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RESEARCH ARTICLE

# Altitude-Dependent Variation in Chemical Composition of Essential Oil of *Origanum acutidens* (Hand-Mazz.) Ietswaart

Furkan Coban[1]✉ • Hakan Ozer[1] • Ramazan Cakmakci[2]

[1]Atatürk University, Faculty of Agriculture, Department of Field Crops, Erzurum/Türkiye
[2]Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Field Crops, Çanakkale/Türkiye

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**ABSTRACT**

Altitude significantly influences the yield and composition of essential oils in medicinal plants, with *Origanum acutidens* (Hand-Mazz.) Ietswaart, an endemic species in Eastern Anatolia, Türkiye, showing noticeable variations. Known for its traditional medicinal uses and aromatic qualities, this species was studied at three different altitudes (1150, 1650, and 2150 m) in the Eastern Black Sea Region. The results showed that essential oil yield increased with altitude, with yields at 0.75%, 0.86%, and 1.03% at each altitude, respectively. Key components of the oil, carvacrol and *p*-cymene, also varied with altitude. Carvacrol content increased significantly from 38.30% to 58.76% as altitude increased, while *p*-cymene content decreased from 35.47% to 17.12%. These results suggest that higher altitudes, which provide conditions like lower temperature, reduced air pressure, and higher UV exposure, stimulate secondary metabolite production in *O. acutidens*. It is recommended that further research be conducted to explore this plant's chemical diversity across varied topography and climate conditions.

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## 1. Introduction

Türkiye is home to a diverse array of plant species harvested for purposes such as food, medicine, and various other applications, thereby contributing significantly to both domestic and foreign markets. Among these, members of the Lamiaceae family, which includes approximately 220 genera and 4,000 species globally, are of particular importance. Türkiye hosts 48 genera and 873 species from this family, showcasing the country's rich botanical diversity (Güner et al., 2012). Within this family, plants colloquially referred to as "thyme" are especially valued for their medicinal and aromatic properties. These plants, from genera like *Thymus*, *Origanum*, *Satureja*, *Tymbra*, and *Coridothymus*, are widely used across Türkiye, often known by various local names depending on the region. Notably, a large portion of the Lamiaceae species in Türkiye is endemic, with endemicity rates of 44.2% for the family, 65.2% for *Origanum*, and 52.6% for *Thymus*, emphasizing Türkiye's role as a gene center for these genera (Baser et al., 1997; Davis, 1970; Tumen et al., 1995), (Figure 1). Many taxa belonging to the genus *Origanum*, commonly known as oregano, are widely used in folk medicine for the treatment of ailments such as headaches, dizziness, cough, flu, gastrointestinal diseases, bronchitis, high cholesterol, diabetes,

✉ Correspondence
E-mail address: furkan.coban@atauni.edu.tr

abdominal pain, hypertension, and toothache (Fidan et al., 2020).

