profile of *Pimpinella anisum* (Anise) and *Foeniculum vulgare* Mill (Fennel) seeds.

cPlant FA	*Pimpinella anisum* FA (%)	*Foeniculum vulgare* Mill FA (%)
C4:0	0.46	< 0.1
C6:0	< 0.1	0.04
C8:0	< 0.1	< 0.1
C10:0	< 0.1	< 0.1
C11:0	< 0.1	< 0.1
C12:0	2.94	0.11
C13:0	< 0.1	0.00
C14:0	0.03	0.05
C14:1	< 0.1	0.07
C15:0	0.05	0.07
C15:1	< 0.1	< 0.1
C16:0	3.79	4.44
C16:1n7t	0.12	0.25
C16:1n7c	0.35	0.10
C17:0	< 0.1	0.05
C17:1	0.06	< 0.1
C18:0	1.38	1.78
C18:1n9t	< 0.1	< 0.1
C18:1n9c	65.05	81.02
C18:1n7	1.41	0.42
C18:2n6t	0.39	0.09
C18:2n6c	23.06	10.29
C18:3n6	0.06	< 0.1
C18:3n3 (ALA)	0.45	0.36
C18:4n3	< 0.1	< 0.1
C20:0	0.18	0.16
C20:1n9	0.10	< 0.1
C20:2n6	< 0.1	< 0.1
C21:0	< 0.1	< 0.1
C20:3n6	< 0.1	< 0.1
C20:4n6	< 0.1	< 0.1
C20:3n3	< 0.1	< 0.1
C20:4n3	< 0.1	< 0.1
C20:5n3 (EPA)	< 0.1	< 0.1
C22:0	0.05	0.06
C22:1n11	< 0.1	< 0.1
C22:1n9	0.08	0.06
C22:2n6	< 0.1	< 0.1
C23:0	< 0.1	0.28
C22:5n3 (DPA)	< 0.1	< 0.1
C24:0	< 0.1	< 0.1
C22:6n3 (DHA)	< 0.1	0.07
C24:1n9	< 0.1	0.25
SFA	8.88	7.04
MUFA	67.16	82.15
PUFA	23.96	10.81

SFA — saturated fatty acids; MUFA — monounsaturated fatty acids; PUFA — polyunsaturated fatty acids; ALA — alpha linolenic acid; EPA — eicosapentaenoic acid; DPA — docosapentaenoic acid; DHA — docosahexaenoic acid; limit of detection (LOD) - 0.1.

(5.6 mg/kg). *Foeniculum vulgare*, fructus contained the highest amounts of Hydroxytyrosol (1069.0 mg/kg), Naringenin (420.3 mg/kg), Epicatechin G. (555.4 mg/kg) and Rutin (70.9 mg/kg) followed with lower amounts of Syringic Acid (27.4 mg/kg), *p*-coumaric acid (26.2 mg/kg), Protocatehuic acid (16.9 mg/kg) and caffeic acid (9.4 mg/kg). Abundant quantities of polyphenol compound Hydroxytyrosol, flavonoids like Naringenin and Rutin, flavanol Epicatechin and phenolic acids (syringic acid, caffeic acid, *p*-coumaric acid, protocatechuic (dihydroxybenzoic) acid might be responsible for potential beneficial activity of these extracts. Antimicrobial activity against *S. aureus* was confirmed for Naringenin, Catechin and Quercetin by alteration of bacterial and host cell membranes [49]. Hydroxytyrosol is a simple phenolic compound mainly found in fruit and leaf of the olive (*Olea europeae* L) in amounts ranging from 2 mg/kg to several hundreds of mg/kg as a free form or as complex compounds (acetates, oleuropein and oleacein). It gained high attention due to the confirmed ability to neutralize harmful free radicals and protect cells from damage. There are several applications in a form of food supplements, functional foods as well as in studies that evaluated anti-inflammatory and cardiovascular benefits. It has been estimated that daily consumption of 5 mg of hydroxytyrosol and its derivatives is necessary for reduction of cardiovascular disease risks in humans, while Food and Drug Administration (FDA) recommended a daily intake of 23 g [50,51]. Therefore, presence of detected polyphenolic compounds might be responsible for confirmed antimicrobial activity of tested plant extracts in our study.

The developed LC-QTOF-MS/MS conditions allowed for improved resolution and sensitivity in the profiling of plant-derived compounds. These improvements represent significant contribution from the perspective of separation science, supporting the broader aim of determining bioactive compounds.

Another important aspect is microbiological safety of plants and their products. This is confirmed by detection of potentially pathogenic fungi from the genera *Aspergillus, Fusarium, Penicillium, Alternaria* and Yeasts in both plant samples in our study. Additionally, all samples contained all tested mycotoxin in diverse range of concentrations.

According to current legislation, among all tested mycotoxins only Ochratoxins are currently included for Herbs with defined maximum tolerable levels (MTL). These levels are defined in Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. Although the quantitative daily intake of potential mycotoxins via plants is considered to be rather low in humans, it shall not be underestimated since mycotoxins can be easily extracted with different solvents and their presence in plants and plant products is inevitable. Therefore, the dose of herbal intake depends upon various factors and each (medicinal) plant should be individually assessed [52]. Considering their potential use in animals as feed additives, acquiring fungi and toxin free plants is of high importance. The research of our study confirmed importance of toxicological testing of plants and careful evaluation of their use in humans and animals.

