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Chemical composition, antifungal, antibacterial, and insecticidal activity of *Echinophora chrysantha* essential oil

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Abstract

This study presents an investigation of the chemical composition and biological activities of the essential oil (EO) extracted from aerial parts of *Echinophora chrysantha* (EC). Gas chromatography-mass spectrometry (GC-MS) analysis revealed the presence of twenty-two components, representing 97.5% of the total oil. Major constituents were determined as α -phellandrene (55.74%), β -phellandrene (17.41%), and *p*-cymene (8.95%). The EO was screened for antimicrobial and antifungal activity against a panel of bacteria and fungi using a broth micro-dilution and fumigant activity assay, respectively. EO sample exhibited better activity against *Listeria monocytogenes*, *Staphylococcus aureus* *Enterococcus faecium* with 31.25, 31.25, and 62.5 μ g/mL MIC values. EOs showed moderate mycelium growth inhibitory activity against *Phytophthora infestans* (32.9 \pm 1.9%), *Verticillium dahliae* (42.3 \pm 1.8%), and *Fusarium oxysporum* f. sp. *lycopersici* (53.0 \pm 2.9%) at 8 μ L/petri concentration. The insecticidal activity of EO was also evaluated against two insects (*Rhyzopertha dominica* and *Tribolium confusum*) showing moderate activity with mortality rates of 25.8 \pm 0.6% and 37.9 \pm 1.2% at a 5% (v/v) concentration. Literature search has not revealed any previous work on the antimicrobial, antifungal, and insecticidal activities of the EO from *E. chrysantha*. In conclusion, this is the first report describing the biological activities of this plant EOs. Additionally, these findings highlight the practical implications of the utilizing essential oil of EC as a potential natural remedy against a spectrum of microbial threats in both pharmaceutical and food preservation applications.

Keywords

Echinophora chrysantha, Essential oil, Biological activity

INTRODUCTION

The *Echinophora* genus represented 6 species, three of them endemic to the flora of Türkiye¹. The *Echinophora* species are edible and used in folk medicine as an appetite enhancer, digestive regulator, and pain reliever in colds by infusion and decoction of herbs in Türkiye². Previous phytochemical studies on *Echinophora* species have revealed the presence of flavonoids, steroids, and organic acids with a wide range of biological activities, including antimicrobial, antiproliferative, and antioxidant.

The introduction of multidrug-resistant bacteria and fungi makes treating illnesses difficult. As a result, there is an urgent need to discover novel antibacterial and antifungal compounds for use in the struggle against these pathogens. Furthermore, the antibacterial and/or antifungal characteristics of EOs have drawn the attention of the food, pharmaceutical, and cosmetic sectors since the use of natural additives has gained prominence as a trend in the replacement of conventional synthetic preservatives. In addition, the EOs have a wide

range of insecticidal effects. Insects negatively impact stored grain affecting food quality, quantity, and safety. The stored grains are lost up to 20-60 percent by insects during storage³. Post-harvest deterioration caused by insects results in economic losses owing to negative changes in odor, quality, texture, and nutritional characteristics. It is important to investigate the potential of using natural essential oils due to volatility and biodegradability as insecticidal since synthetic insecticides are restricted due to customer demands and negative effects on human health.

E. chrysantha, locally known as “Yıldız çördüğü”, is one of the Türkiye endemic species which grown naturally in eastern provinces of the country. The plant is a perennial herb that typically grows 10-30 cm tall. The stems are slender and hairless, often branching near the base. The leaves are finely divided, bipinnate, and have narrow linear segments with a bright green color arranged alternately on the stem. *E. chrysantha* is traditionally used as infusions for gastric ulcers and as food additives for seasoning and flavoring soups and meatballs by the local communities. The previous record by our group reported that the EC extract contains flavonoids and organic acids and has antioxidant activity and remarkable antiproliferative activity against HeLa cell lines with low cytotoxicity toward normal cell lines⁴. GC and GC-MS analysis of essential oils obtained from aerial parts of EC have been reported previously. The major compounds of EC were α -phellandrene (61.06%), *p*-cymene (6.76%), β -phellandrene (7.17%), and α -terpinolene (3.69%). In addition, the chemical composition, and the bioactivity of EOs of other species of the *Echinophora* genus such as antibacterial^{5,6}, antifungal^{7,8}, and insecticidal⁹⁻¹¹ activities were well-studied. However, to the best of our knowledge, the antimicrobial, antifungal, and insecticidal activities of the essential oil of EC have not been studied yet. Therefore, the current study aimed to identify the chemical composition of *Echinophora chrysantha* EOs, collected from Erzincan, Türkiye, based on GC-MS analysis and evaluate the antibacterial, antifungal, and insecticidal activities.