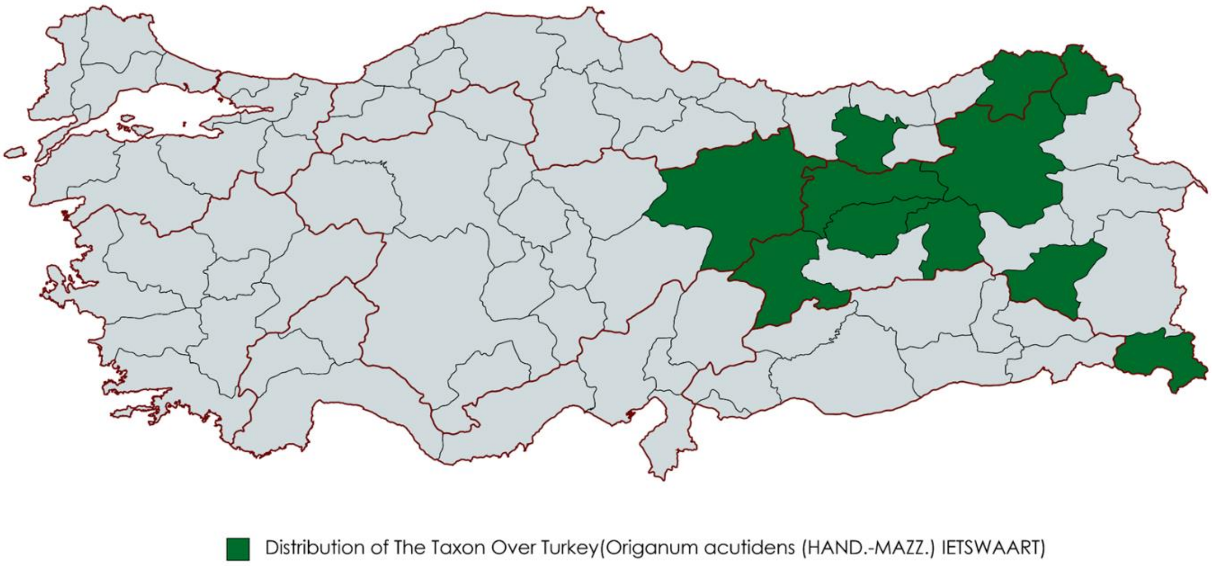


**Figure 1.** Geographic distribution of *Origanum acutidens* in Türkiye (TÜBİVES, 2023).

One noteworthy member of this family is *Origanum acutidens*, a perennial herb endemic to Eastern Anatolia Region in Türkiye (Figure 1). Flowering from July to August in bright pink to white hues, this species thrives on sun-exposed slopes with calcareous or limestone substrates, requiring minimal moisture. *O. acutidens* appealing aroma has made it a candidate for ornamental use in urban landscaping and rock gardens, while its fragrant and disease-protective properties have led to its adoption in the food industry as a natural flavoring and preservative. The essential oil of *O. acutidens* is particularly rich in carvacrol, a phenolic compound known for its antimicrobial properties, which, along with other components like *p*-cymene and thymol, provides a potent, natural alternative to synthetic preservatives (Burt, 2004). This oil has demonstrated significant antibacterial efficacy, notably against strains like *Salmonella typhimurium* and *Escherichia coli*, making it a promising natural antiseptic for applications in health and hygiene sectors (Cosge et al., 2009). As a high-altitude plant endemic to Eastern Anatolia, *O. acutidens* exemplifies the traditional medicinal uses associated with *Origanum* species, which have historically been applied as sedatives, diuretics, digestive aids, and antiseptics (Köse et al., 2021).

Thymol and carvacrol are often confused due to their similar structures; however, these two compounds have different properties and effects. Especially in medical and industrial applications, it is important not to mix up thymol and carvacrol to ensure the correct compound is used (De Vincenzi et al., 2004; Ultee et al., 2002). One of the primary pathways for carvacrol synthesis is through the conversion of *p*-cymene, a compound with the formula $C_{10}H_{14}$. This transformation can occur via different mechanisms. Isomerization allows *p*-cymene to serve as an intermediate, where structural rearrangement of atoms leads to the formation of carvacrol. Oxidation is another pathway; exposure to oxygen or oxidative conditions within the plant modifies *p*-cymene structure, enabling the formation of carvacrol. Additionally, enzymatic reactions in essential oil-producing plant cells play a crucial role, as specific enzymes can catalyze the transformation of *p*-cymene into carvacrol during the plant's natural metabolic processes. These pathways underscore the biochemical complexity behind essential oil composition in plants (Marchese et al., 2016) (Figure 2).

