LC-MS/MS Content Analysis of Ethanol Extracts from Some Naturally Occurring Plants and Their Biological Activity Against Certain Plant Pathogenic Microorganisms Under *In Vitro* Conditions

Erdal Öğün[a], İbrahim Koç[b*], Yunus Güral[c], Ercan Aydoğmuş[d], İnanç Özgen[e]

[a]Department of Molecular Biology and Genetics, Faculty of Science, Van Yüzüncü Yıl University, Van, Türkiye
[b]Vocational School of Health Services, Mardin Artuklu University, 47060, Mardin, Türkiye
[c]Division of Statistics, Faculty of Science, Fırat University, Elazığ, Türkiye
[d]Engineering Faculty, Chemical Engineering, Fırat University, Elazığ, Türkiye
[e]Department of Bioengineering, Engineering Faculty, Fırat University, Elazığ, Türkiye
e-mail: ibrahimkoc@artuklu.edu.tr

DOI: 10.57244/dfbd.1726117
Geliş tarihi/Received: 24/06/2025 Kabul tarihi/Accepted:18/11/2025

Abstract

There is a significant need for environmentally friendly organic pesticides. This study was conducted to analyze the content of ethanol extracts from some naturally occurring plant parts in Elazığ, Türkiye, using LC-MS/MS and to determine their activity against certain plant pathogenic bacteria and microfungi. The study was carried out under *in vitro* conditions using a Randomized Complete Block Design with four replications. Antibacterial activity was assessed using the Agar Well Diffusion Method in Mueller-Hinton medium, while antifungal activity was determined using the Poisoned Food Method in Malt Extract Agar medium. The statistical analysis of the obtained data sets was performed using the Kruskal-Wallis H test. As a result, among the 33 standard compounds investigated in the extracts, none were found in *Asphodelus aestivus* L. and *Melissa officinalis*; three compounds vanillin, gentisic acid, chlorogenic acid) were identified in *Tanacetum parthenium*; and four compounds vanillin, trans-cinnamic acid, p-coumaric acid, hydroxybenzaldehyde) were identified in *Rosa canina* L. *Rosa canina* L. extract showed no activity against the pathogenic test microfungi, while the activity of *Tanacetum parthenium* extract increased with higher concentrations. It was observed that the extracts of *Asphodelus aestivus* L. and *Melissa officinalis* completely inhibited the growth of microfungi at concentrations of 15% and above(v/v). It is believed that the ethanol extracts exhibiting activity have the potential for use as food preservatives and biopesticides.

Keywords: Antimicrobial activity, Bioactive compounds, Biopesticide, *Fusarium oxysporum, Verticillium dahliae*

Doğal Yayılış Gösteren Bazı Bitkilerin Etanol Ekstraktlarının LC-MS/MS İçerik Analizi, *İn Vitro* Şartlarında Bazı Bitki Patojeni Mikroorganizmalar Üzerindeki Biyolojik Aktivitesi

Özet

Çevre dostu organik pestisitlere ciddi derecede gereksinim duyulmaktadır. Bu çalışma, Elazığ'da (Türkiye) doğal yayılış gösteren bazı bitki kısımlarının etanol ekstraktlarının LC-MS/MS ile içerik analizi, bitki patojeni bazı bakteri ve mikrofunguslara karşı aktivitesini belirleme amacıyla yapılmıştır. Çalışma, *in vitro* şartlarında Tesadüf Parselleri Deneme Deseninde ve dört tekerrürlü olarak yürütülmüştür. Antibakteriyel aktivite, Agar Well Diffüzyon Yöntemiyle Mueller-Hinton ortamında; antifungal aktivite, Zehirli Plak Yöntemi kullanılarak Malt Ekstrat Agar ortamında belirlenmiştir. Elde edilen veri setlerinin istatistiksel analizinde Kruskal Wallis H testi kullanılmıştır. Sonuç olarak: Ekstraktlarda araştırılan 33 standart bileşikten *Asphodelus aestivus* L. ve *Melissa officinalis*'te rastlanılmadığı; *Tanacetum parthenium*'da üç (sırasıyla vanillin, gentisic acid, chlorogenic acid); *Rosa canina* L.'da dört (sırasıyla vanillin, trans-cinnamic acid, p-coumaric acid, ydroxybenzaldehyde) bileşik belirlenmiştir. Patojen test mikrofungusları üzerine *Rosa canina* L. ekstraktının aktivite göstermediği; *Tanacetum parthenium*

ekstraktının artan konsantrasyonlarla birlikte aktivitesinin arttığı; *Asphodelus aestivus* L. ve *Melissa officinalis* ekstraktlarının %15 ve üzeri oranlarda (v/v) mikrofungusların çoğalmasını tamamen engellediği görülmüştür. Aktivite gösteren etanol ekstraktlarının gıda koruyucu ve biyopestisit amaçlı kullanılma potansiyelinin olduğu düşünülmektedir.

Anahtar Kelimeler: Antimikrobiyal aktivite, Biyoaktif bileşikler, Biyopestisit, *Fusarium oxysporum, Verticillium dahliae*

Introduction

Plant pathogenic bacteria and fungi cause damage to crops, particularly in agriculture, and pose significant threats to agricultural sustainability and global food security, leading to considerable economic losses (Strange and Scott 2005; Nelson et al. 2018; Martins et al. 2018; Holtappels et al. 2021; Sharma et al. 2022; Anand and Rajeshkumar 2022; Košćak et al. 2023). Important bacterial plant pathogens include *Ralstonia solanacearum*, *Erwinia amylovora*, *Xylella fastidiosa, Agrobacterium tumefaciens, Xanthomonas oryzae* pv. oryzae, *Xanthomonas campestris* pathovars, *Pseudomonas syringae* pathovars, *Dickeya* (*dadantii* and *solani)*, *Pectobacterium carotovorum* (and *Pectobacterium atrosepticum*), *Xanthomonas axonopodis* pathovars, and (Mansfield et al. 2012). Pathogenic fungi of the Fusarium and Verticillium genera are among the pathogens that cause serious damage to plants worldwide (Klosterman et al. 2009; Ferrigo et al. 2016). With the increase in human population, it is essential to reduce crop losses caused by pathogens to meet the rising food demands (Buttimer et al. 2017). Synthetic pesticides are commonly preferred in combating these agents (Sharma et al. 2022; Khan et al. 2023; Košćak et al. 2023). Despite some benefits, numerous studies have identified undesirable effects of synthetic pesticides on non-target organisms and the environment (Arora and Sahni 2016; Buttimer et al. 2017; Kowalska 2021; Tudi et al. 2021; Sharma et al. 2022; Lyubenova et al. 2023; Khan et al. 2023; Košćak et al. 2023). Biopesticides should be developed to manage both pathogens and the undesirable effects of chemical pesticides (Tudi et al. 2021; Košćak, 2023). Natural-based biopesticides are considered alternatives to conventional pesticides. Active compounds from certain plant species, along with beneficial microorganisms, can serve as sources of biopesticides. Among the promising biopesticide sources are plants like *Tanacetum parthenium* (*T. parthenium*) (Lyubenova et al. 2023). The Tanacetum genus belongs to the Asteraceae or Compositae family and has been used as a medicinal agent in traditional medicine worldwide since ancient times. A literature review has identified the presence of numerous chemical compounds in different *Tanacetum species*. More than 200 compounds have been identified in the essential oils obtained from these species. Traditionally, they are used in the production of cosmetics, insecticides, pharmaceuticals, dyes, balms, preservatives, and herbal medicines (Kumar and Tyagi 2013; Ghavam 2021; Michalak et al. 2024; Bandi et al. 2025). Ghavam (2021) reported that the essential oil of *T. parthenium* exhibited activity against *Aspergillus brasiliensis*. Among the plants used in traditional medicine are those from the Asphodelus genus found in the Mediterranean region of Southeast Asia and North Africa. These plants have been used by indigenous peoples for various pathologies, including burns, nephrolithiasis, psoriasis, acne, toothache, alopecia areata, and local inflammation (Malmir et al. 2018; Dioguardi et al. 2019). *Asphodelus aestivus* Brot. (*A. aestivus* Brot.) is recognized as an important wild medicinal plant (Farid et al. 2021; El-Elimat et al. 2024; Zöngür, 2024). It has been observed that different concentrations of *A. aestivus* Brot. essential oils are effective

