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ZIZIPHORA CAPITATA L. ETHANOLIC EXTRACT: EVALUATION OF ANTIOXIDANT PROPERTIES, ENZYME INHIBITION, GENOTOXIC AND ANTIGENOTOXIC ACTIVITIES

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Abstract

Ziziphora capitata L. is used in traditional medicine. The objective of this study was to assess the antioxidant capacity, total phenolic content, elastase, collagenase, and hyaluronidase inhibition, as well as the genotoxic and antigenotoxic properties of the ethanol extract obtained from the aerial parts of *Z. capitata*. The antioxidant activity was evaluated using DPPH and β-carotene bleaching assays, which revealed moderate radical scavenging effects with IC_{50} values of 200 ± 1.13 µg/ml and 138 ± 2.37 µg/ml, respectively. The total phenolic content was determined to be 36 ± 3.7 mg of gallic acid equivalent per gram of extract, thereby substantiating its antioxidative potential. The extract selectively inhibited collagenase (16.51 ± 0.5%) and hyaluronidase (5.94 ± 0.17%). The extract was determined to be non-genotoxic, and it exhibited a reduction in genotoxicity induced by NaN_3 and 4-NPD. Collectively, these findings suggest that *Z. capitata* may represent a promising natural source with potential applications in wound healing.

Introduction

The genus *Ziziphora* L., classified within the family Lamiaceae, comprises approximately 30 aromatic species distributed across the Mediterranean regions of Europe, Asia, and North Africa (Selvi *et al*. 2015, Ilhan *et al*. 2025). In Turkey, *Ziziphora* species are commonly found in the Western, Central, Mediterranean, and Eastern Anatolia regions, and are locally known as "Dağ Reyhanı," or "Nane Ruhu" (Baytop 1999, Abad and Nadaf 2023). These plants are widely recognized for their robust aroma and have traditionally been utilized as wild vegetables or flavoring agents due to their distinctive fragrance and taste (Ilhan *et al*. 2025). Phytochemically, the genus is rich in flavonoids, phenolic acids, and terpenoids, which contribute to a wide spectrum of biological and pharmacological properties (Youssif *et al*. 2024a).

Ziziphora capitata is an annual herbaceous species that thrives in arid, stony terrains, distinguished by its narrow lanceolate leaves and dense capitate inflorescences (Selvi *et al*. 2015). Most existing studies on *Z. capitata* have focused on a limited set of biological activities. For example, Yiğitkan *et al*. (2024) evaluated the antioxidant activity and inhibitory effects of the ethanol extract on elastase, tyrosinase, collagenase, urease, acetylcholinesterase, and butyrylcholinesterase, in addition to performing a preliminary toxicity analysis on a colon cancer cell line. Similarly, Mohammadhosseini *et al*. (2016) reported antioxidant and antimicrobial properties for the methanolic extract, and these findings were later supported by Youssif *et al*. (2024a) using various solvent extracts. The anticancer potential has also been examined in several cell lines (Youssif *et al*. 2024b), and antimicrobial activity was reported by Egamberdieva *et al*. (2017).

No study has been evaluated on the genotoxic, antigenotoxic, and hyaluronidase-inhibitory activities of *Z. capitata*. This study aims to address this knowledge gap by assessing the biological

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activities of the ethanolic extract, alongside its antioxidant capacity, total phenolic content, and collagenase and elastase inhibitory effects. By integrating these analyses, the work provides the first comprehensive insight into the ECM-protective and wound-healing potential of *Z. capitata*, highlighting its relevance as a candidate for natural product-based therapeutic applications.

Materials and Methods

The aerial parts of *Ziziphora capitata* L. were collected in June 2023 from wild populations located in Adana Province, Turkey. The dried aerial parts of *Z. capitata* (20 g) were extracted with 500 ml of 96% ethanol at room temperature for 20 min using an ultrasonic bath (40 kHz, 150 W). The mixture was filtered, and the solvent was removed under reduced pressure to obtain the ethanol extract. The extract was stored at 4°C until further analysis, yielding 2.66% (w/w) based on the dry weight of the plant material.