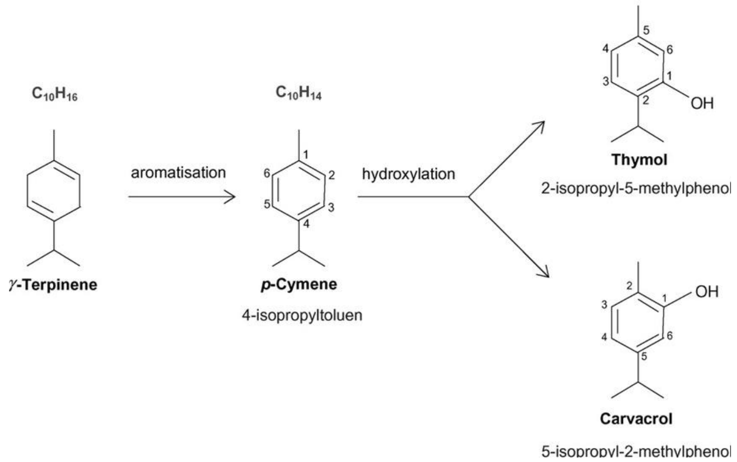


**Figure 2.** Pathways for the synthesis of carvacrol from *γ*-terpinene via *p*-cymene (Taherian et al., 2009).

The composition of essential oils is influenced by numerous factors that determine the chemical profiles and quality of these oils in various plant species. These factors include the geographical location of the plant, climatic conditions, soil composition, and the altitude at which the plant grows (Hussain et al., 2008; Talebi et al., 2019). Altitude plays a critical role in determining the quality and yield of medicinal plants by impacting their biochemical composition, growth rate, and adaptability (Khalil et al., 2020; Mahdavi et al., 2013). For species like *Origanum acutidens*, these altitude-related variations are particularly significant, as changes in altitude can influence essential oil content, concentration of active compounds, and overall plant morphology. Variations in the composition of essential oils with altitude are often complex and depend on multiple interacting factors, including specific plant species, geographic location, climate conditions, soil composition, and the altitude range itself.

Given this context, the present study seeks to investigate how different altitudinal levels influence the essential oil yield and composition in the aerial parts of *Origanum acutidens* (Hand-Mazz.) Ietswaart during its flowering period. This research will examine three distinct altitudes 1150, 1650, and 2150 meters in the Eastern Black Sea Region of Türkiye, aiming to capture how variations in altitude affect the plants chemical profile. By understanding these differences, the study will contribute valuable insights into the medicinal and commercial applications of *O. acutidens*. This information could inform optimal harvesting conditions for maximizing essential oil yield and quality, aiding in the sustainable and efficient use of this valuable plant in industries ranging from food preservation to natural medicine.

## 2. Materials and Methods

### *2.1.* Plant Materials

Aerial parts of *Origanum acutidens* (Hand-Mazz.) Ietswaart were collected from Baba Mountain, İspir, Erzurum, Türkiye, during the flowering season in 2023 at three different altitudes (1150, 1650, and 2150 m) (Figure 3). The samples were collected simultaneously on the same date to ensure consistency, as the content and yield of samples collected at different times may vary. The collected plant material was dried in the shade, ground in a grinder equipped with a 2 mm diameter mesh, and stored. The taxonomic identification of the plant material was verified by Dr. Ali Kandemir, a senior plant taxonomist from the Department of Biology at Erzincan University, Erzincan, Türkiye. Additionally, herbarium specimens were prepared and deposited in the herbarium of the Department of Field Crops, Atatürk University, under the accession numbers Org2023008, Org2023009, and Org2023010, corresponding to the collection sites in ascending order of altitude.