against all tested fungi (Zöngür, 2024). An important plant family is Lamiaceae, which includes 236 genera and 250 species. *Melissa officinalis* (*M. officinalis*) is the most common species in this family (Hassan et al. 2019). *M. officinalis* L. is a perennial plant with various ethnomedical, therapeutic, and culinary applications. Traditionally, it has been used in the treatment of infectious bites, ulcers, herpes, wounds, and parasitic disorders (Abdel-Naime et al. 2019). Another significant plant family is Rosaceae, which consists of approximately 200 species, mostly shrubs (Fayaz et al. 2024). *Rosa canina* L. (Dog rose) (*R. canina* L.) is a plant species that naturally grows in Europe, temperate Eurasia, Western Asia, North Africa, and the northern hemisphere of North and South America (Köroğlu, 2023). The Rosa species exhibit various pharmacological activities, including anti-diabetic, hepatoprotective, antimicrobial, anti-inflammatory, anti-arthritic, anti-proliferative/anti-cancer, neurological, and anti-obesity effects (Öğüt, 2022; Fayaz et al. 2024). *R. canina* contains a large amount of pharmacologically active components (flavonols, carotenoids, tannins, and organic acids) (Öğüt, 2022). Due to the negative effects of chemical pesticides, the search for alternative biopesticides is ongoing. Literature searches indicate that the substances present in plants are effective in various fields, from health to food preservation. Due to their natural distribution and the active compounds they contain, medicinally significant plants play an important role in the search for biopesticides. Undoubtedly, the use of plant-based biopesticides will contribute to the restoration of disrupted ecosystems. Literature searches have shown that parameters such as the location where the plants grow, the substances and methods used in extraction, and the microorganisms used in activity tests significantly affect the results (Abdellatif et al. 2014; Ehsani et al. 2017; Mabrouki et al. 2018; Alizadeh Behbahani and Shahidi 2019; Abdel-Naime et al. 2019). This research was carried out to determine the content analysis of ethanol extracts of some plants (*Asphodelus aestivus* L., *Melissa officinalis*, *Rosa canina* L., *Tanacetum parthenium*) that grow naturally in Elazığ province and to determine their activities against some plant pathogenic microorganisms under *in vitro* conditions.

Materials and Methods

Plants

In this study, the leaves of *Asphodelus aestivus* L. (*A. aestivus* L., Asphodelaceae), the leaves of *Melissa officinalis* (*M. officinalis*, Lamiaceae), the fruits of *Rosa canina* L. (*R. canina* L., Rosaceae), and the flowers of *Tanacetum parthenium* (*T. parthenium*, Asteraceae or Compositae) were used, all of which are naturally occurring and utilized by the local population in Elazığ, Türkiye.

Extraction Process

The plants collected from the natural habitat and identified were dried in the shade. Subsequently, an extraction process was carried out using a 1:1 ethanol/water mixture at a temperature of 60 °C for 10 hours, with a plant/solvent ratio of 1:20 (g/mL). The obtained extracts were stored at -20 °C until used in the experimental study.

Test Microorganisms

The plant pathogenic bacterial isolates *Erwinia amylovora, Pseudomonas syringae* pv. syringae, *Xanthomonas euvesicatoria, Clavibacter michiganensis* subsp. michiganensis, and *Verticillium dahliae, Fusarium oxysporum* fungal isolates, were

obtained from the Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Plant Protection.

Content Analysis by LC-MS/MS

For the chemical analyses of the extracts, 50 mg samples of each extract were taken into 2 mL Eppendorf tubes. To each sample, 1 mL of a mixed solvent (acetonitrile-methanol-water in a 1:1:1 ratio) was added for dissolution. The samples were vortexed until dissolved, and any undissolved material was treated in an ultrasonic bath. Subsequently, 0.8 mL of hexane was added to perform the extraction process. The samples were centrifuged at 7000 rpm for 5 minutes. The lower phase was then collected and diluted at a ratio of 1:4. Finally, the samples were filtered through a 0.25 µm filter before LC-MS/MS analysis was conducted (Atalar et al. 2021). Additional information regarding the analysis procedure is summarized in Table 1.

Table 1. LC-MS/MS conditions

	Time (min)	A (%)	B (%)	Features
1	0	85	15	**System**: Agilent 6460 Agilent 6460 Triple Quad LCMS
2	5	75	25	**Column**: Poroshell 120 EC-C18 (50 mm 4.6 mm I.D., 2.7 µm)
3	15	25	75	**Injection volume**: 4.00 µl
4	16	0	100	**Flow**: 0.400 mL/min
5	20	0	100	**Temperature**: 30.00 °C
6	22	85	15	**Method time**: 40.00 min
7	40	85	15	**A**: Water 0.1% Formic acid, 5mM ammonium formate
				B: Acetonitrile 0.1% Formic acid

Determination of Antimicrobial Activity

Antibacterial Activity Studies

The antibacterial activity of the plant extracts was determined using the Agar Well Diffusion Method (Balouiri et al. 2016). In this study, the turbidity of the 24-hour cultures of the pathogenic bacterial strains used was adjusted according to the 0.5 McFarland standard (Anonymous, 2019). A volume of 100 µL from the cultures with standard turbidity was spread onto the surface of Mueller-Hinton Agar (MERCK) using a sterile glass rod. Subsequently, wells of 10 mm diameter were created using a Cork Borer. To these wells, 50 µL of solutions diluted with distilled water at concentrations of 5%, 10%, 15%, 20%, and 25% (v/v) were applied. Chloramphenicol (30 µg) and Ciprofloxacin (5 µg) standard disks were used as control antibiotics. After incubating the Petri plates at 35ºC for 24 hours, the zones of inhibition around the wells and disks were evaluated as positive results.

Antifungal Activity Studies

The antifungal activities of the plant extracts were determined using the Poisoned Food Method reported by Balouiri et al. (2016). For this purpose, the final concentrations of the extracts were prepared by adding them to Malt Extract Agar (MERCK) at concentrations of 5%, 10%, 15%, 20%, and 25% (v/v) in Petri plates. Ten millimeter diameter sections from cultures of *Verticillium dahliae* (*V. dahliae*) and *Fusarium oxysporum* (*F. oxysporum*) fungal isolates were transferred to the extract-containing Petri plates. For positive control, 50 µL of Cycloheximide (prepared by dissolving at a concentration of 35 mg/mL in ethanol) was added to Malt Extract Agar plates, and sections of 10 mm diameter from both microfungus cultures were aseptically transferred

to the negative control Malt Extract Agar plates. After inoculation, the Petri plates were incubated at 27ºC for 7 days. The study was conducted using a Randomized Complete Block Design with four replications. After incubation, the microfungi in the Petri dishes were checked, and the colony diameters were measured and recorded. The percentage of mycelium growth inhibition was calculated using the following formula (Yahyazadeh et al. 2008).

$$MGI\ (\%) = [(C - T)/C] \times 100$$

MGI (%) is the percent mycelial growth inhibition, C the colony radius of the pathogen when growing (negative control); T the colony radius of the pathogen for each extract concentration.