Companion animal ownership has significantly increased over the past decades. It has been estimated that around 166 million European households have at least one animal. In the year 2023, the cats were the most popular pet and from approximately 129,11 million cats, the number of household cats was over 79 million [53]. Although the results of the studies on positive health effects of cat ownership are not easily comparable and often, they are not fortified with high quality data, it is considered that cats as companion animals can enhance the physical and mental health of their owners and feline therapy is encouraged in certain human diseases [54–56]. However, the coin of cats as pets has the other side too. Namely, one of the potential problems are zoonotic infections [57,58]. To avoid this problem, the plants are considered as one of the best sources of effective antimicrobials. It has already been shown that plants and their extracts exhibit antimicrobial activity against pathogens like bacteria, moulds and viruses. This is especially important in a search of natural alternatives to conventional antibiotics due to alarming rise of antimicrobial resistance and addressing important economic issues

In plants, this activity is related to their (secondary) metabolites that act as a natural defense against pathogens, insects and herbivores. These phytochemicals display antimicrobial activity by forming complexes and intercalating into the cell walls, altering the permeability of microbial cell membranes, inhibiting DNA, RNA and protein synthesis, inactivating bacterial enzymes, interactions with the proteins, inducing substrates deficiency, and hinder the formation of bacterial and fungal biofilms. Known plant derived compounds with antimicrobial properties are alkaloids, lecitins and polypeptides, phenolics (phenolic acids, flavones, flavonoids, flavonols, quinones, tannins, coumarins and some simple phenols), polyacetylenes terpenoids and essential oils [59, 60]. Furthermore, a vast literature data describe the antimicrobial activity of phenolic compounds that have a C_3 side chain at a lower level of oxidation and does not contain oxygen, the class of essential oils, that in line with consumer safety concerns need to be used at

Table 6
Results of polyphenol profile of *Pimpinella anisum* (Anise) and *Foeniculum vulgare* Mill (Fennel) seeds.

Quantification results (mg/kg)	*Pimpinella anisum, seeds*	*Foeniculum vulgare*, fructus
Syringic Acid	19.1	27.4
Hesperidin	5.6	<LOQ
Epicatechin	–	–
Rutin	76.3	70.9
Quercetin	13.6	<LOQ
Protocatechuic	123.2	16.9
Caffeic	32.8	9.4
Ferulic	110.1	–
P.Coumaric	83.0	26.2
Apigenin	360.4	–
Hydroxy Tyrosol	268.7	1069
Catechin	<LOQ	<LOQ
Myricetin	–	–
Luteolin	360.4	–
Ellagic Acid	–	<LOQ
Naringenin	667.3	420.3
Coutaric Acid	328.9	–
Caftaric	317.7	–
Taxifolin	–	–
Epicatechin G.	154	555.4
Isorhamnetin	<LOQ	<LOQ

Table 7
Determination of mycotoxins in *Pimpinella anisum* (Anise) and *Foeniculum vulgare* (Fennel) seeds.

Mycotoxin (μg/kg)	*Pimpinella anisum*	*Foeniculum vulgare*	*EU-MTL* (μg/kg)
Total Aflatoxin	2,00 ± 0.06	3,98 ± 0.06	n/a
Deoxynivalenol	294,13 ± 0.11	349,85 ± 0.12	n/a
Diacetoxyscirpenol	20,00 ± 0.01	20,00 ± 0.01	n/a
Total Fumonisin	2,65 ± 0.27	71,46 ± 0.21	n/a
Ochratoxin	3,50 ± 0.15	3,27 ± 0.15	10
T2/HT-2 toxin	87,71 ± 0.33	77,02 ± 0.24	n/a
Zearalenone	76,62 ± 0.16	19,99 ± 0.09	n/a

EU-MTL — Maximum tolerable levels of Mycotoxins according to Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006 (Text with EEA relevance). SD — Standard deviation.

Table 8
Microbiological evaluation of samples of seeds of *Pimpinella anisum* (Anise) and *Foeniculum vulgare* Mill (Fennel).

Microorganisms		*Pimpinella anisum*	*Foenicullum vulgare*
	1	$20{,}1 \times 10^6$	$20{,}1 \times 10^6$
Mesophilic aerobic bacteria (cfu/g)	2	$20{,}1 \times 10^6$	$20{,}1 \times 10^6$
	3	ND	ND
Other bacteria		ND	ND
	4	$20{,}1 \times 10^3$	$20{,}1 \times 10^3$
Moulds (cfu/g)	5	$20{,}1 \times 10^3$	$20{,}1 \times 10^3$
	6	ND	ND
Yeasts (cfu/g)		$20{,}1 \times 10^3$	$20{,}1 \times 10^3$

According to VDLUFA method: 1 — Yellow pigmented bacteria (*Erwinia* spp.), *Pseudomonas/Enterobacteriaceae,* saprophytic coryneform bacteria; 2 — Indicator microorganisms: *Bacillus* spp, *Staphylococcus* spp., *Micrococcus* spp.; 3 — Indicator microorganisms: *Streptomycetes;* 4 — Saprophytic (field) moulds: *Acremonium* spp., *Aerobasidium* spp., *Dematinaceae, Fusarium* spp., *Verticillium* spp.; 5 — Indicator microorganisms (storage) moulds: *Aspergillus* spp., *Scopulariopsis* spp., *Penicillium* spp., *Wallemias pp*.; 6 — Indicator microorganisms: *Mucorales* like *Rhizopus* spp., *Mucor* spp. [30]. Other bacteria — determination of the presence of members of the genera: *Clostridium, Listeria, Salmonella, Staphylococcus* and bacteria *E. coli* O157.

very low concentrations to avoid adverse effects and organoleptic impacts [61]. For example, antimicrobial activity of *Foenicullum vulgare* has been described for essential oils against bacterial strains from the genera *Acinetobacter, Agrobacterium, Bacillus, Clavibacter, Erwinia, Escherichia, Klebsiella, Listeria, Micrococcus, Pseudomonas, Salmonella, Shigella, Staphylococcus, Streptococcus, Xanthomonas* and various fungal genera [6,62–67]. In a case of *Staphylococcus* strains, essential oils can suppress the production of exotoxins, enterotoxins (SEs) and toxic shock syndrome toxin 1 (TSST-1) by the action of **anethole.** These findings indicate that anethole is a candidate for alternative treatment of infections caused by antimicrobial resistant strains of these genera [63,68,69]. Therefore, this compounds should also be analysed in future research.