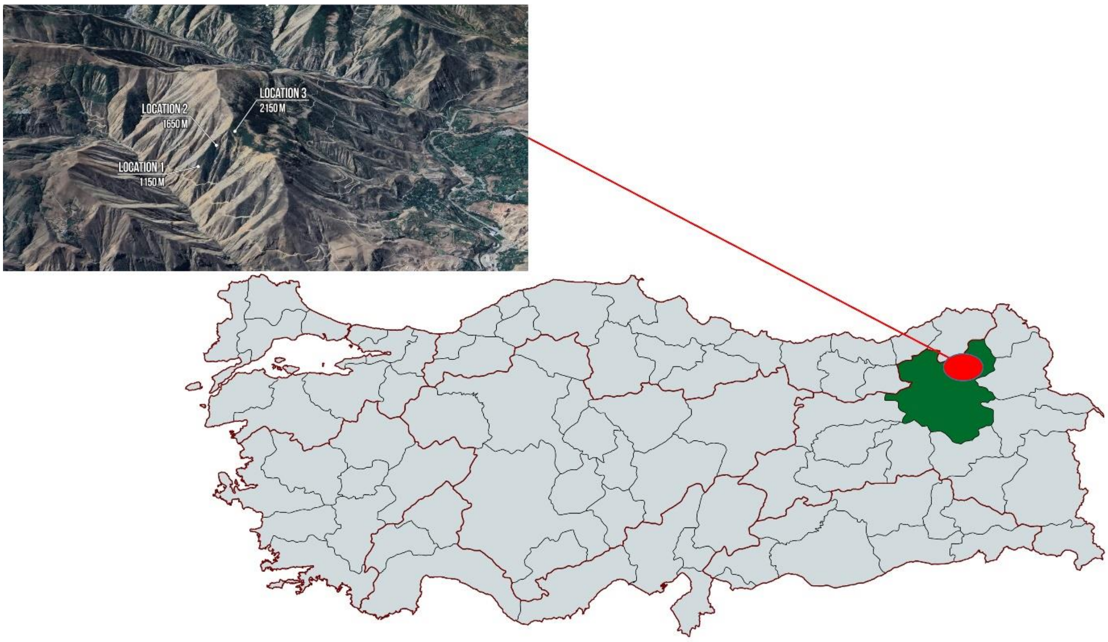


**Figure 3.** Collection sites of *Origanum acutidens* at different altitudes on Baba Mountain, İspir, Erzurum, Türkiye.

### *2.2.* Essential Oil Extraction

The air-dried aerial parts of the plant samples were extracted via hydrodistillation using a Clevenger-type apparatus for a duration of 3 hours. The extraction process was repeated three times to ensure reproducibility. The resulting essential oil was then dehydrated using anhydrous sodium sulfate, filtered, and stored at +4°C until further testing and analysis.

### *2.3.* GC Analysis Conditions

Essential oil analysis was conducted on a Thermofinnigan Trace GC/A1300 system equipped with an SGE/BPX5 MS capillary column (30 m x 0.25 mm i.d., 0.25 µm). Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector temperature was set to 220°C. The temperature program started at 50°C, increased to 150°C at 3°C/min, held isothermal for 10 minutes, and then raised to 250°C at 10°C/min. Diluted samples (1/100, v/v in methylene chloride) of 1.0 µL were manually injected in splitless mode. Quantitative data were obtained from the FID area percentage.

### *2.4.* GC-MS Analysis Conditions

The essential oils were analyzed using a Thermofinnigan Trace GC/Trace DSQ/A1300 system (E.I. Quadrupole) with an SGE-BPX5 MS capillary column (30 m x 0.25 mm i.d., 0.25 µm). Detection was carried out via electron ionization at 70 eV, with helium as the carrier gas at 1 mL/min. Injection and MS transfer line temperatures were 220°C and 290°C, respectively. The temperature program was set from 50°C to 150°C at 3°C/min, held for 10 minutes, and then increased to 250°C at 10°C/min. A 1.0 µL volume of diluted sample (1/100, v/v in methylene chloride) was injected in splitless mode. Component identification was based on retention times, mass spectra comparison with Wiley7N and TRLIB databases, and literature data. Identification was further validated by matching relative retention indices with non-polar phase values reported in the literature (Adams, 2007).