Statistical Analysis

The normality of the obtained data was assessed using the Shapiro-Wilk Goodness of Fit Test and the Kolmogorov-Smirnov Test. It was determined that the data did not conform to a normal distribution. Accordingly, the colony diameters were compared using the Kruskal-Wallis H test. The statistical significance level was considered to be $p<0.05$. The analyses were performed using the SPSS (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL) version 21 software.

Results

Content Analysis

The content analysis of the extracts was investigated for 33 standard compounds using LC-MS/MS. No such compounds were detected in the extracts of *A. aestivus* L. and *M. officinalis*. In the extract of *R. canina* L., four compounds were identified (namely vanillin, trans-cinnamic acid, p-coumaric acid, and hydroxybenzaldehyde), while three compounds (namely vanillin, gentisic acid, and chlorogenic acid) were identified in the extract of *T. parthenium* (Table 2).

Table 2. LC-MS/MS analysis (µg/L)

No	Standards	*R.T. (min)	*R. canina* L.	*A. aestivus* L.	*M. officinalis*	*T. parthenium*
1	Gallic acid	2.227	*N.D.	N.D.	N.D.	N.D.
2	Epigallocatechin	2.576	N.D.	N.D.	N.D.	N.D.
3	Chlorogenic acid	2.572	N.D.	N.D.	N.D.	**43.07**
4	Catechin	2.826	N.D.	N.D.	N.D.	N.D.
5	Gentisic acid	3.082	N.D.	N.D.	N.D.	**125.19**
6	Caffeic Acid	3.321	N.D.	N.D.	N.D.	N.D.
7	Syringic acid	3.495	N.D.	N.D.	N.D.	N.D.
8	Vanillic acid	3.628	N.D.	N.D.	N.D.	N.D.
9	Rutin	2.885	N.D.	N.D.	N.D.	N.D.
10	Isoquercitrin	4.486	N.D.	N.D.	N.D.	N.D.
11	Polydatin	4.601	N.D.	N.D.	N.D.	N.D.
12	Hydroxybenzaldehyde	4.802	**8.77**	N.D.	N.D.	N.D.
13	p-coumaric acid	4.737	**75.09**	N.D.	N.D.	N.D.
14	Sinapic acid	5.406	N.D.	N.D.	N.D.	N.D.
15	Vanillin	5.375	**481.23**	N.D.	N.D.	**271.10**
16	trans-ferulic acid	5.635	N.D.	N.D.	N.D.	N.D.
17	Taxifolin	5.930	N.D.	N.D.	N.D.	N.D.
18	Salicylic Acid	7.589	N.D.	N.D.	N.D.	N.D.
19	o-coumaric acid	7.824	N.D.	N.D.	N.D.	N.D.
20	Baicalin	8.079	N.D.	N.D.	N.D.	N.D.
21	Protocatehuic ethyl ester	8.353	N.D.	N.D.	N.D.	N.D.

Table 2. LC-MS/MS analysis (µg/L) (Continued)

22	Protocatechuic acid	8.529	N.D.	N.D.	N.D.	N.D.
23	Kaempferol	9.975	N.D.	N.D.	N.D.	N.D.
24	Trans-cinnamic acid	10.995	**125.55**	N.D.	N.D.	N.D.
25	Naringenin	11.735	N.D.	N.D.	N.D.	N.D.
26	Morin	12.077	N.D.	N.D.	N.D.	N.D.
27	Quercetin	11.839	N.D.	N.D.	N.D.	N.D.
28	7-Hydroxyflavone	12.113	N.D.	N.D.	N.D.	N.D.
29	Chrysin	13.619	N.D.	N.D.	N.D.	N.D.
30	Luteolin	14.042	N.D.	N.D.	N.D.	N.D.
31	Biochanin A	13.719	N.D.	N.D.	N.D.	N.D.
32	5-Hydroxyflavone	15.762	N.D.	N.D.	N.D.	N.D.
33	Diosgenin	20.976	N.D.	N.D.	N.D.	N.D.

*__R.T.:__ Retention Time, *__N.D.:__ Not detected

Antimicrobial Activity

Antibacterial Activity

It was determined that the plant extracts did not exhibit any activity against the tested bacteria.

Antifungal Activity

The extract of *R. canina* L. showed no activity against the tested microfungi (*F. oxysporum* and *V. dahliae*); however, the activity of the *T. parthenium* extract increased with higher concentrations. The extracts of *M. officinalis* and *A. aestivus* L. completely inhibited the tested microfungi at concentrations of 15% and above (v/v) (Table 3, Figures 1-7).

Upon examining Table 3, it is observed that there is a statistically significant difference in the colony diameters of *F. oxysporum* and *V. dahliae* microfungi among the plant extracts, except for *R. canina* L. (p<0.001).

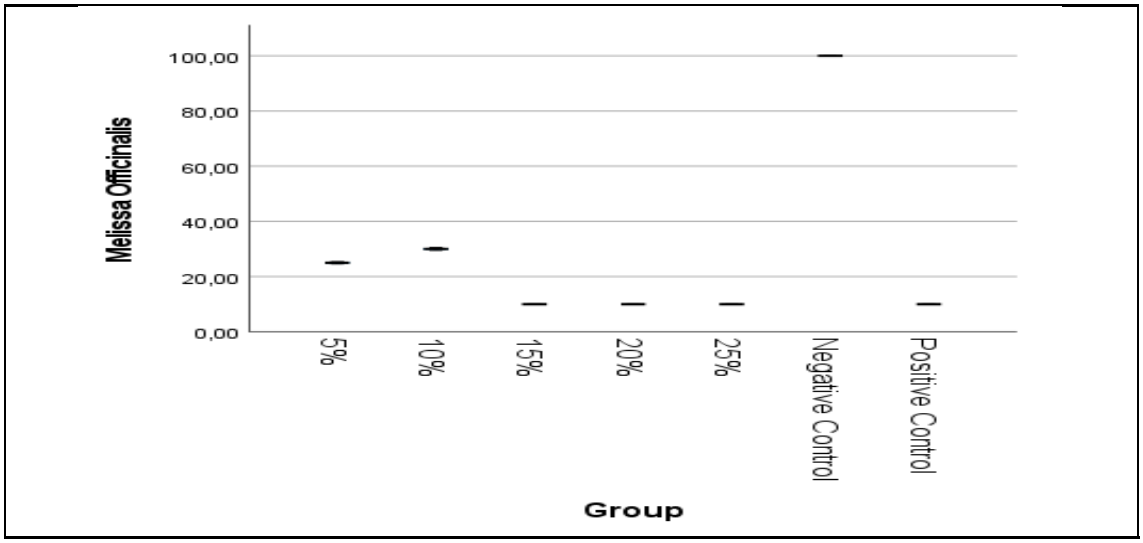

Figure 1. Comparison of the antifungal activity of *M. officinalis* extract against the *F. oxysporum* microfungus in terms of colony diameters.

Upon examining Figure 1, it is observed that the colony diameters of the *F. oxysporum* microfungus in the positive control group, as well as in the *M. officinalis*

extract groups at 15%, 20%, and 25%, are significantly lower than that of the negative control group (p<0.05). This indicates that the positive control and the 15%, 20%, and 25% (v/v) applications are significantly more effective compared to the negative control group (Figure 1).