Likewise, phytochemical profiles and antimicrobial activity of fennel and anise have been investigated by several authors [70–73]. For example, AlBalawi et al. [70] confirmed antimicrobial activity of methanolic extracts of aniseed against Gram positive and Gram negative bacterial isolates from humans. However, their polyphenol profiling of aniseed extracts showed different profile. The most abundant were Estragole (4422.39 mg/kg), cis-anethole (3150.11 mg/kg) and trans-anethole (2312.11 mg/kg), while naringenin (211,23 mg/kg), luteolin (23,65 mg/kg) and apigenin in a form of Apegenin-7-O-glucosed (55.62 mg/kg) were detected in significantly lower amounts than in our study. Unfortunately, comparison of different studies is still difficult because of insufficient information about plants (e.g. geographical origin, climate conditions during culturing and harvesting, storage, etc.), different types of plant materials (fresh, lyophilized, etc.), and procedures

Table 9
Antimicrobial activity of plant extracts prepared from *Pimpinella anisum* (Anise) and *Foeniculum vulgare* (Fennel) seeds against selected bacterial pathogens.

Test organism	*Pimpinella anisum extracts*				*Foenicllum vulgare extracts*			
	H_2O	EtOH/ H_2O	MeOH	DMSO	H_2O	EtOH/ H_2O	MeOH	DMSO
S. aureus ATCC 6538	S	S	S	S	S	S	S	S
S. aureus ATCC 29213	S	S	S	S	S	S	S	S
S. aureus ATCC 256923	S	S	S	S	S	S	S	S
E. coli ATCC 25922	R	R	R	R	R	R	R	R
Multidrug Resistant Isolated Wild Strains from Cats								
S. aureus	S	S	S	S	S	S	S	S
S. aureus	S	S	S	S	S	S	S	S
S. aureus	S	S	S	S	S	S	S	S
S. epidermidis	S	S	S	S	S	S	S	S
S. epidermidis	S	S	S	S	S	S	S	S
S. epidermidis	S	S	S	S	S	S	S	S
S. hominis	S	S	S	S	S	S	S	S
S. hominis	S	S	S	S	S	S	S	S
S. hominis	S	S	S	S	S	S	S	S
S. haemolyticus	R	S	S	R	R	S	S	S
S. haemolyticus	R	S	S	R	R	S	S	S
S. saprophyticius	R	S	S	S	S	S	S	S
E. hirae	S	S	S	R	R	S	S	R
E. gallinarum	S	S	S	R	R	S	S	R

S — denotes observed zone greater than 7 mm; R — denotes no zone, or zone less than 7 mm.
EtOH/H_2O — Ethanol/water extracts, MeOH — methanol extracts, DMSO — imethyl sulfoxide extracts.
All bacterial strains were cultured on Brain Heart Infusion liquid medium at 37 °C ± 1 °C for six hours. The strains were cultured on the Mueller-Hinton agar plantes at concentration of 10^6cells/mL on which filter paper discs of 6 mm were placed. Different extracts were prepared for both plants in Methanol, Ethanol/H2O, DMSO and deionized water) and 50 microlitre of each extract was placed on the filter paper disc. After 24 ± 1 h of incubation at 37 °C ± 1 °C, the zones were measured. The controls were the solvents without plants and commercial antibiotic discs: Cefoxitin, chloramphenicol, gentamicin and vancomycin.

that are used for preparation of extracts and their testing (e.g. sample preparation, solvents, extraction conditions, detection instruments, etc.). In the recent research of Boubker et al. [73], antimicrobial activity of plant extracts against gram positive *Staphylococcus* was less pronounced for anise extract, than for fennel extracts. Nevertheless, these differences highlight the importance of further more-detailed research on polyphenol profile and antimicrobial activity of plant extracts.

In our study, we have confirmed antimicrobial potential of fennel and anise seeds prepared with each of the selected solvents. Additional value of these results is in evaluation of their activity on wild isolated strains with confirmed multi-drug resistance profile. Considering the available data in the literature, such activity of plant extracts prepared with water, alcohols, and other solvents is still not comprehensively confirmed [74]. Therefore, our results might enhance knowledge in this field of research. Since a number of factors affect extraction yield and quality, the choice of extracts for further antimicrobial testing was made according to the polarity closest to the polarity of the biomolecules (i.e. expected polyphenols and other biomolecules), stability of extracted components, fluidity, toxicity, and ease of use. Among commonly used solvents like acetonitrile, dimethyl-formamide, dimethyl sulfoxide (DMSO), ethanol, methanol, we have selected water, methanol and binary mixtures (50/50, v/v) of water and ethanol expecting the highest yield and antimicrobial activity in methanol and the lowest in water [75]. According to the presented results, the highest antimicrobial effects of both tested plants was achieved with methanol and water/ethanol (50/50, v/v) while the results were less pronounced when other solvents were used. In line with these are the results of the total phenolic content determined with Folin-Ciocalteu assay. Chemical composition and phenolic compounds are characteristics of each plant species. However, total phenolic content varies with growing conditions (plant variety, geographic region, soil, etc.) and other factors (maturation, harvest time, processing, etc.) [76–78]. In our study, the F-C Assay was applied to evaluate extraction efficacy of the tested solvents prior the use of extract for antimicrobial testing. Thus, the noticed difference might be explained with the lower extraction efficiency of the phenolic compounds that possess antimicrobial properties when different solvents are used.

5. Conclusion

Nutritional, microbiological and toxicological quality of plants like *Pimpinella anisum* and *Foeniculum vulgare* constitute important factors in assuring their beneficial characteristics and usage. Understanding the profile of compounds like polyphenols provides insights into their potential phytochemical, antioxidant and antimicrobial effects. Considering the evolutionary trend of increasing antimicrobial resistance of pathogenic bacteria in multiple environments, exploring novel approaches and search of alternative treatments in plant world has gained increasing attention.

