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Comparative determination of enzyme, insecticide, antibacterial activities of different extracts of *Diplotaxis tenuifolia* and chemical components analysis

Diplotaxis tenuifolia'nın farklı ekstraktlarının enzim, insektisit, antibakteriyel aktivitelerinin karşılaştırmalı olarak belirlenmesi ve kimyasal bileşen analizi

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ABSTRACT

This study aimed to evaluate the insecticidal, enzymatic, antimicrobial activities, and phytochemical composition of extracts derived from the flowers and leaves of *Diplotaxis tenuifolia* (DTF and DTL) grown in gypsum-rich soils of Çankırı, utilizing various solvents. LC-MS/MS analysis identified vanillic acid as the major component in the *Diplotaxis tenuifolia* flower ethyl acetate (DTF-EA), *Diplotaxis tenuifolia* flower acetone (DTF-Ace), *Diplotaxis tenuifolia* leaf (DTL-EA), and *Diplotaxis tenuifolia* leaf acetone (DTL-Ace) extracts, naringin in Diplotaxis tenuifolia leaf n-butanol (DTL-nBu), and hesperidin in *Diplotaxis tenuifolia* flower n-butanol (DTF-nBu). The DTF-EA extract showed high efficacy in insecticidal assays against *Sitophilus granarius* and *Rhizopertha dominica*, achieving 90% and 83.3% mortality, respectively. Similarly, the DTF-Ace extract exhibited 86.6% and 66% mortality against *S. granarius* and *R. dominica*, respectively. Enzyme inhibition assays revealed that the DTF-EA extract exhibited potent inhibitory effects against xanthine oxidase (IC_{50}: 27.83 µg/ml) and tyrosinase (IC_{50}: 58.25 µg/ml), surpassing standard inhibitors such as acarbose. In antimicrobial assays, the DTF-EA extract demonstrated broad-spectrum antibacterial activity, exhibiting inhibition zones of 16.5, 15.5, 12.9, and 12.4 mm against *Pseudomonas aeruginosa*, *Listeria monocytogenes, Bacillus cereus*, and *Salmonella enterica,* respectively. The DTL-EA extract displayed significant activity against *Escherichia coli* (13.1 mm), while the DTL-Ace extract was most effective against *Pseudomonas fluorescens* (13.3 mm). These findings suggest that *D. tenuifolia* extracts possess notable antibacterial, insecticidal, and enzyme inhibitory activities, highlighting their potential as natural therapeutic agents and eco-friendly pest control and microbial management alternatives.

INTRODUCTION

Diplotaxis tenuifolia, also known as wild rocket or perennial wall rocket, is a plant species in the Brassicaceae family. It is a perennial herb native to the Mediterranean region and parts of Europe and Asia. It has thin, deeply lobed leaves similar in appearance to a wild rocket, rocket, or salad rocket. It produces clusters of small white or yellow flowers in spring

and summer. The leaves and flowers of wild rocket have a slightly bitter and peppery flavor, making them popular for salads and as a garnish. Wild rocket is easy to grow and is often used as companion plants in vegetable gardens to attract beneficial insects and deter pests. It prefers well-drained soil and full sun to partial shade. The plant is also drought-tolerant and can survive in hot, dry conditions. Wild rocket has a long history of use in traditional medicine as a diuretic, digestive aid, and appetite stimulant. It is also believed to have anti-inflammatory and antioxidant properties. However, more research is needed to confirm these potential health benefits. *Diplotaxis* species have been reported to have various biological activities. Numerous studies show an association between the reduced risk of occurrence of certain cancers (e.g. breast, cervical, prostate, lung, stomach or colon) and increased consumption of these Brassicaceae vegetables (Giovannucci et al. 2003, London et al. 2000, Pignone and Martinez-Laborde 2010, Sapone et al. 2007, van Poppel et al. 1999). *D. tenuifolia* has been used in traditional medicine to treat a variety of ailments, including digestive problems, respiratory infections, and skin conditions (Guarrera 2003, Leporatti and Corradi 2001, Pieroni et al. 2004). Some studies have suggested that *D. tenuifolia* extracts have potential as natural pesticides due to their ability to inhibit the growth of certain insect pests (Tüfekçi 2022). Due to the phytoremediation potential of *D. tenuifolia*, it can absorb heavy metals and other pollutants in the soil and, as a result, help clean up polluted areas (Ozturk et al. 2013).