Table 3. Comparison of colony diameters of extracts using the Kruskal-Wallis Test

Test microorganisms	Extracts	Applications (v/v)	N	$\bar{X}$± *SD*	Median	Average inhibition rate (mm)	*P* value
F. oxysporum	*M. officinalis*	5%	4	25.00±0.33	25.00	75	**<0.001**
		10%	4	30.00±0.41	30.00	70	
		15%	4	10.00±0.00	10.00	90	
		20%	4	10.00±0.00	10.00	90	
		25%	4	10.00±0.00	10.00	90	
		Control (Positive)	4	10.00±0.00	10.00	90	
		Control (Negative)	4	100.00±0.00	100.00	0	
	T. parthenium	5%	4	60.00±1.42	60.40	40	**<0.001**
		10%	4	25.00±0.24	25.00	75	
		15%	4	20.13±0.35	20.15	79.88	
		20%	4	12.00±0.71	12.20	88	
		25%	4	11.75±0.33	11.85	88.02	
		Control (Positive)	4	10.00±0.00	10.00	90	
		Control (Negative)	4	99.75±0.50	100.00	0.25	
	A.aestivus L.	5%	4	55.50±0.56	55.35	44.50	**<0.001**
		10%	4	19.50±0.41	19.50	80.50	
		15%	4	10.00±0.00	10.00	90	
		20%	4	10.00±0.00	10.00	90	
		25%	4	10.00±0.00	10.00	90	
		Control (Positive)	4	10.00±0.00	10.00	90	
		Control (Negative)	4	100.00±0.00	100.00	0	
V. dahliae	*M. officinalis*	5%	4	30.00±0.71	29.80	70	**<0.001**
		10%	4	24.82±0.40	24.80	74.92	
		15%	4	10.00±0.00	10.00	90	
		20%	4	10.00±0.00	10.00	90	
		25%	4	10.00±0.00	10.00	90	
		Control (Positive)	4	10.00±0.00	10.00	90	
		Control (Negative)	4	100.00±0.00	100.00	0	
	T. parthenium	5%	4	55.00±0.71	55.25	45	**<0.001**
		10%	4	25.00±0.57	25.00	75	
		15%	4	20.00±0.33	20.00	80	
		20%	4	11.03±0.31	11.10	88.97	
		25%	4	11.00±0.71	11.20	89	
		Control (Positive)	4	10.00±0.00	10.00	90	
		Control (Negative)	4	100.00±0.00	100.00	0	
	A. aestivus L.	5%	4	45.00±0.41	45.00	55	**<0.001**
		10%	4	20.00±0.71	19.80	80	
		15%	4	10.00±0.00	10.00	90	
		20%	4	10.00±0.00	10.00	90	
		25%	4	10.00±0.00	10.00	90	
		Control (Positive)	4	10.00±0.00	10.00	90	
		Control (Negative)	4	100.00±0.00	100.00	0	

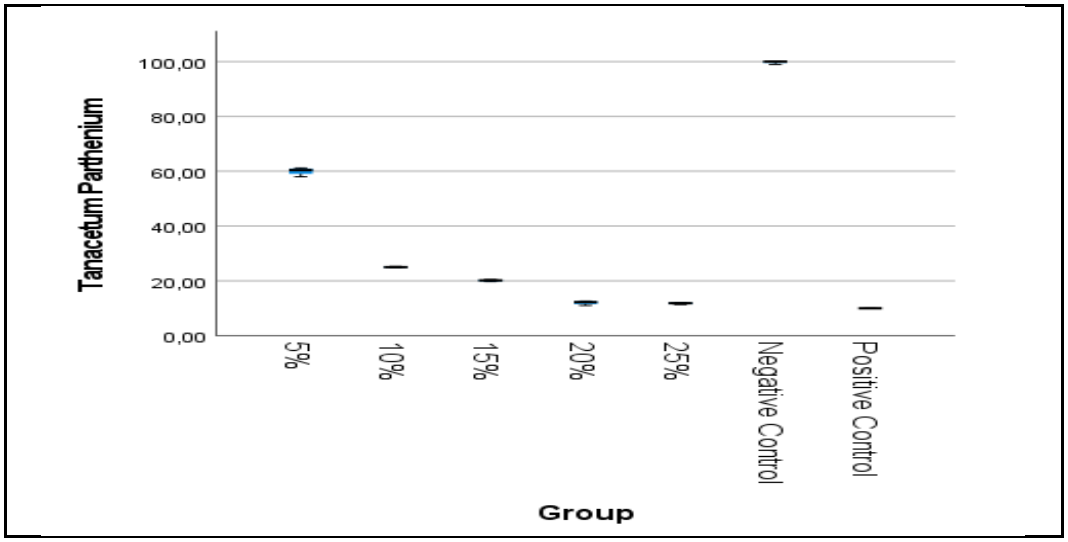

Figure 2. Comparison of the antifungal activity of *T. parthenium* extract against the *F. oxysporum* microfungus in terms of colony diameters

Upon examining Figure 2, it is observed that the colony diameters of the *F. oxysporum* microfungus in the positive control, as well as in the *T. parthenium* extract groups at 20% and 25%, are significantly lower than that of the negative control group (p<0.05). This indicates that the positive control and the 20% and 25% (v/v) groups are significantly more effective compared to the negative control group. Additionally, in the *T. parthenium* extract, the colony diameter of the positive control group is significantly lower than that of the 5% group (p<0.05). Thus, the positive control group is significantly more effective compared to the 5% group.

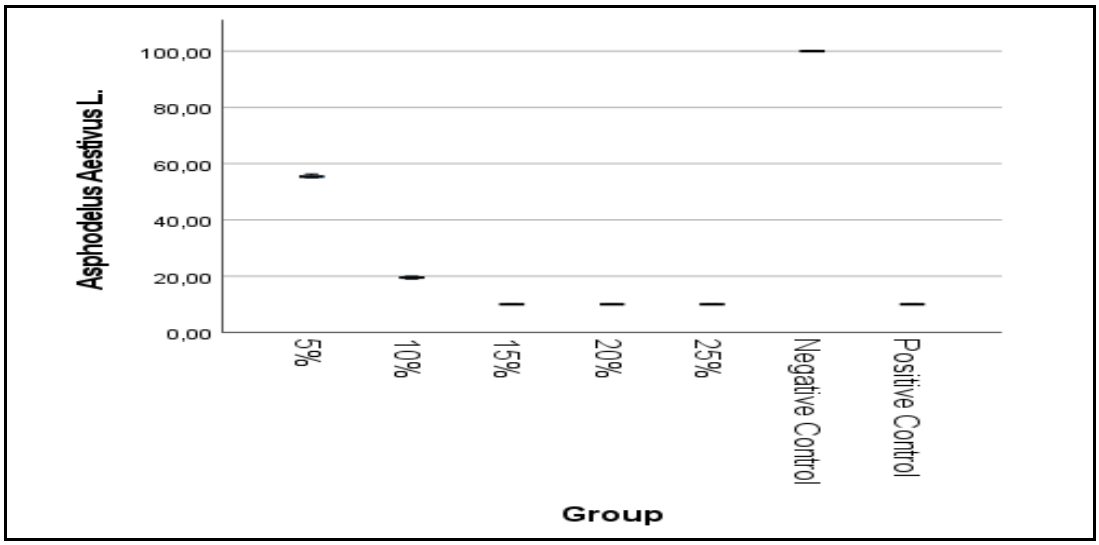

Figure 3. Comparison of the antifungal activity of *A. aestivus* L. extract against the *F. oxysporum* microfungus in terms of colony diameters

Upon examining Figure 3, it is observed that the colony diameters of the *F. oxysporum* microfungus in the positive control, as well as in the *A. aestivus* L. extract groups at 15%, 20%, and 25%, are significantly lower than that of the negative control

group (p<0.05). This indicates that the positive control and the 15%, 20%, and 25% (v/v) groups are significantly more effective compared to the negative control group.