MATERIALS AND METHODS

Plant materials

The aerial parts of *E. chrysantha* (3 kg) were collected from Pöske Mountain, 8th-kilometer Erzincan-Kelkit Road, southern slopes in June 2021 during the inflorescence period (39°50'49.9"N, 39°22'24.0"E). The samples were authenticated by Prof. Dr. Ali Kandemir (Erzincan Binali Yıldırım University, Faculty of Art and Science, Biology Dept.). A voucher specimen was deposited at EBYU Herbarium (AKSIT-2021-12).

Isolation of volatile components

The aerial parts of plant material were freshly used for essential oil isolation. The fresh aerial parts (400 g) were cut into small pieces and subjected to hydrodistillation using a Neo-Clevenger type apparatus in 2-liter deionized water and maintained for 2 hours as described previously¹². The hydrodistillation process was carried out in triplicate. Decanted and dried over Na₂SO₄ colorless essential oils were kept at +4°C in sealed dark vials.

GC-MS analysis of essential oil

GC-MS analyses were performed using a Thermo Scientific GC-MS instrument coupled with a Thermo Trace 1310 MS detector and an HP-5MS capillary column (30 m x 0.25 mm and 0.25 μ m). The oven temperature was programmed at 60°C for 3 min, ramped to 200°C at 3°C /min, then raised to 240°C at 5°C/min, and maintained for 5 min. The helium was used as a carrier gas in split mode by 50:1 with 1.2 mL/min flow rate. All heated site temperatures (injection site, mass transfer line, and ion source) were set at 280°C. The MS spectra were recorded in the full-scan mode in the range of 40-240 amu at 70 eV according to the previously published method¹³. The retention indexes (RI) were compared with the previous literature for the confirm compounds¹⁴⁻¹⁹. The relative percentages of each compound were calculated normalizing the peak area obtained from the FID chromatogram without using any correction factor.

Antifungal activity

In vitro antifungal activity of the EO was tested for its antifungal activities against three pathogenic fungi of *Phytophthora infestans*, *Verticillium dahliae*, and *Fusarium oxysporum* f. sp. *lycopersici*¹³. Fungus cultures were developed for 7 days at 25±2°C in 90 mm petri dishes containing 20 mL potato dextrose agar (PDA). Essential oil was transferred with a micropipette at a dose of 0 (Control), 1, 2, 4, and 8 µL/petri on filter papers adhered to the lids of the petri dishes. Fungus cultures were incubated for 7 days at 25±2°C. The mycelium growth inhibition rate was calculated by following the formula;

$$\text{Inhibition rate (\%)} = (dc - dt)/dc \times 100,$$

where dc is the mean diameter of mycelium growth in mm of the test material, and dt is the mean diameter of mycelium growth in mm of the test material.

Antibacterial activity

The broth micro-dilution technique, reported by Gözcü et al.²⁰ was utilized in an antimicrobial test to determine the antibacterial characteristics of essential oil of EC against five gram-positive: *Staphylococcus aureus* (ATCC 6538), *Listeria monocytogenes* (ATCC 51774), *Bacillus cereus* (ATCC 10876), *Clostridium perfringens* (ATCC 13124), *Enterococcus faecium* (ATCC 8459), and as well as five gram-negative; *Pseudomonas fluorescens* (ATCC 13525), *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enterica* (ATCC 15442), *Escherichia coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 13076). The results were expressed as MIC (minimal inhibition concentration) in µg/mL. Tetracycline was used as antibiotic standard.

Insecticidal activity

Cultivation of insect cultures

The insect cultures used in the experiment were obtained from the Plant Protection Central Research Institute (Türkiye) stock cultures. A nutrient mixture consisting of soft breadcrumbs and dry yeast (*Saccharomyces cerevisiae*) was used in the cultivation of *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) and *Tribolium confusum* Jacquelin du Val

(Coleoptera: Tenebrionidae). The wheat used in the cultivation of insects was ground to a coarse size in a crusher and then kept in a deep freezer at -18°C for 72 hours to eliminate the risk of possible contamination. Dry yeast was ground in a laboratory mill and mixed with 5% of cracked wheat. Adults aged 7-14 days were used in the studies. To obtain adults at the desired age, adult emergence was followed daily for approximately one week after the eggs were taken into the food jar.