In our study, the proximate chemical compositions of both seeds were in line previous researches. In fennel and anise seeds, the most abundant group of fatty acids were monosaturated fatty acids followed by polysaturated fatty acids. Microbiological analysis of both plants showed presence of bacteria, fungi, yeasts and tested mycotoxins. This work covers the chromatographic separation of a wide range of phenolic analytes. The chromatographic separation was optimized to achieve high resolution and sensitivity for the target analytes. Specifically, the mobile phase was adjusted to enhance the separation of structurally phenolic acids and flavonoids. The suggested conditions enabled the detection of a great number of polyphenolic constituents. According to the results of this study, evaluation of phytochemical profile, toxicological and microbiological quality and confirmed antimicrobial activity of *Pimpinella anisum* and *Foeniculum* extracts against bacterial pathogens isolated from cats it is clear that both plants and their products (extracts) could be considered for treatment of infections caused by tested bacteria. Finally, the Nature contains an enormous therapeutic potential but it is still not well understood. Our research data highlight the importance of further comprehensive interdisciplinary approach in this research area.

CRediT authorship contribution statement

Marijana Sokolovic: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Natasa P.**

Kalogiouri: Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Victoria F. Samanidou:** Writing – review & editing, Visualization, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Camelia Tulcan:** Writing – review & editing, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Camen Dorin:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Roberta Tripon:** Writing – review & editing, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Snježana Kazazić:** Writing – review & editing, Visualization, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Fani Krstulović:** Writing – review & editing, Investigation, Data curation. **Mirta Balenović:** Writing – review & editing, Investigation, Data curation. **Tajana Amšel Zelenika:** Writing – review & editing, Investigation, Data curation. **Ana Vulić:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Claudia Daniela Serban:** Writing – review & editing, Investigation, Formal analysis. **Marija Berendika:** Writing – review & editing, Investigation, Formal analysis, Data curation.

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. The author V.F. Samanidou is member of the Advisory Editorial Board for Journal of Chromatography Open and was not involved in the editorial review or the decision to publish this article.

Acknowledgements

This study was funded by the European Union NextGenerationEU and supported by the Ministry of Science and Education of the Republic of Croatia through the project No. NPOO 15 of Croatian Veterinary Institute titled “Comprehensive Research on the Impact of Plant Extracts against resistant Staphylococcus (CRISP)”

Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.jcoa.2025.100275.

Data availability

Data will be made available on request.

References

[1] F. Rancé, A. Juchet-Gibon, S. Fejji, F. Brémont, G. Dutau, Sensibilisation aux épices chez l’enfant, Rev. Fr. D’Allergologie et D’Immunologie Clin. (ISSN: 03357457) 34 (6) (1994) 475–479, http://dx.doi.org/10.1016/S0335-7457(05)80391-0.

[2] Katia Gialluisi, Nada El Darra, Maria Giovanna Nicoletti, Michele Solfrizzo, Lucia Gambacorta, Natural occurrence of main mycotoxins in herbs and spices commercialized in Italy, 2025, http://dx.doi.org/10.3390/foods14111889.

[3] Lars P. Christensen, Kirsten Brandt, Bioactive polyacetylenes in food plants of the apiaceae family: Occurrence, bioactivity and analysis, J. Pharm. Biomed. Anal. (ISSN: 07317085) 41 (3) (2006) 683–693, http://dx.doi.org/10.1016/j.jpba.2006.01.057.

[4] Bouchra Sayed-Ahmad, Thierry Talou, Zeinab Saad, Akram Hijazi, Othmane Merah, The apiaceae: Ethnomedicinal family as source for industrial uses, Ind. Crop. Prod. (ISSN: 09266690) 109 (2017) 661–671, http://dx.doi.org/10.1016/j.indcrop.2017.09.027.

[5] Tatyana Pavlova, Mikhail Stepanichev, Natalia Gulyaeva, Pentylenetetrazole kindling induces neuronal cyclin B1 expression in rat hippocampus, Neurosci. Lett. (ISSN: 03043940) 392 (1–2) (2006) 154–158, http://dx.doi.org/10.1016/j.neulet.2005.09.021.

[6] Muhammad Gulfraz, Sajid Mehmood, Nasir Minhas, Nyla Jabeen, Rehana Kausar, Kokab Jabeen, Gulshan Arshad, Composition and antimicrobial properties of essential oil of foeniculum vulgare, Afr. J. Biotechnol. (ISSN: 16845315) 7 (24) (2008) 4364–4368.

[7] Yong Soo Kwon, Won Gyu Choi, Won Jun Kim, Woo Kyung Kim, Myong Jo Kim, Won Hee Kang, Chang Min Kim, Antimicrobial constituents of foeniculum vulgare, Arch. Pharmacal Res. (ISSN: 02536269) 25 (2) (2002) 154–157, http://dx.doi.org/10.1007/bf02976556.

[8] Chi Hoon Lee, Bo Kyung Sung, Hoi Seon Lee, Acaricidal activity of fennel seed oils and their main components against Tyrophagus putrescentiae, a stored-food mite, J. Stored Prod. Res. (ISSN: 0022474X) 42 (1) (2006) 8–14, http://dx.doi.org/10.1016/j.jspr.2004.10.004.

[9] Firas A. Al-Bayati, Synergistic antibacterial activity between Thymus vulgaris and pimpinella anisum essential oils and methanol extracts, J. Ethnopharmacol. (ISSN: 03788741) 116 (3) (2008) 403–406, http://dx.doi.org/10.1016/j.jep.2007.12.003.

[10] Ana Clara Aprotosoaie, Monica Hăncianu, Antonia Poiată, Cristina Tuchiluș, A. Spac, Oana Cioană, Elvira Gille, Ursula Stănescu, In vitro antimicrobial activity and chemical composition of the essential oil of foeniculum vulgare mill, Rev. Medico-Chirurgicală A Societăţii Medici Şi NaturaliŞti Din IaŞi (ISSN: 03008738) 112 (3) (2008) 832–836.

[11] D Yazdani, Sh Rezazadeh, Gh Amin, MA Zainal Abidin, S Shahnazi, H Jamalifar, Antifungal activity of dried extracts of anise (pimpinella anisum l.) and star anise (illicium verum hook. f.) against dermatophyte and saprophyte fungi, J. Med. Plants 8 (29) (2009) 24–29.

[12] Münir Oktay, Ilhami Gülçin, Ö Irfan Küfrevioğlu, Determination of in vitro antioxidant activity of fennel (foeniculum vulgare) seed extracts, LWT - Food Sci. Technol. (ISSN: 0023-6438) 36 (2) (2003) 263–271, http://dx.doi.org/10.1016/S0023-6438(02)00226-8.