The antioxidant activity of the extract was evaluated using DPPH and β-carotene-linoleic acid assays. For DPPH, the extract was incubated with 0.1 mM DPPH solution in the dark for 30 min, and absorbance was measured at 517 nm (Ebrahimabadi *et al*. 2010). For the β-carotene assay, the extract was incorporated into an emulsion and incubated at 50°C, with oxidation inhibition measured at 470 nm (Rauter *et al*. 2012). Total phenolic content was estimated via the Folin-Ciocalteu method and expressed as mg GAE/g dried extract (Singleton *et al*. 1999).

Collagenase, elastase, and hyaluronidase inhibitory activities were measured using standard chromogenic substrates: N-[3-(2-furyl)-acryloyl]-Leu-Gly-Pro-Ala for collagenase (Barrantes and Guinea 2003), Suc-Ala-Ala-Pro-Phe-pNA for elastase, and hyaluronic acid for hyaluronidase (Lee *et al*. 1999). Absorbances were recorded at 335, 410, and 585 nm, respectively. Percent inhibition was calculated relative to negative controls (DMSO), and standard inhibitors (EGCG tannic acid) were included for comparison.

The Ames test was performed using *Salmonella typhimurium* TA98 and TA100 strains, which detect frameshift and base-pair substitution mutations, respectively. Spontaneous revertants were counted, and non-cytotoxic concentrations of the extract were determined (Mortelmans and Zeiger 2000). The mutagenic effect of the ethanol extract of *Z. capitata* was then assessed using the plate/incorporation method (Maron and Ames 1983). Positive controls were 4-NPD (3 µg/plate) for TA98 and NaN3 (8 µg/plate) for TA100, while DMSO served as the negative control. Plates were incubated at 37°C for 48 hrs, and His^+ revertants were counted. Antigenotoxicity was evaluated by co-incubating the extract at 1000, 100, 10, 1 and 0.1 µg/plate with the mutagens, followed by 72 hrs incubation at 37°C, and the percentage of mutagenic inhibition was calculated.

The experiments were performed in three replicates, with two parallel plates per group. Data are expressed as mean ± standard deviation (SD). Statistical analyses were conducted using SPSS version 16.0 for Windows (SPSS, Munich, Germany). Normality was assessed using the Shapiro-Wilk test, and homogeneity of variances using Levene's test ($P > 0.05$). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used to determine significant differences among groups. Statistical significance was set at $P < 0.05$.

Results and Discussion

This study assessed the antioxidant potential of the ethanol extract from the aerial parts of *Z. capitata*, employing DPPH and β-carotene bleaching methods (Table 1). The extract demonstrated IC_{50} values of 200 ± 1.13 µg/ml and 138 ± 2.37 µg/ml for the DPPH and β-carotene assays, respectively, which were considerably higher than those of ascorbic acid (34.8 ± 0.3 µg/ml for DPPH and 30.4 ± 0.3 µg/ml for β-carotene, $p < 0.05$). These values classify the extract as having moderate antioxidant potential compared to well-known medicinal plants ($IC_{50} < 50$ µg/ml).

The results are consistent with previous findings, which reported a DPPH IC_{50} value of 206.6 µg/ml for the methanolic extract of *Z. capitata* (Mohammadhosseini *et al*. 2016). In contrast, Youssif *et al*. (2024a) observed stronger antioxidant effects in the ethyl acetate and 95% ethanol extracts (IC_{50}: 18.6 µg/ml and 30.4 µg/ml, respectively), while water, chloroform, and hexane extracts showed weaker activity (IC_{50}: 134.4, 620, and 1137.8 µg/ml). Yiğitkan *et al*. (2024) also reported potent DPPH scavenging activity in ethanol extracts from the flower and root (IC_{50}: 37.18 and 37.40 µg/ml). Antioxidant activity is particularly important for wound healing because excessive ROS can damage lipids, proteins, and fibroblasts, thereby delaying tissue regeneration. Antioxidants neutralize free radicals by donating electrons or hydrogen atoms, preventing ROS from oxidizing critical biomolecules. This protective mechanism reduces oxidative damage and inflammation, creating a favorable microenvironment for fibroblast proliferation, angiogenesis, and extracellular matrix remodeling (Comino-Sanz *et al*. 2021). Particularly important for wound healing, antioxidants such as *Z. capitata* extract may promote tissue regeneration during the initial inflammatory phase by scavenging excessive ROS and maintaining ECM integrity.