Contact toxicity bioassay

The contact toxicity of essential oil of EC was conducted according to Alkan et al.¹⁴. Briefly, 1 µL of diluted essential oil with acetone in 1%, 2%, and 5% (v/v) concentrations were applied to the dorsal surface of the thorax using a micro-applicator. 1 µL of acetone was applied to the control group. The insects were transferred to 25 mL glass tubes containing approximately 10 g of cracked wheat and yeast mixture. The dead individuals were recorded after 24 and 48 hours. These tubes were kept in the incubator at 27±2°C under 65% relative humidity conditions. The experiment was set up in a randomized block design with three replications and two parallels.

Statistical analysis

The obtained data were analyzed by analysis of variance and Tukey multiple comparison test. All statistical analyzes were carried out with the help of the MINITAB Release 18 package program.

RESULTS AND DISCUSSION

Composition of essential oil of EC analyzed by GC-MS

The essential oil was yielded by 1.5% and obtained as a colorless liquid. The GC-MS profiling of the EC essential oil led to the identification of 22 components representing 97.68% of the total oil that is rich in monoterpenes (95.62%) (Table 1). α-Phellandrene (54.74%), p-cymene (8.95%), β-phellandrene (17.41%), and α-terpinolene (6.52%) were found as major constituents of essential oil, supporting the previous report^{21,22} with the small qualitative differences attributed to environmental conditions, harvest period,

Table 1. The chemical composition of *E. chrysantha* essential oil

Comp. No.	RT	RI*	RI lit	Component	% Composition**
1	6.05	929	931	Thujene	1.08±0.1
2	6.26	939	935	α-Pinene	4.36±0.0
3	6.73	954	951	Camphene	0.06±0.0
4	7.55	975	977	Sabinene	0.64±0.0
5	7.66	981	986	β-Pinene	0.38±0.0
6	8.16	993	991	α-Myrcene	0.57±0.0
7	8.75	1005	1005	α-Phellandrene	54.74±0.6
8	8.89	1010	1011	3-Carene	0.38±0.1
9	9.11	1018	1018	α-Terpinene	0.10±0.01
10	9.42	1024	1027	p-Cymene	8.95±0.1
11	9.61	1032	1034	β-Phellandrene	17.41±0.9
12	9.95	1048	1050	cis-Ocimene	0.07±0.0
13	12.01	1084	1088	α-Terpinolene	6.52±0.2
14	12.68	1099	1102	Nonanal	0.21±0.1
15	12.97	1130	1130	Cosmene	0.36±0.1
16	15.05	1162	1160	Borneol	0.49±0.1
17	15.83	1177	1180	4-Terpineol	0.07±0.0
18	16.89	1256	1252	Sabinyl acetate	0.07±0.0
19	28.61	1473	1477	Germacrene-D	0.67±0.0
20	29.24	1507	1509	α-Farnesene	0.06±0.0
21	30.22	1522	1520	Myristicin	0.39±0.1
22	31.56	1565	1560	Globulol	0.09±0.0
Monoterpenes (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 15)					95.62
Oxygenated monoterpenes (16, 17 and 18)					0.14
Sesquiterpenes (19 and 20)					0.73
Oxygenated sesquiterpenes (21, 22)					0.48
Total identified					97.68

*RI, retention index as determined on an HP-5MS column using the homologous series of n-hydrocarbons; ** Percent compositions were expressed as mean ± standard deviation of three independent measures

and other climatic factors. After a detailed literature review, no *Echinophora* species was found containing both α- and β-phellandrene predominantly except *E. cinerea* collected from Iran^{23,24}. Therefore, the co-occurrence of α- and β-phellandrenes has chemotaxonomic importance for EC. Despite the chemical composition of EC essential oils being documented by only two records^{21,22}, the biological activities have not been evaluated. In this study, the antifungal, antibacterial, and insecticidal activity of EC essential oil was first reported.