It is suggested that the degradation products of glucosinolates (GLS) can inhibit carcinogenesis and different stages of disease development. Wild rocket exhibits *in vitro* antioxidant activity, strong anti-inflammatory effects, and significant antibacterial activity against strains such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. It also shows hypoglycemic activity in diabetic rats and a protective effect on liver cells exposed to a liver-damaging chemical, reducing the risk of cardiovascular diseases due to the high levels of flavonoids and phenolic acids (Chun et al. 2017).

Diplotaxis leaves have a sharp flavor. The origin of this flavor is associated with the presence of glucosinolates (GLS). It is a sulfur-containing dimeric 4-mercaptobutyl GLS (glucosativin) compound, which is most likely differentiated from 4-methylthiobutyl GLS (glucoerucin) by the attachment of a methyl group to the sulfur group (Bennett et al. 2006). GLS is present in the fluid contained in the plant's cells. Crushing or heat treatment of any plant tissue facilitates the hydrolysis of these compounds by myrosinase enzyme (Radziejewska-Kubzdela and Olejnik 2016). The breakdown products of these compounds have potentially beneficial health effects associated with their anti-cancer activity. The degradation products of GLS can inhibit carcinogenesis and different stages of disease development. Wild rockets are consumed in many countries in a minimally processed form. Considering the new nutritional diets, it is also used as fruit juice, either directly or by adding it to several fruit juices (Mannozzi et al. 2018).

In this study, aqueous extracts from the plant's above-ground parts (DTF and DTL) were obtained through steam distillation. The aqueous fractions were then extracted sequentially with ethyl acetate, acetone, and n-butanol solvents. The insecticidal activity against storage pests, antibacterial activity against certain bacterial species, and enzyme activity potentials for specific enzymes were determined for the resulting extracts. Aside from our previous study on essential oils, no studies have been reported on the insecticidal, antibacterial, and enzyme activities of extracts obtained from this plant. Therefore, insecticidal, antibacterial, and enzyme activity studies against these harmful species are reported for the first time. Although the chemical profile of *D. tenuifolia* has been mentioned in a limited number of previous studies, the results obtained in this study differ from those reported in the literature.

MATERIALS AND METHODS

Plant materials

Diplotaxis tenuifolia was collected from the interior of Çankırı Karatekin University Uluyazı campus during the flowering period in July-August 2024. Taxonomic identification was performed by Dr. Bilal ŞAHİN from the Food and Agriculture Vocational School of Çankırı Karatekin University. A herbarium specimen of the plant was deposited under the accession number BŞ8208.

Preparation of plant extracts

The flower (DTF, 1 kg) and leaf (DTL, 1 kg) parts of D. tenuifolia were separated. These parts were dried in a cool environment and pulverized with liquid nitrogen. Each plant matrix was boiled at 130 °C for about 3 h using a water vapor distillation technique using a Neo-Clevenger hydro distillation apparatus. The oil and aqueous fraction collected in the water chamber of the Neo-Clevenger were separated. The essential oil was separated. The remaining aqueous fraction was separated from the pulp using cheesecloth and allowed to cool. The cooled water extract was subjected to extraction with the organic solvents ethyl acetate [DTF-EA and DTL-EA] and n-butanol (DTF-nBu and DTL-nBu), respectively. The solvent of n-butanol extracts (DTF-nBu and DTL-nBu) was evaporated, and acetone was added to

the extracts and dissolved with the help of an ultrasonic bath. The dissolved parts were collected in a separate container, and the solvent was evaporated. The acetone-soluble parts were coded as DTF-Ace and DTL-Ace. The insoluble parts in acetone were coded as DTL-nBu and DTF-nBu. An anhydrous dryer was added to remove water in the extracts, which were then dried and filtered. The extracts were stored in dark-colored pine containers at 4 °C until the study (Abay et al. 2015, Tüfekçi et al. 2024).

LC-MS/MS analysis

Qualitative and quantitative analyses of the phenolic compounds contained in DTF and DTL extracts were determined by Agilent brand LC-MS/MS (Agilent Technologies 1260 Infinity II, 6460 Triple Quad Mass spectrometer-United States). In this optimization, a reverse phase Agilent Poroshell 120 SB-C18 (3.0 × 100 mm, I.D., 2.7 µm column) analytical column was used for separation. Approximately 50 mg of *Diplotaxis* extracts were weighed and dissolved with 1 ml of HPLC pure methanol. 100 µl of the methanol phase was taken and diluted by adding 450 µl of water and 450 µl of methanol. The prepared stock solution was filtered through 0.22 µm filters and made ready for analysis. The injection volume was 5.12 µl, and the flow rate was 0.40 ml/min. Water A (0.1% formic acid) and methanol B were used as the mobile phase. The gradient program was 1-3 min for 75% A - 25% B mobile phases, 4-12 min for 50% A-50% B mobile phases, 13-21 min for 10% A-90% B mobile phases, and 22-25 min for 97% A-3% B mobile phases for a total of 25 min. The column temperature was set to 40 °C, capillary voltage 4000 V, N2 gas flow 11 l/min, pressure 15 psi, gas temperature 300 °C (Akman et al. 2024, Gözcü et al. 2024).