[13] Ullagaddi Rajeshwari, Bondada Andallu, Medicinal benefits of coriander(Coriandrum Sativum L), Spatula DD - Peer Rev. J. Complement. Med. Drug Discov. (ISSN: 1309-9914) 1 (1) (2011) 51, http://dx.doi.org/10.5455/spatula.20110106123153.

[14] V. Neveu, J. Perez-Jiménez, F. Vos, V. Crespy, L. du Chaffaut, L. Mennen, C. Knox, R. Eisner, J. Cruz, D. Wishart, A. Scalbert, Phenol-explorer: an online comprehensive database on polyphenol contents in foods, Database : J. Biological Databases Curation (ISSN: 17580463) 2010 (2010) 1–9, http://dx.doi.org/10.1093/database/bap024.

[15] Munawar Abbas, Farhan Saeed, Faqir Muhammad Anjum, Muhammad Afzaal, Tabussam Tufail, Muhammad Shakeel Bashir, Adnan Ishtiaq, Shahzad Hussain, Hafiz Ansar Rasul Suleria, Natural polyphenols: An overview, Int. J. Food Prop. (ISSN: 15322386) 20 (8) (2017) 1689–1699, http://dx.doi.org/10.1080/10942912.2016.1220393.

[16] Olalla López-Fernández, Rubén Domínguez, Mirian Pateiro, Paulo E S Munekata, Gabriele Rocchetti, José M Lorenzo, Determination of polyphenols using liquid chromatography-tandem mass spectrometry technique (LC-ms/MS): A review, Antioxidants 9 (479) (2020) 1–27, http://dx.doi.org/10.3390/antiox9060479, URL www.mdpi.com/journal/antioxidants.

[17] Mohamed Hussein Hamdy Roby, Mohamed Atef Sarhan, Khaled Abdel-Hamed Selim, Khalel Ibrahim Khalel, Antioxidant and antimicrobial activities of essential oil and extracts of fennel (Foeniculum vulgare L.) and chamomile (Matricaria chamomilla L.), Ind. Crop. Prod. (ISSN: 09266690) 44 (2013) 437–445, http://dx.doi.org/10.1016/j.indcrop.2012.10.012.

[18] Roja Rahimi, Mohammad Reza Shams Ardekani, Medicinal properties of foeniculum vulgare mill. in traditional Iranian medicine and modern phytotherapy, Chin. J. Integr. Med. (ISSN: 1672-0415) 19 (1) (2013) 73–79, http://dx.doi.org/10.1007/s11655-013-1327-0.

[19] Valentina Lubomirova Christova-Bagdassarian, Kristine Samvel Bagdassarian, Maria Stefanova Atanassova, M Ayaz Ahmad, Comparative analysis total phenolic and total flavonoid contents , rutin , tannins and antioxidant capacity in apiaceae and lamiaceae families comparative analysis of total phenolic and total flavonoid contents , rutin , tannins and antioxidant capacity i, Indian Hortic. J. 4 (3/4) (2014) 131–140.

[20] Noelia Pallarés, Houda Berrada, Guillermina Font, Emilia Ferrer, Mycotoxins occurrence in medicinal herbs dietary supplements and exposure assessment, J. Food Sci. Technol. (ISSN: 09758402) 59 (7) (2022) 2830–2841, http://dx.doi.org/10.1007/s13197-021-05306-y.

[21] Valérie O. Baede, Els M. Broens, Mirlin P. Spaninks, Arjen J. Timmerman, Haitske Graveland, Jaap A. Wagenaar, Birgitta Duim, Joost Hordijk, Raw pet food as a risk factor for shedding of extended-spectrum beta-lactamase-producing Enterobacteriaceae in household cats, PLoS One (ISSN: 19326203) 12 (11) (2017) http://dx.doi.org/10.1371/journal.pone.0187239.

[22] Francesca Paola Nocera, Francesca Pizzano, Angelo Masullo, Laura Cortese, Luisa De Martino, Antimicrobial resistant staphylococcus species colonization in dogs, their owners, and veterinary staff of the veterinary teaching hospital of Naples, Italy, Pathogens (ISSN: 2076-0817) 12 (8) (2023) 1016, http://dx.doi.org/10.3390/pathogens12081016.

[23] Ricardo Antonio Pilegi Sfaciotte, Leandro Parussolo, Fernanda Danielle Melo, Luis Henrique Pompelli, Naiara Dognani Israel, David Germano Gonçalves Schwarz, Ubirajara Maciel da Costa, Sandra Maria Ferraz, Prevalence, identification and antimicrobial sensitivity profile of methicillin resistant staphylococcus colonizing dogs, cats and humans in a veterinary hospital, Brazil, Res. Vet. Sci. (ISSN: 00345288) 193 (2025) 105793, http://dx.doi.org/10.1016/j.rvsc.2025.105793.

[24] Ragini Pandey, Puneet Gupta, Reviewing the therapeutic potential of plant-based natural products against multidrug-resistant bacteria (MDR), Curr. Drug Res. Rev. (ISSN: 25899775) 17 (2) (2025) 191–205, http://dx.doi.org/10.2174/0125899775267934240308065823.

[25] SeyedAhmad SeyedAlinaghi, Esmaeil Mehraeen, Pegah Mirzapour, Soudabeh Yarmohammadi, Soheil Dehghani, Sarina Zare, Shayan Gholami, Niloofar Attarian, Amene Abiri, Farid Farahani Rad, Amir Tabari, Fatemeh Afroughi, Amirhossein Gholipour, Mohammad Mahdi Roozbahani, Shayesteh Jahanfar, A systematic review on natural products with antimicrobial potential against WHO's priority pathogens, Eur. J. Med. Res. (ISSN: 2047-783X) 30 (1) (2025) 525, http://dx.doi.org/10.1186/s40001-025-02717-x.