Antifungal activity of essential oil

The antifungal effect of EC essential oil on plant pathogens is given in Table 2. It was determined that the essential oil of EC had a statistically significant antifungal effect on the tested plant pathogenic fungi. Different effects on mycelium growth were observed depending on the fungi cultures and application dose. EC essential oil reduced the mycelium growth of FORL compared to the control, and the highest effect was seen at 8 μL/Petri dose. However, similar results prevented the mycelial growth of *P. infestans* and

V. dahliae pathogens at different rates depending on the dose. The 8 µL/Petri dose inhibited the mycelial growth of *P. infestans* and *V. dahliae* by 32.9% and 41.3%, respectively. *P. infestans* was determined as the most tolerant pathogen to EC essential oil, and the lowest mycelium growth was seen in this fungus only at the highest dose.

The LC₅₀ doses for the EO against test microorganisms are given in Table 3. The LC₅₀ values of the essential oil against FORL, *V. dahliae*, and *P. infestans* as 7.3, 8.8, and 10.6 µL/Petri dish, respectively. It was observed that FORL showed the highest sensitivity to essential oil, while *P. infestans* showed the greatest resistance when compared LC₅₀ values. However, further research is needed to utilize the potential of EC essential oils as a biocontrol method for the above-mentioned plant pathogens.

Despite the essential oil of EC was not assessed for antifungal activity on phytopathogenic fungi some other *Echinophora* species were tested previously. For example, *E. platyloba* essential oil containing *p*-cymene, α -pinene, β -phellandrene, and α -phellandrene as major constituent was reported to display great potential for antifungal activity inhibiting mycelial growth of phytopathogenic fungi including *Alternaria alternata*, *Culvularia fallax*, *Macrophomina*

phaseolina, *Fusarium oxysporum*, *Cytospora sacchari*, and *Colletotrichum tricbellum*⁷. The Turkish pickling herb (*E. tenuifolia* subsp. *sibthorpiana*) essential oil was reported as a potential mycelial growth inhibitor of *Alternaria alternata* fungus cultures²⁵.

Antimicrobial activity of essential oil

Several studies have summarized the relationship between MIC and microbial action as follows: the antibacterial activity of an extract or plant-derived essential oil was regarded to be good if the MIC was less than 100 µg/mL; moderate if it was between 100 and 500 µg/mL; weak if it was between 500 and 1000 µg/mL; and inactive if it was over 1000 µg/mL²⁶⁻²⁸. Based on the results given in Table 4, the most sensitive tested microorganisms were *L. monocytogenes* and *S. aureus* with a MIC value of 31.25 µg/mL, and *E. faecium* with 62.5 µg/mL MIC value. The EC essential oil was found moderately active against *B. cereus*, *S. enterica*, and *C. perfringens* with a 125 µg/mL MIC concentration. On the other hand, it was determined that essential oil showed weak antibacterial activity against *P. fluorescence*, *P. aeruginosa*, and *E. coli* at a concentration greater than 1000 µg/mL. Generally, the essential oil of EC was found to be more active against gram-

Table 2. Antifungal activity of *Echinophora chrysantha* essential oil (percent growth inhibitory)

Doses (µL/petri)	<i>Phytophthora infestans</i> *	<i>Verticillium dahliae</i>	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>
Control	0.0±0.0 ^c	0.0±0.0 ^c	0.0±0.0 ^c
1	0.0±0.0 ^c	0.0±0.0 ^c	0.0±0.0 ^c
2	0.0±0.0 ^c	0.0±0.0 ^c	5.3±3.9 ^c
4	14.6±2.5 ^b	20.8±1.2 ^b	24.5±1.9 ^b
8	32.9±1.9 ^a	41.3±1.8 ^a	53.0±2.9 ^a

*Percent growth inhibitions were expressed as mean ± standard deviation of three independent measures; ^{a-c} Values presented by the same letter within same columns are not statistically different ($p < 0.05$)

Table 3. LC₅₀ values of essential oil on plant pathogenic fungi

	<i>P. infestans</i>	<i>V. dahliae</i>	<i>F. oxysporum</i> f. sp. <i>lycopersici</i>
LC ₅₀ (µL/Petri dish)	10.6	8.8	7.3
Slope	3.0±0.5	3.1±0.4	2.9±0.3
Chi-square	4.3	6.8	1.1