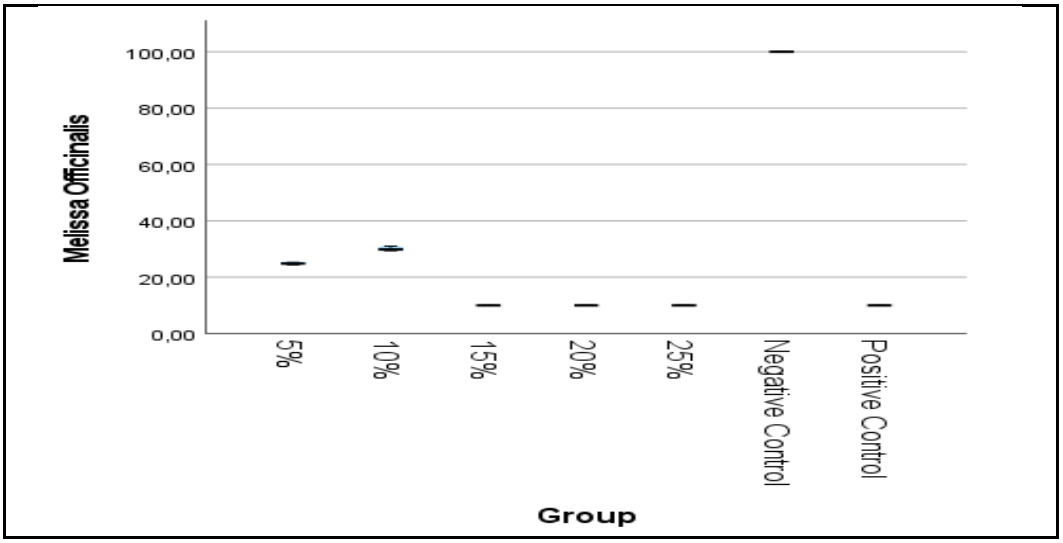

Figure 4. Comparison of the antifungal activity of *M. officinalis* extract against the *V. dahliae* microfungus in terms of colony diameters

Upon examining Figure 4, it is observed that the colony diameters of the *V. dahliae* microfungus in the positive control, as well as in the *M. officinalis* extract groups at 20% and 25%, are significantly lower than that of the negative control group (p<0.05). This indicates that the positive control and the 15%, 20%, and 25% (v/v) groups are significantly more effective compared to the negative control group.

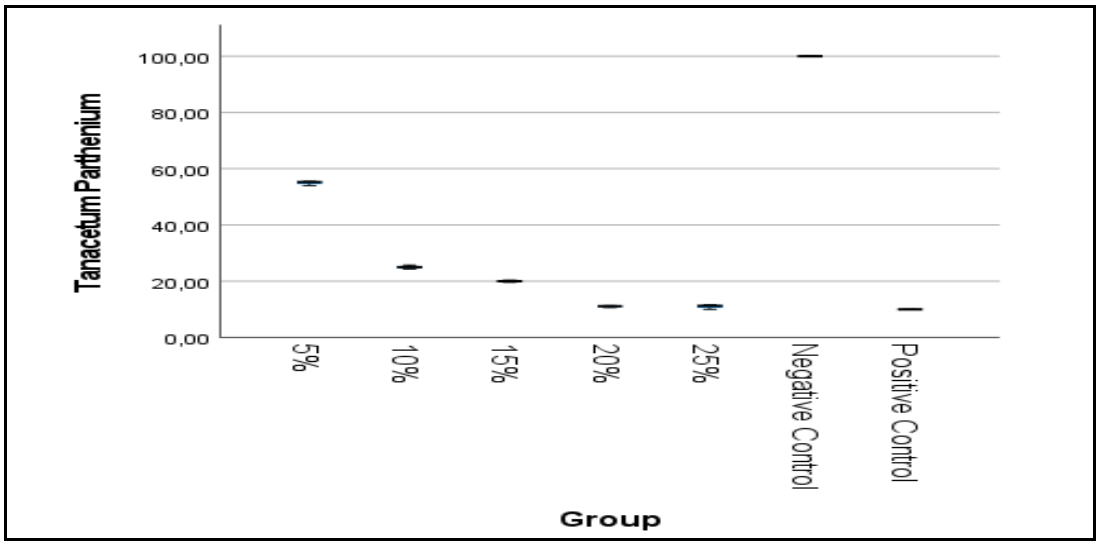

Figure 5. Comparison of the antifungal activity of *T. parthenium* extract against the *V. dahliae* microfungus in terms of colony diameters

Upon examining Figure 5, it is observed that the colony diameters of the *V. dahliae* microfungus in the positive control, as well as in the *T. parthenium* extract groups at 20% and 25%, are significantly lower than that of the negative control group (p<0.05).

This indicates that the positive control and the 20% and 25% dosage groups are significantly more effective compared to the negative control group. Additionally, the colony diameter of the positive control group is significantly lower than that of the 5% group in the *T. parthenium* extract (p<0.05). Thus, the positive control group is significantly more effective compared to the 5% group.

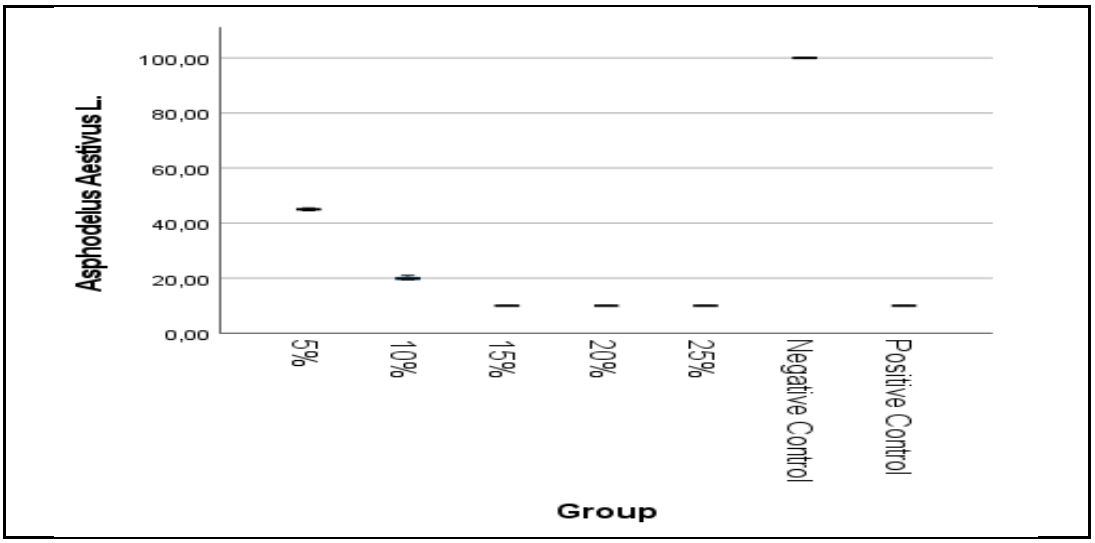

Figure 6. Comparison of the antifungal activity of *A. aestivus* L. extract against the *V. dahliae* microfungus in terms of colony diameters

Upon examining Figure 6, it is observed that the colony diameters of the *V. dahliae* microfungus in the positive control, as well as in the *A. aestivus* L. extract groups at 15%, 20%, and 25%, are significantly lower than that of the negative control group (p<0.05). This indicates that the positive control and the 15%, 20%, and 25% (v/v) groups are significantly more effective compared to the negative control group.