[26] Norton Nelson, A photometric adaptation of the somogyi method for the determination of glucose, J. Biol. Chem. (ISSN: 00219258) 153 (2) (1944) 375–380, http://dx.doi.org/10.1016/S0021-9258(18)71980-7.

[27] M. Somogyi, Notes on sugar determination., J. Biol. Chem. (ISSN: 00219258) 195 (1) (1952) 19–23, http://dx.doi.org/10.1016/s0021-9258(19)50870-5.

[28] Marijana Sokolović, Borka Šimpraga, Survey of trichothecene mycotoxins in grains and animal feed in Croatia by thin layer chromatography, Food Control (ISSN: 09567135) 17 (9) (2006) 733–740, http://dx.doi.org/10.1016/j.foodcont.2005.05.001.

[29] Marijana Sokolovic, Marija Berendika, Tajana Amšel Zelenika, Borka Šimpraga, Fani Krstulović, Determination of fumonisins in grains and poultry feedstuffs in Croatia: A 16-year study, Toxins (ISSN: 20726651) 14 (7) (2022) http://dx.doi.org/10.3390/toxins14070444.

[30] C. Naumann, R. Bassler, Die chemische Untersuchung von Futtermitteln, Methodenbuch, two thousand twelfth ed., VDLUFA - Verlag, Darmstadt, ISBN: 978-3-941273-04-7, 2012, p. 640.

[31] Vernon L. Singleton, Rudolf Orthofer, Rosa M. Lamuela-Raventós, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, Methods Enzymol. (ISSN: 00766879) 299 (1999) 152–178, http://dx.doi.org/10.1016/S0076-6879(99)99017-1.

[32] Gislene G.F. Nascimento, Juliana Locatelli, Paulo C. Freitas, Giuliana L. Silva, Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria, Braz. J. Microbiol. (ISSN: 1517-8382) 31 (4) (2000) http://dx.doi.org/10.1590/S1517-83822000000400003.

[33] M. Stegger, P.S. Andersen, A. Kearns, B. Pichon, M.A. Holmes, G. Edwards, F. Laurent, C. Teale, R. Skov, A.R. Larsen, Rapid detection, differentiation and typing of methicillin-resistant staphylococcus aureus harbouring either mecA or the new mecA homologue mecALGA251, Clin. Microbiol. Infect. (ISSN: 14690691) 18 (4) (2012) 395–400, http://dx.doi.org/10.1111/j.1469-0691.2011.03715.x.

[34] European Food, Safety Authority, Marc Aerts, Antonio Battisti, Rene Hendriksen, Jesper Larsen, Oskar Nilsson, José Jos', José Cortiñascorti, Cortiñas Abrahantes, Beatriz Guerra, Alexandra Papanikolaou, Pierre-Alexandre Beloeil, Technical specifications for a baseline survey on the prevalence of methicillin-resistant staphylococcus aureus (MRSA) in pigs, EFSA J. 20 (10) (2022) 7620, http://dx.doi.org/10.2903/j.efsa.2022.7620.

[35] CLSI, ClsI. Performance standards for antimicrobial susceptibility testing. 35th ed. CLSI supplement M100., 2025.

[36] EURL-AR, Laboratory protocol: MRSA multiplex PCR-2 (mecA, mecC, PVL and SPA) - version 1.1, (August 2012) 2024, pp. 1–7.

[37] V. Tavallali, Antioxidant activity, polyphenolic contents and essential oil composition of composition of Pimpinella anisum L. as affected by zinc fertilizer, J. Sci. Food Agric. 97 (14) (2017) 4883–4889, http://dx.doi.org/10.1002/jsfa.8360.

[38] Edgar B. Cahoon, Yonghua Li-Beisson, Plant unusual fatty acids: learning from the less common, Curr. Opin. Plant Biol. (ISSN: 13695266) 55 (2020) 66–73, http://dx.doi.org/10.1016/j.pbi.2020.03.007.

[39] Abbas Besharati-Seidani, Ali Jabbari, Yadollah Yamini, Headspace solvent microextraction: a very rapid method for identification of volatile components of Iranian pimpinella anisum seed, Anal. Chim. Acta (ISSN: 00032670) 530 (1) (2005) 155–161, http://dx.doi.org/10.1016/j.aca.2004.09.006.

[40] Michal Gross, Efraim Lewinsohn, Nativ Dudai, Yael Cohen, Jacob Friedman, Flowering dynamics and crossability of different populations of bitter fennel (foeniculum vulgare mill. var. vulgare, apiaceae), Isr. J. Plant Sci. (ISSN: 07929978) 56 (3) (2008) 215–226, http://dx.doi.org/10.1560/IJPS.56.3.215.

[41] Lillian Barros, Ana Maria Carvalho, Isabel C.F.R. Ferreira, The nutritional composition of fennel (Foeniculum vulgare): Shoots, leaves, stems and inflorescences, Lwt (ISSN: 00236438) 43 (5) (2010) 814–818, http://dx.doi.org/10.1016/j.lwt.2010.01.010.

[42] Bo Kyeong Yoon, Joshua A Jackman, Min Chul Kim, Nam-Joon Cho, Spectrum of membrane morphological responses to antibacterial fatty acids and related surfactants, Langmuir : ACS J. Surfaces Colloids (ISSN: 1520-5827) 31 (37) (2015) 10223–10232, http://dx.doi.org/10.1021/acs.langmuir.5b02088.

[43] Abbas Abel Anzaku, Josiah Ishaku Akyala, Adeola Juliet, Ewenighi Chinwe Obianuju, Antibacterial activity of lauric acid on some selected clinical isolates, Ann. Clin. Lab. Res. 05 (02) (2017) 1–5, http://dx.doi.org/10.21767/2386-5180.1000170.

[44] Satoshi Nagase, Miki Matsue, Yumiko Mori, Mariko Honda-Ogawa, Kayo Sugitani, Sumitomo Tomoko, Masanobu Nakata, Shigetada Kawabata, Shigefumi Okamoto, Comparison of the antimicrobial spectrum and mechanisms of organic virgin coconut oil and lauric acid against bacteria, J. Wellness Heal. Care 41 (1) (2017) 87–95.