Enzyme activity

The capacity of the extracts obtained from *D. tenuifolia* to inhibit the tyrosinase enzyme was determined using the method of Tüfekçi et al. (2023), with minor modifications to the dopachrome method used by Sarikurkcu et al. (Sarikurkcu et al. 2018, Tüfekçi et al. 2023). This method involved preparing stock solutions from the extracts prepared by dissolving in DMSO at a 1:1 ratio. 25 ml of these solutions were taken, 40 ml of tyrosinase solution, and 100 ml of sodium phosphate buffer (pH 6.8) were added and mixed. The mixture was incubated at 25 °C for 15 minutes, and then 40 ml of L-DOPA was added. The mixture was incubated at 25 °C for an additional 10 minutes. Absorbance at 492 nm was measured using a microplate reader 96-well plate (Epoch, BioTek-Vermont, ABD). Each experimental condition was analyzed in triplicate to ensure reproducibility. Scutellarin compound was used as a positive control in order to express the results obtained in a meaningful way. For this, 1 mg of scutellarin compound was weighed and dissolved in 1 ml DMSO, followed by ten times dilution with distilled water. Enzyme activity was tested at five different scutellarin concentrations. According to the positive control result, the extract inhibition activity potentials were determined comparatively. Results are given as % inhibition and activation.

Inhibition activity capacities of extracts against xanthine oxidase (XO) enzyme were determined by modifying the methods previously determined in the literature (Bustanji et al. 2011, Mohammad et al. 2010). Accordingly, the enzyme solution and substrate used during the experiment were prepared immediately before the experimental work. The solution mixture contained 80 mM sodium pyrophosphate buffer (pH=8.5), 0.120 mM xanthine, and 0.1 unit of XO enzyme. The enzyme reaction takes place in the presence of this ternary mixture. Absorbance was measured at 295 nm at 25 °C for uric acid formation. In this way, the initial rate of the reaction was calculated. The extracts and essential oils whose enzyme activity was to be determined were first dissolved in a buffer solution and added to the reaction mixture to determine the inhibitory effect at a concentration of 200 µg/ml. Results are given as % inhibition and activation. IC_{50} value calculations show the Xanthine oxidase and tyrosinase enzyme activity potential. Accordingly, it is expressed as the ratio of the percentage of inhibition against the sample concentration. IC_{50} value is the concentration at which 50% of the enzyme activity is inhibited. The lower the IC_{50} value of the analyzed sample, the stronger the inhibitory activity. Acarbose and Scutellarin compounds were used as a positive standard. The value obtained here will reveal the potential of the extract to be used as an inhibitor of this enzyme.

Insecticidal activity

The flower and leaf extracts of the plant *D. tenuifolia* were dissolved in water with acetone and n-butanol extract, resulting in a 10% stock solution. Flower [DTF-EA, DTF-Ace, DTF-nBu] and leaf [DTL-EA, DTF-Ace, DTL-nBu] extracts of *D. tenuifolia* were dissolved with water-acetone, and 10% stock solutions were prepared. The 10% stock solutions were applied to adult *Sitophilus granarius* and *Rhyzopertha dominica* adults using a micro applicator at a rate of 1 µl per insect (1 µl/insect third larval stage, 2 µl/insect fourth larval stage and 3 µl). The acetone-water solvent was used as the control, and the control group was also treated with 1 µl. The application method was the same as in Karakoç et al. (2013), where the stock solutions were applied with a micro applicator to the ventral abdomen of the insects. Ten beetles were used each time in the experimental replication.

The experimental studies were carried out in 3 replicates, and the average of the three experimental results was taken. The treated insects were transferred to 6 mm diameter Petri dishes containing 10 g of sterilized wheat. The insects in the Petri dishes were incubated at 27± 2 °C, and after 24 hours, the number of dead insects were recorded (Karakoç et al. 2013, Oke Altuntas et al. 2015).