Table 1. Biological activities of the ethanol extract of *Ziziphora capitata*.

Samples	Antioxidant activity		Total phenolic content	Enzyme inhibition activity (1 mg/ml)		
	IC_{50} (µg/ml)		mg GAE/g extract	% inhibition		
	DPPH	β-carotene bleaching		Elastase	Collagenase	Hyaluronidase
Z. capitata	200 ± 1.1[a]	138 ± 2.3[a]	36 ± 3.7	0 (ns)	16.51 ± 0.5*	5.94 ± 0.1*
Ascorbic acid	34.8 ± 0.3[b]	30.4 ± 0.3[b]	-	-	-	-
EGCG	-	-	-	25.3 ± 0.1	20.36 ± 0.2	-
Tannic acid	-	-	-	-	-	62.8 ± 0.2

a, b indicate statistically significant differences ($p < 0.05$). Data are expressed as mean ± standard deviation ($n = 3$). "*" indicates a statistically significant difference compared to the negative control ($p < 0.05$), while "ns" indicates no significant difference.

It was determined that the phenolic content of the ethanol extract amounts to 36 ± 3.7 mg of gallic acid equivalent (GAE) per gram of extract (Table 1). Yiğitkan *et al*. (2024) reported phenolic content values for ethanol extracts of *Z. capitata* ranging from 33.93 to 63.49 µg PEs/mg extract, with the highest value in the root. These findings underscore the significance of phenolic compounds in the antioxidant activity of plant extracts. Phenolic compounds, including flavonoids, phenolic acids, and tannins, function as free radical scavengers, thereby protecting cellular structures such as lipids, proteins, and DNA. By impeding lipid peroxidation, these compounds assist in maintaining cellular integrity, decelerating the aging process, accelerating wound healing, and providing protection against chronic diseases (Tungmunnithum *et al*. 2018).

The ethanol extract was also evaluated for its inhibitory effects on key enzymes that degrade the ECM at a concentration of 1 mg/ml. The extract exhibited no elastase inhibition; however, it demonstrated collagenase (16.51 ± 0.5%) and hyaluronidase (5.94 ± 0.17%) inhibition ($p < 0.05$) (Table 1). Positive controls showed higher inhibition, with EGCG inhibiting elastase and collagenase by 25.3 ± 0.1 and 20.36 ± 0.2%, respectively, and tannic acid inhibiting hyaluronidase by 62.8 ± 0.2%.

*Ziziphor*a *capitata*'s bioactive phytochemicals, including phenolic acids, flavonoids, and tannins, have been shown to possess inhibitory effects by modulating ECM-degrading enzymes

(Murata *et al*. 2010, van Strijp *et al*. 2015, Mohammadhosseini *et al*. 2016, Yiğitkan et al. 2024). While studies on enzyme inhibition in *Ziziphor*a species are limited, Yiğitkan *et al*. (2024) reported similarly low collagenase inhibition (7.28-10.47%) and no elastase inhibition in *Z. capitata*, while Çavuşoğlu *et al*. (2024) observed similarly no elastase and only mild collagenase inhibition (10.04%) in *Z. clinopodioides* extracts. Inhibition of collagenase, elastase, and hyaluronidase is important because too much enzyme activity can cause skin problems. *Z. capitata* extract may be an effective ingredient for wound healing and anti-aging by controlling enzymes.