[45] Giancarlo Casillas-Vargas, Carlimar Ocasio-Malavé, Solymar Medina, Christian Morales-Guzmán, René García Del Valle, Néstor M. Carballeira, David J. Sanabria-Ríos, Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents, Prog. Lipid Res. (ISSN: 18732194) 82 (2021) http://dx.doi.org/10.1016/j.plipres.2021.101093.

[46] Phuc Nguyen, Thien Le, Andrew P. Desbois, Antibacterial effect of eicosapentaenoic acid against Bacillus cereus and staphylococcus aureus: Killing kinetics, selection for resistance, and potential cellular target, Mar. Drugs 15 (2017) 334, http://dx.doi.org/10.3390/md15110334.

[47] Ummara Butt, Amr Elshaer, Lori A S Snyder, Athina Chaidemenou, Raid G Alany, Fatty acid microemulsion for the treatment of neonatal conjunctivitis: quantification, characterisation and evaluation of antimicrobial activity, Drug Deliv. Transl. Res. 6 (2016) 722–734, http://dx.doi.org/10.1007/s13346-016-0338-3.

[48] Pradeep Pushparaj Selvadoss, Jayshree Nellore, Malathy Balaraman Ravindrran, Uma Sekar, Jayakrishna Tippabathani, Enhancement of antimicrobial activity by liposomal oleic acid-loaded antibiotics for the treatment of multidrug-resistant Pseudomonas aeruginosa, Artif. Cells Nanomedicine Biotechnol. (ISSN: 2169-1401) 46 (2) (2018) 268–273, http://dx.doi.org/10.1080/21691401.2017.1307209.

[49] Artem G. Veiko, Ewa Olchowik-Grabarek, Szymon Sekowski, Anna Roszkowska, Elena A. Lapshina, Izabela Dobrzynska, Maria Zamaraeva, Ilya B. Zavodnik, Antimicrobial activity of quercetin, naringenin and catechin: Flavonoids inhibit staphylococcus aureus-induced hemolysis and modify membranes of bacteria and erythrocytes, Molecules (ISSN: 14203049) 28 (3) (2023) http://dx.doi.org/10.3390/molecules28031252.

[50] Cristina Vilaplana-Pérez, David Auñón, Libia A García-Flores, Angel Gil-Izquierdo, Hydroxytyrosol and potential uses in cardiovascular diseases, cancer, and aids, Front. Nutr. (ISSN: 2296-861X) 1 (2014) 18, http://dx.doi.org/10.3389/fnut.2014.00018.

[51] Zhong Wang, Ziteng Lei, Haijing Zhang, Zheng Liu, Wei Chen, Yan Jia, Ruoyu Shi, Chengtao Wang, From biosynthesis to legislation: A review of hydroxytyrosol's biological functions and safety, Int. J. Mol. Sci. 26 (4470) (2025) 1–20, http://dx.doi.org/10.3390/ijms26104470,

[52] Samina Ashiq, Mubbashir Hussain, Bashir Ahmad, Natural occurrence of mycotoxins in medicinal plants: A review, Fungal Genet. Biol. (ISSN: 10960937) 66 (2014) 1–10, http://dx.doi.org/10.1016/j.fgb.2014.02.005.

[53] Statista, Pet ownership and pet care market in europe, 2020, URL https://www.statista.com/study/51871/pet-ownership-and-pet-care-market-in-europe/.

[54] Human Services, Intro power of pets, (February) 2018, NIH.

[55] Susan Little, The health benefits of cat ownership, (6) 2020, pp. 6–8, VMX2020.

[56] Michelle Cleary, Sancia West, Deependra K. Thapa, Rachel Kornhaber, Putting cats on the spectrum: A scoping review of the role of cats in therapy and companionship for autistic adults and children, Issues Ment. Heal. Nurs. (ISSN: 10964673) 44 (6) (2023) 505–516, http://dx.doi.org/10.1080/01612840.2023.2195509.

[57] Breck Peterson, Amber N. Barnes, Feline-human zoonosis transmission in North Africa: A systematic review, Vector-Borne Zoonotic Dis. (ISSN: 15577759) 20 (10) (2020) 731–744, http://dx.doi.org/10.1089/vbz.2019.2591.

[58] Sarah M.L. Tan, Anastasia C. Stellato, Lee Niel, Uncontrolled outdoor access for cats: An assessment of risks and benefits, Animals (ISSN: 20762615) 10 (2) (2020) 1–13, http://dx.doi.org/10.3390/ani10020258.

[59] Marjorie Murphy Cowan, Plant products as antimicrobial agents, Clin. Microbiol. Rev. (ISSN: 08938512) 12 (4) (1999) 564–582, http://dx.doi.org/10.1128/cmr.12.4.564.

[60] F.J. Álvarez-Martínez, E. Barrajón-Catalán, M. Herranz-López, V. Micol, Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action, Phytomedicine (ISSN: 1618095X) 90 (2021) http://dx.doi.org/10.1016/j.phymed.2021.153626.

[61] Joshua B. Gurtler, Christina M. Garner, A review of essential oils as antimicrobials in foods with special emphasis on fresh produce, J. Food Prot. (ISSN: 19449097) 85 (9) (2022) 1300–1319, http://dx.doi.org/10.4315/JFP-22-017.

[62] N.H. Jazani, M. Zartoshti, H. Babazadeh, N. Ali-Daiee, S. Zarrin, S. Hosseini, Antibacterial effects of iranian fennel essential oil on isolates of acinetobacter baumannii, Pak. J. Biol. Sci. (ISSN: 10288880) 12 (9) (2009) 738–741, http://dx.doi.org/10.3923/pjbs.2009.738.741.

[63] Jiazhang Qiu, Hongen Li, Hongwei Su, Jing Dong, Mingjing Luo, Jianfeng Wang, Bingfeng Leng, Yanhong Deng, Juxiong Liu, Xuming Deng, Chemical composition of fennel essential oil and its impact on staphylococcus aureus exotoxin production, World J. Microbiol Biotechnol 28 (2012) 1399–1405, http://dx.doi.org/10.1007/s11274-011-0939-4.