Table 4. Antimicrobial activity of *Echinophora chrysantha* essential oil

Microorganisms	Essential oil MIC (µg/mL)	Tetracycline MIC (µg/mL)
Gram-negative		
<i>Pseudomonas fluorescence</i>	>1000	3.91
<i>Pseudomonas aeruginosa</i>	>1000	7.81
<i>Escherichia coli</i>	>1000	7.81
<i>Salmonella enterica</i>	125	3.91
<i>Salmonella enteritidis</i>	250	3.91
Gram-positive		
<i>Listeria monocytogenes</i>	31.25	3.91
<i>Staphylococcus aureus</i>	31.25	1.95
<i>Bacillus cereus</i>	125	7.81
<i>Clostridium perfringens</i>	125	3.91
<i>Enterococcus faecium</i>	62.5	1.95

positive strains than gram-negative ones. The observed antimicrobial activity of essential oils of EC agreed with previous reports that essential oils of the other *Echinophora* species selectively inhibited the growth of different microorganisms^{5,6,29,30}. The antimicrobial activity of the essential oil can be attributed to the major compounds reported above, as well as to its synergistic effect on other minor compounds. It is often difficult to define the mechanism of action of essential oils due to their complexity, but it is very important to highlight that chemical diversity is a good feature that can limit the development of microbial resistance.

Insecticidal activity of essential oil

EC essential oil showed contact activity against *Rhyzopertha dominica* at varying rates depending on the application dose and incubation time (Table 5). At the end of the 24th hour, the highest activity was observed at the 5% (v/v) concentration of the essential oil with a mortality rate of 16.1% (F=17; df=3.3; p<0.05). The mortality rate at this concentration was statistically separated from other application doses. At 48 hours, most EC essential oils had a contact activity of 25.8% (F=31.2; df=3.3; p<0.05) at the 5% (v/v) concentration. Plant essential oil showed varying levels of activity

Table 5. Insecticidal activities of EC essential oils against *Rhyzopertha dominica* and *Tribolium confusum*

Essential oil	Concentration (% v/v)	Mortality (%)*	
		24 HAT	48 HAT
<i>Rhyzopertha dominica</i>	1	0.1±0.4 ^{cd}	0.1±0.4 ^d
	2	2.5±1.2 ^c	15.6±1.0 ^c
	5	16.1±0.3 ^b	25.8±0.6 ^{bc}
<i>Tribolium confusum</i>	1	12.5±0.9 ^a	13.4±1.0 ^a
	2	22.7±0.4 ^a	27.1±0.5 ^a
	5	31.4±0.7 ^a	37.9±1.2 ^a
Control	Acetone	0.0±0.0 ^d	0.0±0.0 ^d

* Percent mortality values were expressed as mean ± standard deviation of three independent measures; ^{a-d} Values presented by the same letter within same columns are not statistically different (p < 0.05); HAT: Hours after treatment

against the *Tribolium confusum* (Table 5). EC essential oil showed the highest activity of 37.9% at the end of 48 hours and had the highest activity in this period ($F=22.0$; $df=3.3$; $p<0.05$). *Tribolium confusum* was found to be more sensitive to EC essential oil than *Rhyzopertha dominica*.

When the literature is examined in terms of insecticidal activity; many reports exist that attempt usage of *Echinophora* essential oils against some insects to find eco-friendly and safe active ingredients for the preparation of green insecticides. For example, *Echinophora orientalis* essential oil was tested against *Tribolium castaneum* by Delazar et al.³¹ and a notable insecticidal activity was not observed. On the other hand, the myristicin and terpinolene-dominated root essential oil of *Echinophora spinosa* exhibited promising toxicity against *Culex quinquefasciatus*, *Spodoptera littoralis*, and *Musca domestica*⁹. It was reported that the *E. platiloba* essential oil showed insecticidal activity against *Tribolium castaneum*, *Callosobruchus maculatus*, and *Rhyzopertha dominica* with increasing mortality depending on dose and exposure time¹⁰.

CONCLUSION

In conclusion, the essential oil was found to be rich in monoterpenes including, α -phellandrene, β -phellandrene, *p*-cymene, and α -terpinolene according to GC-MS analysis. The antimicrobial activity of essential oil *Echinophora chrysantha*, especially towards *L. monocytogenes*, *S. aureus*, and *E. faecium*, highlights its usefulness in traditional medicine for the treatment of wounds or disorders caused by gram-positive bacterial strains. The essential oil was also found to be a mycelium growth inhibitor for tested phytopathogenic fungi, which can be used to develop eco-friendly, green antifungal agents. The insecticidal activity of essential oil was found moderately active on the tested insects. This study has demonstrated potential applications against bacteria, fungi, and insects of *E. chrysantha* EOs although further pharmacological and phytochemical studies are needed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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