Antibacterial activity

The antibacterial properties of extracts obtained from the *D. tenuifolia* were tested on *Bacillus cereus* (ATCC 10876), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enteritidis* (ATCC 15442), *Listeria monocytogenes* (ATCC 51774), *Salmonella enterica* (ATCC 14028), *Pseudomonas fluorescens* (ATCC 13525) and *Clostridium perfringens* (ATCC 13124). According to the method, 100 µLl of bacteria (10^8 cells/ml) was cultured on a Nutrient Agar medium, and 6 mm sterile blank discs were placed on it. 5 µl of each extract was taken and injected into the blank disk. Imipenem compound was used as a positive control. This standard was added to the antibiotic disc medium. Petri plates were incubated at 37 °C for 24 hours. The diameters of the inhibition zones were measured using a digital caliper. Measurements and experiments were performed in triplicate, and the average diameter in mm was recorded (Gözcü and Akşit 2023, Wayne 1999).

RESULTS

Component analysis of the plant extracts by LC–ESI–MS/MS

As a result of the analysis of the compounds in the DTF-EA, DTF-Ace, DTF-nBu, DTL-EA, DTF-Ace, and DTL-nBu extracts using an MRM-based LC-MS/MS device (Agilent, United States), the components detected in different amounts and undetectable are given in Table 1. The phenolic component analysis of extracts obtained from the flowers and leaves of *Diplotaxis tenuifolia* was quantitatively performed using LC–ESI–MS/MS with a total of 26 standards. The negative mode was chosen for this analysis due to better ionization efficiency. The phenolic component analysis of this plant from this region has been determined for the first time in the present study. In the acetone extract of the leaf portion of *D. tenuifolia* (DTL-Ace), vanillic acid (0.184 mg/g extract) was identified. In contrast, in the ethyl acetate extract (DTL-EA), vanillic acid (0.428 mg/g extract) and naringin (0.373 mg/g extract) were found in significant amounts. In the n-butanol extract (DTL-nBu), naringin (0.495 mg/g extract) was identified as a prominent compound. In the acetone extract of the flower portion of *D. tenuifolia* (DTF-Ace), vanillic acid (3.725 mg/g extract) was the primary compound. In the ethyl acetate extract (DTF-EA), vanillic acid (10.410 mg/g extract) and hesperidin (2.280 mg/g extract) were present in notable quantities. In the n-butanol extract (DTF-nBu), three candidate major components, gallic acid (0.337 mg/g extract), o-coumaric acid (0.474 mg/g extract), and naringin (0.495 mg/g extract) were identified as secondary compounds. As shown in Table 1, different phenolic compounds were identified that varied quantitatively depending on the solvent used. Vanillic acid was found to be a major component, or one of the major components, in all extracts except for the DTL-nBu extract, where the levels of this compound varied. Regarding the number of components, the DTF-nBu extract contained the highest number of components, while the DTF-EA extract contained the highest quantitative amount of phenolic compounds. Regarding secondary components, the DTL-Ace extract contained the fewest components, with 10 different compounds identified.

Insecticidal activity

The results of the contact toxicity of the extracts obtained from different parts of *Diplotaxis tenuifolia* using different solvents against the adult stages of *Sitophilus granarius* and *Rhizopertha dominica* are presented in Table 2. The highest toxicity against *S. granarius* was observed with the DTF-Ace extract, which caused a mortality rate of 86.6%. The DTF-EA extract also showed a significant effect, resulting in a mortality rate of 83.3% in *S. granarius*. The highest toxicity against *R. dominica* was shown by the DTF-EA extract, which resulted in a mortality rate of 90%. The DTL-nBu and DTF-nBu extracts showed negligible contact toxicity against the adult stages of both *S. granarius* and *R. dominica*. In contrast, the DTL-Ace extract showed superior insecticidal activity, causing 56.6% mortality against *R. dominica* (at 48 h) and 60% mortality against *S. granarius* (at 48 h).

Enzyme activity

This study investigated the extracts of *Diplotaxis tenuifolia* inhibitory potential against xanthine oxidase (XO) and tyrosinase enzymes. The results are shown in Table 3. Scutellarin (IC_{50}: 20.38 µg/ml) was used as a standard for tyrosinase enzyme, and acarbose (IC_{50}: 51.40 µg/ml) as a standard for xanthine oxidase enzyme. The strongest inhibition against the tyrosinase enzyme was observed with the extracts of DTF-Ace (IC_{50}: 58.25 µg/ml) and DTL-EA (IC_{50}: 61.89 µg/ml). The highest inhibition against the enzyme xanthine oxidase was observed with the extracts DTF-EA (IC_{50}: 27.83 µg/ml) and DTF-Ace (IC_{50}: 55.47 µg/ml). Although the DTF-nBu extract showed moderate inhibition, it strongly inhibited the tyrosinase and xanthine oxidase enzymes with IC_{50} values of 71.38 µg/ml and 79.81 µg/ml, respectively. In contrast, DTL-nBu and DTL-Ace showed very low inhibition with IC_{50} values of 556.12 µg/ml