[64] Wen Rui Diao, Qing Ping Hu, Hong Zhang, Jian Guo Xu, Chemical composition, antibacterial activity and mechanism of action of essential oil from seeds of fennel (Foeniculum vulgare Mill.), Food Control (ISSN: 09567135) 35 (1) (2014) 109–116, http://dx.doi.org/10.1016/j.foodcont.2013.06.056.

[65] G Singh, I.P.S. Kapoor, S.K Pandey, U.K Singh, R.K. Singh, Studies on essential oils: part 10; antibacterial activity of volatile oils of some spices, Phytother. Res. : PTR (ISSN: 0951-418X) 16 (7) (2002) 680–682, http://dx.doi.org/10.1002/ptr.951.

[66] M. Kazemi, E. Mousavi, H. Kharestani, Chemical compositions and antimicrobial activities of essential oils of varthemia persica, foeniculum vulgare and ferula lycia, Curr. Res. Bacteriol. (ISSN: 19945426) 5 (2) (2012) 42–52, http://dx.doi.org/10.3923/crb.2012.42.52.

[67] Ankita Wal, Divyanshi Gupta, Amin Gasmi, Abida Khan, Astik Manju Ashesh, Ajay Kumar, Dileep Kumar, Unveiling the chemical composition, bioactive properties, and potential applications of fennel essential oil, S. Afr. J. Bot. (ISSN: 0254-6299) 185 (2025) 617–630, http://dx.doi.org/10.1016/J.SAJB.2025.08.020.

[68] Bülent Çeti, Hakan Özer, Ahmet Cakir, Taşkin Polat, Atilla Dursun, Ebru Mete, Erdoğan Öztürk, Melek Ekinci, Antimicrobial activities of essential oil and hexane extract of florence fennel [foeniculum vulgare var. azoricum (Mill.) Thell.] against foodborne microorganisms, J. Med. Food (ISSN: 1096620X) 13 (1) (2010) 196–204, http://dx.doi.org/10.1089/jmf.2008.0327.

[69] M Martins, M. Rosário Tinoco, A.S. Teresa Almeida, J. Cruz-Morais, Chemical composition, antioxidant and antimicrobial properties of three essential oils from portuguese flora, J. Pharmacogn. 3 (1) (2012) 39–44.

[70] Aisha Nawaf AlBalawi, Alaa Elmetwalli, Dina M. Baraka, Hadeer A. Alnagar, Eman Saad Alamri, Mervat G. Hassan, Chemical constituents, antioxidant potential, and antimicrobial efficacy of pimpinella anisum extracts against multidrug-resistant bacteria, Microorganisms (ISSN: 20762607) 11 (4) (2023) http://dx.doi.org/10.3390/microorganisms11041024.

[71] I. Bettaieb Rebey, S. Bourgou, W. Aidi Wannes, I. Hamrouni Selami, M. Saidani Tounsi, B. Marzouk, M.L. Fauconnier, R. Ksouri, Comparative assessment of phytochemical profiles and antioxidant properties of Tunisian and Egyptian anise (Pimpinella anisum L.) seeds, Plant Biosyst. (ISSN: 17245575) 152 (5) (2018) 971–978, http://dx.doi.org/10.1080/11263504.2017.1403394.

[72] Jiajia Wu, Zhen Cao, Syed Shams ul Hassan, Haozhen Zhang, Muhammad Ishaq, Xu Yu, Shikai Yan, Xue Xiao, Hui Zi Jin, Emerging biopharmaceuticals from pimpinella genus, Molecules (ISSN: 14203049) 28 (4) (2023) http://dx.doi.org/10.3390/molecules28041571.

[73] Aicha Boubker, Abdelmoula El Ouardi, Taha El Kamli, Adnane El Hamidi, Mohammed Kaicer, Faouzi Kichou, Najia Ameur, Khaoula Errafii, Rachid Ben Aakame, Aicha Sifou, Phytochemical analysis, antioxidant and antibacterial activities, minerals element profiling, and identification of bioactive compounds by UPLC-HRMS orbitrap in four aromatic and medicinal plants, Molecules (ISSN: 14203049) 30 (6) (2025) http://dx.doi.org/10.3390/molecules30061279.

[74] Rahul Yadav, Madhulika Pradhan, Krishna Yadav, Anand Mahalvar, Homesh Yadav, Present scenarios and future prospects of herbal nanomedicine for antifungal therapy, J. Drug Deliv. Sci. Technol. (ISSN: 17732247) 74 (2022) http://dx.doi.org/10.1016/j.jddst.2022.103430.

[75] Meriem Soussi, Wissal El Yaagoubi, Houria Nekhla, Laila El Hanafi, Wafae Squalli, Meryem Benjelloun, Lahsen El Ghadraoui, A multidimensional review of pimpinella anisum and recommendation for future research to face adverse climatic conditions, Chem. Afr. (ISSN: 25225766) 6 (4) (2023) 1727–1746, http://dx.doi.org/10.1007/s42250-023-00633-3, URL https://doi.org/10.1007/s42250-023-00633-3,

[76] Vincenzo Fogliano, Raffaele Sacchi, Oleocanthal in olive oil: Between myth and reality, Mol. Nutr. Food Res. (ISSN: 16134125) 50 (1) (2006) 5–6, http://dx.doi.org/10.1002/mnfr.200690002.

[77] Kashif Ameer, Hafiz Muhammad Shahbaz, Joong Ho Kwon, Green extraction methods for polyphenols from plant matrices and their byproducts: A review, Compr. Rev. Food Sci. Food Saf. (ISSN: 15414337) 16 (2) (2017) 295–315, http://dx.doi.org/10.1111/1541-4337.12253.

[78] Lenka Burdejova, Blanka Tobolkova, Martin Polovka, Jarmila Neugebauerova, Differentiation of medicinal plants according to solvents, processing, origin, and season by means of multivariate analysis of spectroscopic and liquid chromatography data, Molecules (ISSN: 14203049) 28 (10) (2023) http://dx.doi.org/10.3390/molecules28104075.