To evaluate the genotoxicity of these samples, the Ames test method was employed, utilizing the *S. typhimurium* strains TA98 and TA100. The extract demonstrated no mutagenic activity across the range of concentrations (0.1-1000 µg/plate), indicating no significant difference compared to the negative control and confirming its genotoxic safety. In addition, the extract exhibited substantial antigenotoxic effects, reducing sodium azide (NaN_3)-induced mutations in TA100 and 4-NDP-induced mutations in TA98. The extract exhibited antigenotoxic activity in both tester strains. In TA98, the highest inhibition was observed at 1000 µg/mL (34.59%), indicating a measurable protective effect against 4-NPD-induced mutagenicity. In TA100, the extract also reduced NaN_3-induced mutagenicity, showing 34.49% inhibition at 1000 µg/ml and inhibition rates of 38.2, 28.0, 26.58, and 22.00% at 100, 10, 1, and 0.1 µg/mL, respectively. These values suggest that the extract can mitigate mutation-inducing damage in both frameshift (TA98) and base-pair substitution (TA100) models (Fig. 1).

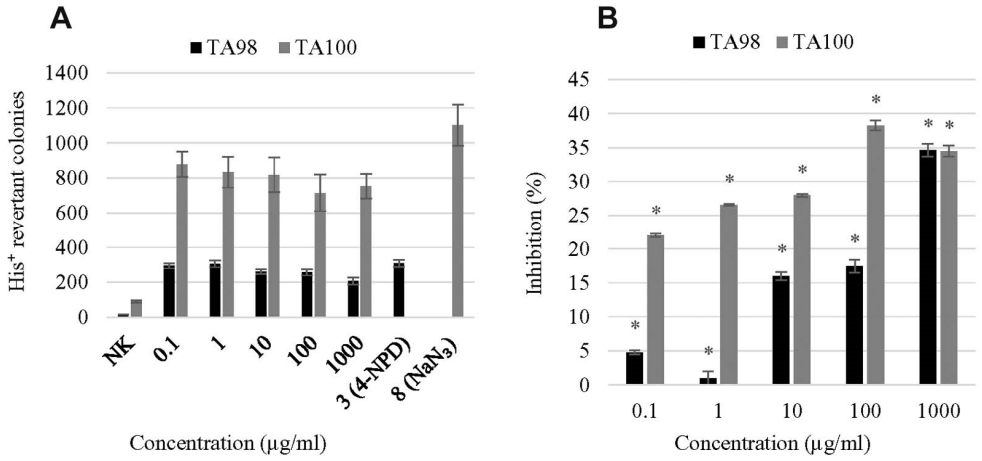

Fig. 1. Antigenotoxic activity *Ziziphora capitata*. A: number of His^+ revertant colonies, and B: percentage of inhibition compared to the positive controls. Data are presented as mean ± standard deviation of three independent replicates, with two parallel plates per group. $P < 0.05$ compared to the positive control. 4-NPD was used as a positive control for the TA98 strain, and NaN_3 for the TA100 strain. Bars marked with an asterisk (*) indicate a statistically significant difference from the positive control.

These findings are consistent with those reported for another *Ziziphora* species. The ethanolic extract of *Z. clinopodioides* did not exhibit mutagenic activity against *S. typhimurium* TA98 and TA100 strains in the Ames test across concentrations of 0.25-1 mg/ml (Ahmadi *et al*. 2021). No studies have investigated the genotoxicity or antigenotoxicity of *Z. capitata*, and available information on enzyme inhibition within the genus remains limited. Extracts from various members of the Lamiaceae family have generally been reported to be non-mutagenic in the Ames assay (Boran and Ugur 2015, 2018). These findings support the interpretation that the absence of

mutagenicity observed in *Z. capitata* aligns with its congeners and the broader genotoxic safety profile commonly recognized in lamiaceae species.

The ethanol extract of *Z. capitata* demonstrated antioxidant capacity and inhibitory effects on collagenase and hyaluronidase. The extract also exhibited antigenotoxic activity without inducing genotoxicity, supporting its safety and indicating a potential protective effect against mutagenic agents. Overall, these findings strengthen the limited existing data on *Z. capitata* and highlight its pharmacological relevance, particularly in addressing skin-related problems and supporting wound healing processes.

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