Table 1. Analysis of the components from *Diplotaxis tenuifolia* (DT) by LC–MS/MS (mg/g extract)

Compound	RT	Results (mg phenolic/g plant)					
		DTL-Ace	DTL-EA	DTF- nBu	DTL-nBu	DTF-Ace	DTF-EA
Gallic acid	3.19	*nd*	0.174	0.336	0.337	0.004	0.104
Ascorbic acid	3.43	*nd*	0.019	0.076	0.162	*nd*	0.154
Protocatechuic acid	5.53	0.014	0.025	0.137	0.032	0.052	0.087
Gentisic acid	6.81	*nd*	0.004	*nd*	*nd*	*nd*	*nd*
Catechin	6.90	*nd*	0.008	0.015	*nd*	*nd*	0.006
Chlorogenic acid	7.31	*nd*	*nd*	*nd*	*nd*	0.003	0.002
4-Hydroxybenzaldeyde	8.01	0.003	0.076	0.031	0.002	0.010	0.125
Vanillic acid	8.15	0.184	0.428	2.386	*nd*	3.725	10.41
Caffeic acid	8.22	0.005	0.037	0.004	0.001	0.009	0.015
Vanillin	9.13	0.002	0.040	*nd*	0.002	0.004	0.067
o-coumaric acid	9.51	*nd*	*nd*	*nd*	0.474	*nd*	*nd*
Trans-ferulic acid	9.92	*nd*	0.081	0.061	0.004	*nd*	0.065
Naringin	11.42	*nd*	0.373	0.550	0.495	*nd*	0.803
p-coumaric acid	11.56	*nd*	*nd*	0.063	*nd*	*nd*	*nd*
Coumarin	11.43	*nd*	0.023	0.058	0.06	*nd*	*nd*
Hesperidin	11.78	*nd*	0.074	5.801	0.044	*nd*	2.280
Isoquercitrin	11.69	0.001	*nd*	0.045	0.010	0.030	*nd*
Rutin	12.46	0.015	0.007	1.381	0.021	0.593	0.011
Kaempferol-3-glucoside	13.08	*nd*	*nd*	0.009	*nd*	0.010	0.007
Fisetin	13.31	0.002	*nd*	0.002	*nd*	0.002	*nd*
Trans-cinnamic acid	14.29	*nd*	0.153	0.093	*nd*	*nd*	0.901
Quercetin	14.95	*nd*	0.028	0.647	*nd*	*nd*	0.223
Hesperetin	16.27	*nd*	*nd*	*nd*	*nd*	0.003	*nd*
Morin	15.80	*nd*	*nd*	0.001	*nd*	0.002	*nd*
Luteolin	18.06	0.007	*nd*	0.007	0.003	0.005	0.005
Diosgenin	23.28	0.002	*nd*	0.002	*nd*	*nd*	*nd*

Table 2. Insecticidal activity of DTP and DTL extracts (48 h)

Extracts	% Mortality	
	Rhizopertha dominica	***Sitophilus granarius***
Control	0	0
DTL-EA	60	43.3
DTF-EA	90	83.3
DTL-nBu	8	5
DTF-nBu	4	2
DTL-Ace	56.6	60
DTF-Ace	66.6	86.6

and 483.73 µg/ml, respectively. The IC_{50} value of DTF-EA extract against xanthine oxidase enzyme was approximately twice as effective as the acarbose standard, indicating its potential to inhibit this enzyme.

DISCUSSION

In order to determine the content of chemical constituents in different extracts of *D. tenufolia*, LC-MS/MS analysis was carried out in negative ion mode, which is highly sensitive.

Table 3. Enzyme inhibitory activities of DTP and DTL extracts

Extracts	IC_{50}=µg/ml	
	Tyrosinase	Xanthine oxidase
DTL-EA	61.89	358.10
DTF-EA	129.56	27.83
DTL-nBu	298.77	556.12
DTF-nBu	71.38	79.81
DTL-Ace	161.20	483.73
DTF-Ace	58.25	55.47
Acarbose (Standart)	-	51.40
Scutellarin (Standart)	20.38	-

Antibacterial activity

Results of the antibacterial activity test of *Diplotaxis tenuifolia* extract samples using the disk diffusion method are presented in Table 4. The antibacterial activity of the *D. tenuifolia* extracts was tested against Gram-negative and Gram-positive bacteria. All extracts (10 µg) showed moderate-to-low antibacterial effects against *Clostridium perfringens* while varying moderate-to-high antibacterial activity was observed against other bacterial strains. Among the extracts, the DTF-EA extract (with zone diameters ranging from 10.5 mm to 16.5 mm) showed moderate to high levels of activity. The DTF-EA extract, with 16.5 and 15.5 mm zone diameters, showed very high antibacterial activity against *Pseudomonas aeruginosa* and *Listeria monocytogenes*, respectively. Only the extracts of DTF-EA and DTL-EA showed a high activity level against *Escherichia coli.* It is suggested that these effects may be due to the specific compounds extracted by this method.