## 3. Results and Discussion

The essential oil (EO) yield of *Origanum acutidens* varied significantly with altitude, indicating that altitude plays a role in enhancing oil production in this plant. At 1150 m, the essential oil yield was recorded at 0.75 ± 0.01%, increasing to 0.86 ± 0.02% at 1650 m, and reaching a peak of 1.03 ± 0.02% at 2150 m (Table 1). Increasing altitude positively impacts essential oil (EO) yield, mainly due to environmental stresses such as elevated UV radiation, lower temperatures, and fluctuating light intensity. These conditions stimulate the production of secondary metabolites, including essential oils, in plants. The heightened UV exposure at higher altitude promotes the production of protective compounds with antioxidant properties, such as phenolics and terpenoids (Chrysargyris et al., 2020). These compounds enhance the plants defense against UV-induced oxidative stress, thereby boosting EO yield and effectiveness. Temperature fluctuations in mountainous areas also influence EO composition, specifically by promoting the biosynthesis of oxygenated monoterpenes, which are crucial for aroma and medicinal properties (Chrysargyris et al., 2020; Zhang et al., 2021). Furthermore, studies on medicinal plants indicate that both light and temperature stress enhance EO yield and alter antioxidant activity, emphasizing the critical role of altitude in plant bioactivity (Thoma et al., 2020). The EO yield we obtained, ranging from 0.75% to 1.03%, aligns closely with the findings reported by Karagöz et al. (2022), who identified EO yields within the range of 0.42% to 1.13%. This consistency suggests that our results fall within the expected yield range under similar conditions, supporting the reliability of our findings in the context of established research.

Carvacrol and *p*-cymene are two primary monoterpene compounds that play a crucial role in the chemical composition of plant essential oils. However, the concentrations of these compounds vary depending on the altitude at which the plants grow and the environmental stress factors they experience. As observed in the study, *p*-cymene is found in high levels at moderate altitudes (1150-1650 m), while its concentration decreases at higher elevations (2150 m). In contrast, the Carvacrol content shows a significant increase with altitude; at 1150 m, the Carvacrol level is 38.30%, which rises to 41.58% at 1650 m and further to 58.76% at 2150 m (Table 1).

**Table 1.** Chemical profiling of essential oil of *Origanum acutidens* from different altitudes of Eastern Black Sea Region, Türkiye.

| Constituents | RI* | Composition (%) | | | Method of identification |
|---|---|---|---|---|---|
| | | Altitude | | | |
| | | 1150m | 1650m | 2150m | |
| *α*-thujene | 924 | 0.87 | 3.07 | 0.70 | GC-MS- RI |
| *α*-pinene | 932 | 3.08 | - | 2.18 | GC-MS- RI |
| camphene | 946 | 1.30 | 0.47 | 0.79 | GC-MS- RI |
| *β*-pinene | 974 | - | 0.32 | 0.41 | GC-MS- RI |
| myrecene | 988 | 3.05 | 4.19 | 3.28 | GC-MS- RI |
| *α*-phellandrene | 1002 | - | 0.61 | 0.44 | |
| *δ*-3-Carene | 1008 | 0.55 | - | - | |
| *α*-terpinene | 1014 | 1.80 | 3.38 | 1.78 | GC-MS- RI |
| *p*-cymene | 1020 | 35.47 | 22.75 | 17.12 | GC-MS- RI |
| trans-*β*-ocimene | 1044 | - | 0.35 | - | GC-MS- RI |
| *γ*-terpinene | 1054 | 5.53 | 15.89 | 7.69 | GC-MS- RI |
| cis-sabinenehydrate | 1065 | 0.43 | 0.22 | 0.30 | MS- RI |
| terpinolene | 1086 | 0.36 | - | 0.13 | GC-MS- RI |
| linalool | 1095 | 2.45 | - | 0.71 | GC-MS- RI |
| borneol | 1165 | 2.46 | 0.55 | 1.88 | GC-MS- RI |
| terpinen-4-ol | 1174 | 0.76 | 0.83 | 0.95 | GC-MS- RI |
| *α*-terpineol | 1186 | 0.57 | 0.33 | 0.44 | GC-MS- RI |
| cis-dihydrocarvone | 1191 | - | - | 0.13 | |
| carvocrol methyl ether | 1241 | - | 2.58 | 0.09 | |
| ascaridole | 1234 | - | - | 0.14 | |
| thymol | 1289 | 0.16 | 2.13 | 0.36 | GC-MS- RI |
| carvacrol | 1298 | 38.30 | 41.58 | 58.76 | GC-MS- RI |
| carvacrol acetate | 1370 | - | 0.26 | - | |
| geranyl acetate | 1379 | 0.39 | - | 0.14 | GC-MS- RI |
| *β*-caryphyllene | 1417 | 2.12 | 0.49 | 1.39 | GC-MS- RI |
| aromadendrene | 1439 | - | - | 0.19 | GC-MS- RI |
| EO yield | | 0.75 ± 0.01 | 0.86 ± 0.02 | 1.03 ± 0.02 | |
| Total | | 99.65 | 100 | 100 | |