Applications (v/v)	***M. officinalis***	***T. parthenium***	***A. aestivus* L.**
5%			
10%			

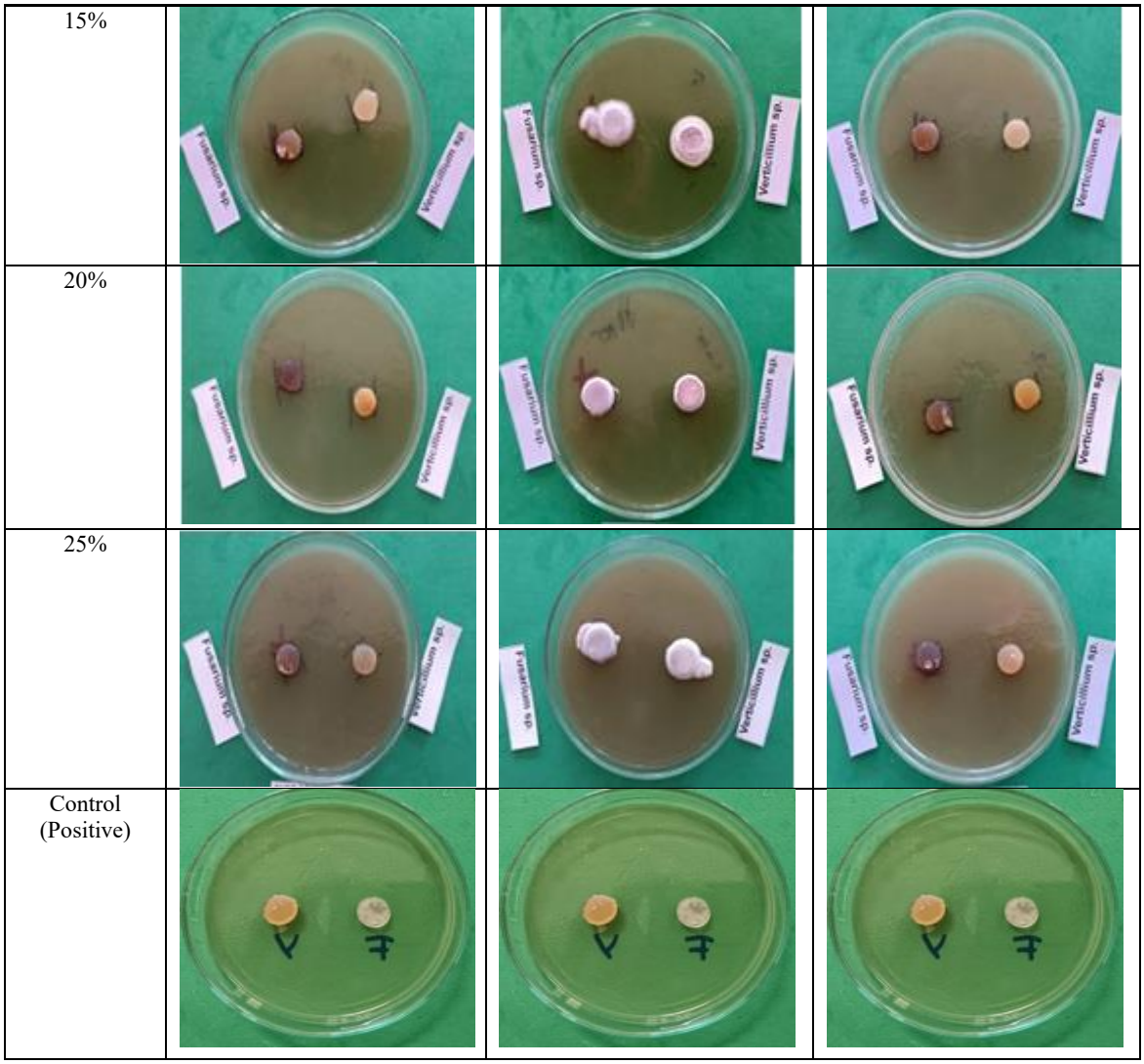

Figure 7. Sample ımages related to the antifungal activity of plant extracts at different concentrations and positive control (50 µl cycloheximide) applications (Continued).

Discussion

In the current study conducted by us, the ethanol extracts of different parts (such as leaves, fruits, and flowers) of four plant species were evaluated for 33 standard compounds investigated by LC-MS/MS. It was found that some of the analyzed compounds were not present in certain extracts (*M. officinalis* and *A. aestivus* L.); four compounds (namely vanillin, p-coumaric acid, trans-cinnamic acid, and hydroxybenzaldehyde) were detected in *R. canina* L., while three compounds (vanillin, gentisic acid, and chlorogenic acid) were identified in *T. parthenium*. In a similar study conducted in Jordan, seven compounds were identified in the LC-MS analysis of *A. aestivus* Brot. The authors of this study attributed this result to the presence of a wide variety of chemical compounds in the plant and different chemical variations influenced by regional environmental factors (El-Elimat et al. 2024). Due to the lack of sufficient studies in the literature regarding the content analysis of the studied plant extracts using LC-MS/MS, studies conducted with various methods such as GC/MS were evaluated. For example, 22 different components were identified in the extract produced from *A. aestivus*

Brot. using hydrodistillation (GC/MS) (Zöngür, 2024). In a study regarding the extract content produced from the hydrodistillation of *M. officinalis* L. leaves grown in Algeria (GC/MS and GC-FID), 63 compounds were detected (Abdellatif et al. 2014). In a study aimed at characterizing the polyphenolic profile and bioactive properties of the boiled, infused, and hydroalcoholic extracts of *M. officinalis* L., the main phenolic compound identified was rosmarinic acid (Silva et al. 2023). Alizadeh Behbahani and Shahidi (2019) reported that geranyl acetate, citral, z-citral, citronellal, and citronellol were the main components in the GC-MS analysis of *M. officinalis* essential oil. Ehsani et al. (2017) identified components such as citronellal, thymol, citral, and β-caryophyllene in their analyses of *M. officinalis* essential oil (GC-MS). Differences were observed between the findings of our study and the comparative studies. It is estimated that these differences arise from parameters such as the location where the plants are grown, the parts used, the extraction method, and the devices and methods used in content analyses. In the current study conducted by us, it was found that the extracts did not show activity against the plant pathogens *Xanthomonas euvesicatoria*, *Clavibacter michiganensis* subsp. michiganensis, *Erwinia amylovora*, and *Pseudomonas syringae* pv. syringae; however, different results were obtained regarding their activities against the microfungi *F. oxysporum* and *V. dahliae*. Among these, the extracts of *M. officinalis* and *A. aestivus* L. completely inhibited the test microfungi at concentrations of 15% and above; the activity of *T. parthenium* extract increased with increasing concentrations, while *R. canina* L. extract showed no activity at all. Literature searches revealed that some studies had been conducted on the antimicrobial activities of the plant species used by us. However, there are some differences between these studies and the study we conducted regarding the extraction method, the location where the plants were grown, and the parts used. For instance, Zöngür (2024) reported that the extract produced from *A. aestivus* Brot. was effective against all test species (*Aspergillus flavus, Rhizopus oryzae, Fusarium oxysporum, Alternaria solani, Penicillium expansum,* and *Aspergillus parasiticus*) at different concentrations of volatile oils. Zöngür and Buzpinar (2023) stated that the inhibition rates of *A. aestivus* leaf extracts against *Aspergillus flavus* and *Aspergillus parasiticus* ranged from 3.13% to 89.94%. It appears that the results obtained in terms of antifungal activity of *A. aestivus* leaf extracts overlap with the findings of both studies. Rabbani et al. (2016) reported that the hydroalcoholic extract of *M. officinalis* showed the highest antibacterial activity against *Staphylococcus aureus* among various test bacteria. Mabrouki et al. (2018) stated that the ethanol extract produced from *M. officinalis* L. using various solvents exhibited a strong inhibitory effect against *Staphylococcus aureus*. Ehsani et al. (2017) determined that *Staphylococcus aureus* was the most sensitive bacterial species in their activity determination tests conducted on *M. officinalis* essential oil against four foodborne bacteria. In a study by Abdel-Naime et al. (2019), it was reported that *M. officinalis* L. has significant antimicrobial potential, especially against gram-positive bacteria and yeasts. Abdellatif et al. (2014) indicated that the extract produced from the hydrodistillation of *M. officinalis* L. leaves showed high antimicrobial activity against five human pathogenic bacteria, one yeast, and two phytopathogenic fungi. Alizadeh Behbahani and Shahidi (2019) reported that *M. officinalis* has greater inhibitory effects on commercial bacterial strains that cause infections compared to clinical bacterial strains, with the greatest effect occurring against gram-positive bacteria. Yu et al. (2022) reported that the essential oil of *M. officinalis* L. (MOEO) exhibited antimicrobial activity against *Vibrio parahaemolyticus*, attributing this effect to the damage caused by MOEO to the morphology of the cell membrane. Additionally, they