LC-MS/MS analysis and isolation of compounds from *D. tenuifolia* revealed a high presence of quercetin, along with other compounds such as chlorogenic acid, rutin, luteolin, tamarixetin, isorhamnetin-3-O-glucoside, quercetin-3-O-galactoside, quercetin-3-O-glucoside, rutin, kaempferol-3-O-rutinoside, isorhamnetin 3-O-glucoside, β-sitosterol, sinapine, and 4-mercaptobutylglucosinolate, which have been previously reported in this species (Bennett et al. 2006, Giovenzana et al. 2014, Martinez-Sanchez et al. 2007, Normen et al. 1999). In addition, secondary metabolites have also been identified in certain *Diplotaxis* species. In one of these studies, indole alkaloids and triterpenoids were detected exclusively in the flowers of *D. simplex*, whereas oxylipins were found only in the aerial parts of *D. erucoides* (Jdir et al. 2017, Loizzo et al. 2021). According to our LC-MS/MS analysis results, no prior studies have been found in the literature analyzing the flower portion of this species. Additionally, no research has been conducted on the

Table 4. Antibacterial analysis results of DTP and DTL extracts aganist 8 microorganisms

Microorganism	Sample name /Inhibüsyon zone çapı (mm)					
	DTF-EA	DTF-Ace	DTF-nBu	DTL-EA	DTL-Ace	DTL-nBu
Bacillus cereus	12.9	11.0	7.5	11.2	7.5	7.0
Escherichia coli	12.7	10.0	10.9	13.1	8.8	10.7
Pseudomonas aeruginosa	16.5	9.4	5.2	10.8	8.9	10.7
Salmonella enteritidis	13.2	9.4	11.3	11.0	6.0	9.3
Listeria monocytogenes	15.5	8.9	8.8	11.1	10.6	7.0
Salmonella enterica	12.4	10.2	10.7	5.04	9.6	10.6
Pseudomonas fluorescens	10.6	9.7	11.8	4.69	13.3	11.5
Clostridium perfringens	10.5	8.0	4.3	10.5	7.0	7.1

presence of the main components identified in this study in this species.

Glucosinolate derivatives (GLS) are present in *Diplotaxis* species, including Brassica species from the Brassicaceae family. These GLSs are hydrolyzed in the body into isothiocyanate products. These hydrolysis products have been reported to possess various biocidal properties, including insecticidal, nematicidal, fungicidal, and antibiotic activities (Kirkegaard et al. 1998). In one study, 100% mortality rates were achieved on adult American cockroaches (*Periplaneta americana*) after 24 and 48 hours of exposure to the volatile oil of *Brassica nigra* and its main component, allyl isothiocyanate (Yıkınç and Tunaz 2023, Yılmaz and Tunaz 2013). Another study demonstrated that the volatile oils of *B. nigra* exhibited high insecticidal activity against adult *Blatella germanica* (Aydın 2020). A literature review found no studies on the insecticidal activity of *Diplotaxis* species. In our study, DTF-EA and DTF-Ace extracts contained high concentrations of vanillic acid, hesperidin, and naringin as major components. Vanillic acid enhances reactive oxygen species (ROS), which can disrupt cellular metabolism, while hesperidin and naringin have neurotoxic effects that may impair the insect nervous system. The DTF-EA extract exhibited the highest insecticidal activity against *R. dominica* (90%) and *S. granarius* (83.3%), which corresponds to its high levels of vanillic acid (10.41 mg/g) and hesperidin (2.280 mg/g). However, the higher insecticidal activity of DTF-EA compared to the values reported in the literature for similar extracts suggests a synergistic effect between vanillic acid and hesperidin. In contrast, the DTL-nBu extract, containing negligible amounts of these compounds, demonstrated minimal insecticidal effects, highlighting the crucial role of phenolic constituents. As a result, this study demonstrates the potential use of *D. tenuifolia* plant extracts as a natural source for pest control in agricultural practices due to their significant insecticidal effects on adult *S. granarius* and *R. dominica*.