*RI, retention index: Compounds listed in order of elution from a BPX5 MS column.

The carvacrol content of *Origanum acutidens* and its related species exhibits considerable variability across different regions of Türkiye, with documented values ranging between 49.4% and 87.0%. This variation is closely associated with geographical and environmental factors that significantly influence the composition of essential oils (Baser et al., 1997; Cosge et al., 2009; Gulec et al., 2014; Kordali et al., 2008; Sökmen et al., 2004). Among these factors, altitude emerges as a critical determinant, shaping the biosynthesis of major constituents such as carvacrol and *p*-cymene.

High-altitude regions expose plants to unique environmental stressors, including increased UV radiation and lower temperatures. These conditions stimulate the production of secondary metabolites, particularly phenolic compounds, which play a vital role in enhancing the plant's defense mechanisms. The increase in carvacrol content at higher altitudes is attributed to the upregulation of the mevalonate (MVA) pathway, which governs the biosynthesis of terpenoids and related compounds (Khoshbakht et al., 2020; Pirigharnaei et al., 2012; Tsoumani et al., 2022). Furthermore, the conversion of precursor molecules, such as *p*-cymene, into carvacrol is believed to intensify in response to these stressors (Hosseini et al., 2024).

These adaptive biosynthetic responses are not merely survival mechanisms but also illustrate the complex relationship between environmental factors and the chemical profile of oregano essential oils. Understanding these dynamics provides a basis for optimizing cultivation strategies aimed at enhancing carvacrol content. For instance, deliberate exposure to moderate environmental stress, as observed at higher altitudes, may be strategically utilized to improve the therapeutic and commercial value of oregano essential oils.

## 4. Conclusion

This study demonstrates that altitude significantly affects the essential oil yield and composition of *Origanum acutidens* (Hand-Mazz.) Ietswaart, an endemic species to Türkiye's Eastern Anatolia Region. As altitude increases, there is a corresponding increase in essential oil yield and notable shifts in chemical composition, particularly in carvacrol and *p*-cymene content. Higher altitudes stimulate the production of carvacrol while reducing *p*-cymene levels, likely due to increased UV radiation, lower temperatures, and reduced atmospheric pressure. These environmental factors activate biosynthetic pathways such as the mevalonate (MVA) pathway, enhancing the production of secondary metabolites that strengthen the plant's defense mechanisms. The findings suggest that by strategically cultivating *O. acutidens* at higher altitudes, it may be possible to optimize carvacrol levels, thereby improving its antimicrobial potency and commercial value for applications in food preservation, medicine, and natural products.

Further research on the influence of altitude and other environmental factors across various topographies and climates could provide deeper insights into the adaptive mechanisms of *O. acutidens*. This knowledge could inform more sustainable harvesting and cultivation practices, enhancing the quality and yield of essential oils from this valuable species.

## Acknowledgment

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## Conflict of Interest

The authors declare that they have no conflict of interest.

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