suggested that MOEO has potential as a natural food preservative. It was determined that the studies provided regarding the antimicrobial activity of *M. officinalis* overlap with the antifungal activity of the study conducted by us. In our study, differences were detected between the LC-MS/MS content and antimicrobial activity results. For instance, it was found that *R. canina* L. and *T. parthenium*, which contain the highest phenolic compounds, showed no activity; however, *A. aestivus* L. and *M. officinalis* extracts, which did not have phenolic compounds detected by LC-MS/MS, showed activity only against microfungi. This situation indicates the presence of compounds exhibiting antifungal activity that could not be detected by LC-MS/MS standards. Furthermore, the antimicrobial effects of the compounds identified by LC-MS/MS may vary depending on their concentrations, the type of microorganisms, and many other factors.

Conclusion

In this study, out of 33 standard compounds investigated by LC-MS/MS, four compounds were determined in *R. canina* L. extract (vanillin, trans-cinnamic acid, p-coumaric acid and ydroxybenzaldehyde, respectively) and three compounds were determined in *T. parthenium* extract (vanillin, gentisic acid and chlorogenic acid, respectively). Among the tested pathogenic microfungi, the best activity on *F. oxysporum* and *V. dahliae* was determined to be from *M. officinalis* and *A. aestivus* L. plant species extracts. In particular, it is reported that ethanol extracts of *M. officinalis* and *A. aestivus* L. plant species at 15% and above can be evaluated in terms of antifungal activity. Additionally, it is anticipated that the ethanol extracts exhibiting activity may contribute economically, with potential applications in biotechnology, food preservation, biopesticide development, and the pharmaceutical industry.

Conflict of Interest

The authors declare that they have no conflict of interest

References

Abdel-Naime, W. A., Fahim, J. R., Fouad, M. A., Kamel, M. S. (2019). Antibacterial, antifungal, and GC–MS studies of *Melissa officinalis*. *South African Journal of Botany*, *124*, 228-234. https://doi.org/10.1016/j.sajb.2019.05.011

Abdellatif, F., Boudjella, H., Zitouni, A., Hassani, A. (2014). Chemical composition and antimicrobial activity of the essential oil from leaves of Algerian *Melissa officinalis* L. *EXCLI journal*, *13*, 772.

Alizadeh Behbahani, B., Shahidi, F. (2019). *Melissa officinalis* essential oil: Chemical compositions, antioxidant potential, total phenolic content and antimicrobial activity. *Nutrition and Food Sciences Research*, *6*(1), 17-25.

Anand, G., Rajeshkumar, K. C. (2022). *Challenges and threats posed by plant pathogenic fungi on agricultural productivity and economy.* In Fungal diversity, ecology and control management (pp. 483-493). Singapore: Springer Nature Singapore.

Anonymous. 2019. *Mcfarland standard for in vitro use only Dalyn Biologicals, Catalogue No. TM50-TM60,* http://www.dalynn.com/dyn/ck_assets/files/tech/TM53.pdf, (Access date: May 10, 2019).

Arora S, Sahni D, (2016). Pesticides effect on soil microbial ecology and enzyme activity-an overview. *Journal of Applied and Natural Science, 8*(2), 1126-1132.

Atalar, M. N., Aras, A., Türkan, F., Barlak, N., Yildiko, Ü., Karatas, O. F., Alma, M. H. (2021). The effects of *Daucus carota* extract against PC3, PNT1a prostate cells, acetylcholinesterase, glutathione S-transferase, and α-glycosidase; an in vitro–in silico study. *Journal of Food Biochemistry, 45*(12). e13975. https://doi.org/10.1111/jfbc.13975

Balouiri, M., Sadiki, M., Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

Bandi, N., Sarvepalli, R., Venugopalaiah, P. (2025). Review on *Tanacetum parthenium*. *International Journal of Indigenous Herbs and Drugs*, 7-13. https://doi.org/10.46956/ijihd.v10i1.674

Buttimer, C., McAuliffe, O., Ross, R. P., Hill, C., O'Mahony, J., Coffey, A. (2017). Bacteriophages and bacterial plant diseases. *Frontiers in microbiology*, *8*, 34.

Dioguardi, M., Campanella, P., Cocco, A., Arena, C., Malagnino, G., Sovereto, D., Lo Muzio, L. (2019). Possible uses of plants of the genus Asphodelus in oral medicine. *Biomedicines*, *7*(3), 67. https://doi.org/10.3390/biomedicines7030067

Ehsani, A., Alizadeh, O., Hashemi, M., Afshari, A., Aminzare, M. (2017). Phytochemical, antioxidant and antibacterial properties of *Melissa officinalis* and *Dracocephalum moldavica* essential oils. *In Veterinary Research Forum, 8,* 3.

El-Elimat, T., Olimat, S., Agha, A. S. A., Aburjai, A., Aburjai, T. (2024). LC-MS Analysis of Secondary Metabolites of *Asphodelus aestivus* Brot.(Asphodelaceae) grown wild in Jordan. *Jordan Journal of Pharmaceutical Sciences*, *17*(2), 333-343. https://doi.org/10.35516/jjps.v17i2.1850

Farid, M., Salem, M., Elatif, R., Elkhateeb, A., Marzouk, M., Hussein, S. (2021). Chemical analysis and cytotoxic evaluation of *Asphodelus aestivus* Brot. flowers. *Egyptian Journal of Chemistry*, *64*(9), 5167-5174. https://dx.doi.org/10.21608/ejchem.2021.70226.3546

Fayaz, F., Singh, K., Gairola, S., Ahmed, Z., Shah, B. A. (2024). A comprehensive review on phytochemistry and pharmacology of *Rosa species* (Rosaceae). *Current Topics in Medicinal Chemistry*, *24*(4), 364-378. https://doi.org/10.2174/0115680266274385231023075011