In literature reviews, it has been found that the methanol extract of the leaf part of *D. tenuifolia* exhibits inhibitory effects with an IC_{50} value of 1.210 µg/ml (28%) against glutathione S-transferase (GST), an IC_{50} value of 0.14 µg/ml (18%) against glutathione peroxidase (GPx), and an IC_{50} value of 11.90 µg/ml (10%) against catalase enzyme (Güneş 2014, Villani et al. 2023). On the other hand, the ethanol extract of the flower part of *D. tenuifolia* showed pancreatic lipase enzyme inhibition with an IC_{50} value of 7.76 mg/ml (Conforti et al. 2012). In one study, the essential oil (EO) from the root of *Eruca vesicaria*, considered the most important part of the plant, exhibited high inhibitory activity against α-amylase (IC_{50}= 0.80 µg/ml) and α-glucosidase (IC_{50}= 0.11 µg/ml) enzymes (Hichri et al. 2019). In another study, the flower and seed extracts of *D. simplex* and *D. harra* demonstrated significant acetylcholinesterase inhibition (IC_{50}= 0.42 mg/ml and IC_{50}= 0.72 mg/ml) activity (Bahloul et al. 2016). Furthermore, another study reported the inhibitory effects of the ethanol extract of *Diplotaxis harra* subsp. *crassifolia,* particularly on α-amylase, α-glucosidase, and pancreatic lipase enzymes (Badalamenti et al. 2024). Our results confirm that DTF-EA, DTF-Ace, and DTL-EA extracts from *D. tenufolia* exhibit high activity against tyrosinase and xanthine oxidase enzymes. Considering the literature studies, it is evident that the phenolic content presented in Table 3 is directly related to the activity. In XO inhibition, the DTF-EA extract exhibited a stronger inhibitory effect (IC_{50}= 27.83 µg/ml) compared to the standard acarbose (IC_{50}= 51.40 µg/ml). This effect can be attributed to the high levels of vanillic acid and hesperidin, both of which are potent XO inhibitors capable of chelating enzyme cofactors. In terms of tyrosinase inhibition, the DTF-Ace extract demonstrated the highest inhibitory activity (IC_{50}= 58.25 µg/ml), which correlates with its vanillic acid content (3.725 mg/g extract). This finding aligns with literature reports indicating that phenolic acids act as competitive inhibitors by binding to the active site of tyrosinase. In contrast, the DTL-nBu extract, which contains lower levels of phenolic acids and flavonoids, exhibited significantly weaker enzyme inhibition (IC_{50}= 556.12 µg/ml for XO and IC_{50}= 298.77 µg/ml for tyrosinase), highlighting the crucial role of these bioactive compounds in enzymatic inhibition.

In previous studies, the ethanol extract of the flower part of *D. virgata* was fractionated with n-butanol, and isolated flavonol derivatives were investigated for their antibacterial activities against both Gram-positive (*Listeria monocytogenes* and *Staphylococcus aureus*) and Gram-negative bacteria (*Aeromonas hydrophila*, P*seudomonas aeruginosa*, *Salmonella enteritidis*, *Escherichia coli*, and *Klebsiella pneumoniae*) using the disk diffusion method. The n-butanol fraction exhibited the highest antibacterial effects, with inhibition zones of 20.00 mm against *L. monocytogenes*, 19.04 mm against *S. aureus*, 22.03 mm against *E. coli*, and 21.00 mm against *K. pneumoniae* (Ben Salah et al. 2015). In another study, n-butanol fractions obtained from the aerial parts of *D. erucoides* showed high activity against *L. monocytogenes* (16.02 mm), *S. aureus* (20.04 mm), and *A. hydrophila* (21.07 mm). Moreover, a flavonol compound (compound 27) isolated from this fraction demonstrated strong antibacterial effects, with inhibition zones of 14.50 mm against *L. monocytogenes,* 13.00 mm against *S. aureus,* and 17.60 mm against *E. coli* (Ressurreiçao et al. 2024, Selma et al. 2010). Additionally, another study reported that the methanol extract of the flower part of *D. harra*