Ferrigo, D., Raiola, A., Causin, R. (2016). Fusarium toxins in cereals: Occurrence, legislation, factors promoting the appearance and their management. *Molecules*, *21*(5), 627. https://doi.org/10.3390/molecules21050627

Ghavam, M. (2021). *Tripleurospermum disciforme* (CA Mey.) Sch. Bip., *Tanacetum parthenium* (L.) Sch. Bip, and *Achillea biebersteinii* Afan.: efficiency, chemical profile, and biological properties of essential oil. *Chemical and Biological Technologies in Agriculture*, *8*, 1-17. https://doi.org/10.1186/s40538-021-00245-4

Hassan, R. A., Abotaleb, S. T., Hamed, H. B., Eldeen, M. S. (2019). Antioxidant and antimicrobial activities of *Melissa officinalis* L. (lemon balm) extracts. *Journal of Agricultural Chemistry and Biotechnology*, *10*(9), 183-187. https://dx.doi.org/10.21608/jacb.2019.56823

Holtappels, D., Fortuna, K., Lavigne, R., Wagemans, J. (2021). The future of phage biocontrol in integrated plant protection for sustainable crop production. *Current Opinion in Biotechnology*, *68*, 60-71. https://doi.org/10.1016/j.copbio.2020.08.016

Khan, B. A., Nadeem, M. A., Nawaz, H., Amin, M. M., Abbasi, G. H., Nadeem, M., Ayub, M. A. (2023). *Pesticides: impacts on agriculture productivity, environment, and management strategies.* In Emerging contaminants and plants: Interactions,

adaptations and remediation technologies (pp. 109-134). Cham: Springer International Publishing.

Klosterman, S. J., Atallah, Z. K., Vallad, G. E., Subbarao, K. V. (2009). Diversity, pathogenicity, and management of Verticillium species. *Annual review of phytopathology*, *47*(1), 39-62.

Košćak, L., Lamovšek, J., Đermić, E., Prgomet, I., Godena, S. (2023). Microbial and plant-based compounds as alternatives for the control of phytopathogenic bacteria. *Horticulturae*, *9*(10), 1124. https://doi.org/10.3390/horticulturae9101124

Kowalska, B. (2021). Management of the soil-borne fungal pathogen–*Verticillium dahliae* Kleb. causing vascular wilt diseases. *Journal of Plant Pathology*, *103*(4), 1185-1194. https://doi.org/10.1007/s42161-021-00937-8

Köroğlu, A. (2023). *Rosa canina* L. *in medicinal plants of Turkey* (pp. 225-240). CRC Press.

Kumar, V., Tyagi, D. (2013). Chemical composition and biological activities of essential oils of genus Tanacetum-a review. *J Pharmacogn Phytochem*, *2*(3), 159-163.

Lyubenova, A., Rusanova, M., Nikolova, M., Slavov, S. B. (2023). Plant extracts and Trichoderma spp: possibilities for implementation in agriculture as biopesticides. *Biotechnology & Biotechnological Equipment*, *37*(1), 159-166. https://doi.org/10.1080/13102818.2023.2166869

Mabrouki, H., Duarte, C. M. M., Akretche, D. E. (2018). Estimation of total phenolic contents and in vitro antioxidant and antimicrobial activities of various solvent extracts of *Melissa officinalis* L. *Arabian Journal for Science and Engineering*, *43*, 3349-3357. https://doi.org/10.1007/s13369-017-3000-6

Malmir, M., Serrano, R., Caniça, M., Silva-Lima, B., Silva, O. (2018). A comprehensive review on the medicinal plants from the genus Asphodelus. *Plants*, *7*(1), 20. https://doi.org/10.3390/plants7010020

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Foster, G. D. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular Plant Pathology*, *13*(6), 614-629. https://doi.org/10.1111/j.1364-3703.2012.00804.x

Martins, P. M., Merfa, M. V., Takita, M. A., De Souza, A. A. (2018). Persistence in phytopathogenic bacteria: do we know enough?. *Frontiers in Microbiology*, *9*, 1099.

Michalak, M., Stryjecka, M., Żarnowiec, P., Zagórska-Dziok, M., Kiełtyka-Dadasiewicz, A. (2024). Chemical composition of extracts from various parts of feverfew (*Tanacetum parthenium* L.) and their antioxidant, protective, and antimicrobial activities. *International Journal of Molecular Sciences*, *25*(22), 12179. https://doi.org/10.3390/ijms252212179

Nelson, R., Wiesner-Hanks, T., Wisser, R., Balint-Kurti, P. (2018). Navigating complexity to breed disease-resistant crops. *Nature Reviews Genetics*, *19*(1), 21-33. https://doi.org/10.1038/nrg.2017.82

Öğüt, K. (2022). *Rosa canina* L. *in novel drug targets with traditional herbal medicines: scientific and clinical evidence* (pp. 513-523). Cham: Springer International Publishing.

Rabbani, M., Etemadifar, Z., Karamifard, F., Borhani, M. S. (2016). Assessment of the antimicrobial activity of *Melissa officinalis* and *Lawsonia inermis* extracts against some bacterial pathogens. *Comparative Clinical Pathology*, *25*, 59-65. https://doi.org/10.1007/s00580-015-2140-x

Sharma, A., Abrahamian, P., Carvalho, R., Choudhary, M., Paret, M. L., Vallad, G. E., Jones, J. B. (2022). Future of bacterial disease management in crop production. *Annual Review of Phytopathology*, *60*(1), 259-282.

Silva, B. N., Cadavez, V., Caleja, C., Pereira, E., Calhelha, R. C., Añibarro-Ortega, M., Gonzales-Barron, U. (2023). Phytochemical composition and bioactive potential of *Melissa officinalis* L., Salvia officinalis L. and *Mentha spicata* L. extracts. *Foods*, *12*(5), 947. https://doi.org/10.3390/foods12050947

Strange, R. N., Scott, P. R. (2005). Plant disease: a threat to global food security. *Annu. Rev. Phytopathol.*, *43*(1), 83-116.

Tudi, M., Daniel Ruan, H., Wang, L., Lyu, J., Sadler, R., Connell, D., Phung, D. T. (2021). Agriculture development, pesticide application and its impact on the environment. *International Journal of Environmental Research and Public Health*, *18*(3), 1112. https://doi.org/10.3390/ijerph18031112

Yahyazadeh, M., Omidbaigi, R., Zare, R., Taheri, H. (2008): Effect of some essential oils on mycelial growth of *Penicillium digitatum* Sacc. *World Journal of Microbiology and Biotechnology,* 24, 1445–1450. https://doi.org/10.1007/s11274-007-9636-8

Yu, H., Pei, J., Qiu, W., Mei, J., Xie, J. (2022). The antimicrobial effect of *Melissa officinalis* L. essential oil on *Vibrio parahaemolyticus*: insights based on the cell membrane and external structure. *Frontiers in Microbiology*, *13*, 812792.

Zöngür, A. (2024). *Asphodelus aestivus* Brot. ve *Nigella sativa* L. esansiyel yağının bazı fungus türlerine karşı antifungal etkisi. *Karaelmas Fen ve Mühendislik Dergisi*, *14*(1), 28-35. DOI: 10.7212/karaelmasfen.1395445

Zöngür, A., Buzpinar, M. A. (2023). AI-assisted antifungal discovery of *Aspergillus parasiticus* and *Aspergillus flavus*: investigating the potential of *Asphodelus aestivus, Beta vulgaris*, and *Morus alba* plant leaf extracts. *Modeling Earth Systems and Environment*, *9*(2), 2745-2756. https://doi.org/10.1007/s40808-022-01658-2