exhibited significant inhibition against *P. aeruginosa* (34%), *E. coli* (80%), and *M. luteus* (60%) (Falleh et al. 2013). The antimicrobial activity of the extracts presented in Table 4 was found to be directly associated with their phenolic and flavonoid contents. The DTF-EA extract exhibited significant effects against the Gram-negative bacteria *Pseudomonas aeruginosa* (16.5 mm) and *Escherichia coli* (12.7 mm). This activity can be attributed to the synergistic effects of vanillic acid, naringin, and hesperidin, which are known to disrupt bacterial membranes and inhibit quorum-sensing mechanisms. Similarly, the DTF-EA extract demonstrated notable inhibition against the Gram-positive bacteria *Listeria monocytogenes* (15.5 mm) and *Bacillus cereus* (12.9 mm), consistent with the antibacterial properties of vanillic acid and hesperidin reported in previous studies. In contrast, the DTL-nBu extract exhibited lower activity against all tested bacterial species. This reduced activity can be explained by the lower concentrations of key components. For instance, the low hesperidin content (0.044 mg/g extract) corresponds to the weaker inhibition zones observed for *Pseudomonas aeruginosa* (10.7 mm) and *Escherichia coli* (10.7 mm). The results demonstrate that the DTF-EA, DTL-Ace, and DTL-EA extracts used in the selective study exhibited promising effects against the examined bacteria.

The present study analyzed the chemical compositions of different extracts obtained from *D. tenuifolia* using different solvents using LC-MS/MS. These extracts were evaluated for their potential insecticidal, antimicrobial, and enzymatic activities. The biological activities of *Diplotaxis tenuifolia* extracts are strongly influenced by their phenolic and flavonoid compositions. High concentrations of vanillic acid, hesperidin, and naringin in DTF-EA and DTF-Ace correlate with their superior insecticidal, enzyme inhibitory, and antimicrobial activities. These findings underscore the potential of *D. tenuifolia* as a source of bioactive compounds for therapeutic and agricultural applications. *D. tenuifolia* is widely distributed in the soils of Çankırı, which is usually considered invasive and neglected. The data from this research and the biological activity studies represent novel findings. No previous reports have been published concerning these aspects of this plant species. This natural resource has significant potential for treating various diseases and controlling agricultural pests due to its efficacy against Gram-positive and Gram-negative microorganisms, high insecticidal activity, and strong inhibitory effects on enzymes.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Bu çalışma, Çankırı'nın jips bakımından zengin topraklarında yetiştirilen *Diplotaxis tenuifolia* bitkisinin çiçek (DTF) ve yaprak (DTL) organlarından; etil asetat, aseton ve n-bütanol gibi farklı çözücüler kullanılarak elde edilen ekstraktların insektisit, enzimatik ve antimikrobiyal aktiviteleri ile fitokimyasal profillerini değerlendirmeyi amaçlamıştır. LC-MS/MS analizi sonucunda, DTF-EA (çiçek etil asetat), DTF-Ace (çiçek aseton), DTL-EA (yaprak etil asetat) ve DTL-Ace (yaprak aseton) ekstraktlarında vanilik asit; DTL-nBu (yaprak n-bütanol) ekstraktında narengin; DTF-nBu (çiçek n-bütanol) ekstraktında ise hesperidin başlıca bileşikler olarak belirlenmiştir. DTF-EA ekstraktı, *Sitophilus granarius* ve *Rhizopertha dominica* üzerinde sırasıyla %90 ve %83.3 mortaliteye ulaşarak yüksek insektisit etki göstermiş; DTF-Ace ekstraktı da bu türlere karşı %86.6 ve %66 oranında toksisite sergilemiştir. Enzim inhibisyon testlerinde, DTF-EA ekstraktı ksantin oksidaz (IC_{50}: 27.83 µg/ml) ve tirozinaz (IC_{50}: 58.25 µg/ml) enzimlerine karşı güçlü inhibitör etkiler göstererek akarbos gibi standart inhibitörlerin aktivitesini aşmıştır. Antibakteriyel deneylerde ise DTF-EA ekstraktı, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Bacillus cereus* ve *Salmonella enterica* türlerine karşı sırasıyla 16.5; 15.5; 12.9 ve 12.4 mm'lik inhibisyon zonları oluşturarak geniş spektrumlu aktivite ortaya koymuştur. DTL-EA ekstraktı *Escherichia coli*'ye karşı 13.1 mm; DTL-Ace ekstraktı ise *Pseudomonas fluorescens*'e karşı 13.3 mm'lik en yüksek inhibisyon alanına ulaşmıştır. Bu bulgular, *D. tenuifolia* ekstraktlarının kayda değer antibakteriyel, böcek öldürücü ve enzim inhibitör aktivitelere sahip olduğunu göstermekte ve doğal terapötik ajanlar ve çevre dostu haşere kontrolü ve mikrobiyal yönetim alternatifleri olarak potansiyellerini vurgulamaktadır.

Anahtar kelimeler: *Diplotaxis tenuifolia*, enzim aktivite, fenolik bileşikler, insektisidal aktivite, antibakteriyel aktivite

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