



Research Article

Bioactive Content, Antioxidant Capacity, and Quality Assessment of *Origanum onites* and *Thymus fallax* (Lamiaceae)Pelın Tastañ^{1,*}, Ayten Acar²¹ İzmir Katip Çelebi University, Faculty of Pharmacy, Department of Pharmacognosy, İzmir, Türkiye; <https://orcid.org/0000-0003-0913-5369>² İzmir Katip Çelebi University, Faculty of Pharmacy, İzmir, Türkiye; <https://orcid.org/0009-0003-1413-2741>* Corresponding author: pelin.tastan@ikcu.edu.tr

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Abstract: Lamiaceae family includes approximately 236 genera and 6900 to 7200 species. Medicinal plants belonging to this family have been used for many years in the treatment of various animal and human diseases based on their antibacterial, antioxidant, antitumoral, antifungal, analgesic and insecticidal effects. This research covers the determination of quality control methods and analytical studies for *Thymus fallax* (catrı) and *Origanum onites* (kekik) which were collected from Şırnak and İzmir, respectively. Heavy metal analyses using Wavelength Dispersive X-Ray Spectrometer (WDXRF) method, determination of ash content and loss on drying according to the European Pharmacopoeia 8.0 standards were carried out. Furthermore, the total phenolic contents of aqueous extracts of both plants were tested with the Folin-Ciocalteu colorimetric method to getting first step for determining their antioxidant activities. According to WDXRF results, the amounts of heavy metals determined were within the limits of pharmacopoeia standards for both species. In addition, total ash and water amounts were determined as 7%, 14% and 14%, 16% for *Origanum onites* and *Thymus fallax* respectively and were determined to be in compliance with pharmacopoeia standards. Total phenolic contents (GAE µg/mL) were found to be 28.152 ± 0.389 and 41.009 ± 2.059 for *Origanum onites* and *Thymus fallax*, respectively. This is an evidence that both species contain phenolic compounds that will cause them to display antioxidant activity. The data obtained within the scope of the study showed that if the plants are procured correctly and the water extracts are prepared with appropriate methods, the quality and effectiveness will increase the efficiency.

Keywords: *Origanum onites*, *Thymus fallax*, Lamiaceae, phenolic content, WDXRF.

1. Introduction

The Lamiaceae family, commonly known as the mint family, is one of the largest and most diverse botanical families, comprising approximately 236 genera and more than 6900-7200 species distributed worldwide, particularly across Mediterranean, Asian and temperate regions (Tzima et al., 2018). Members of this family are well recognized for their aromatic properties, high essential oil content and rich phytochemical composition, which includes phenolic acids, flavonoids, terpenoids and other bioactive metabolites. Owing to these chemical characteristics, Lamiaceae species have been extensively utilized in traditional medicine, culinary applications and modern phytotherapeutics (Hossain et al., 2010). In Turkey, the family is represented



by a remarkable diversity, with 46 genera and nearly 600 species, more than 40% of which are endemic—highlighting its ecological and ethnobotanical importance (Davis, 1982).

Among the most economically and pharmacologically significant genera of the Lamiaceae family are *Origanum* and *Thymus*. These genera include numerous species widely employed as culinary herbs, flavoring agents and medicinal plants. *Origanum* species, commonly referred to as oregano, are characterized by their high content of essential oil constituents such as carvacrol and thymol, which contribute to their strong antimicrobial, antioxidant and anti-inflammatory properties. Likewise *Thymus* species, known as thyme, are rich in volatile oils, phenolic compounds and terpenoids, making them valuable resources in food preservation, aromatherapy and traditional health practices (Moshari-Nasirkandi et al., 2023).

The genus *Thymus* L. is divided into eight sections. The Western Mediterranean region is considered the center of origin of the genus. Although the number of species worldwide is uncertain, approximately 215-220 are recorded (Morales, 2002). *Thymus* species are perennial and have fibrous and woody understory parts. In Turkey 41 species, a total of 45 taxa, grow in eight subsections, 17 species are endemic and the endemism rate is 41.42%. It is widely distributed in our country, primarily in Central, Eastern and Southeastern Anatolia (Davis, 1988). Analyses have identified approximately 270 terpenes in *Thymus* species, with one or more reported to be dominant. Thymol, carvacrol, linalool, psimene, geraniol and borneol are the most important terpenes. *Thymus* species are the most important source of monoterpenoid phenols in the plant kingdom (Stahl-Biskup, 2002). *Thymus fallax* species, found especially in the Southeastern and Eastern Anatolia regions of Turkey, is known colloquially as Catri and Catır (Güner et al., 2012).

The genus *Origanum* has 52 species worldwide. In Turkey, 16 of the 32 taxa are endemic (Duman et al., 1995). Approximately 75% of *Origanum* L. species, known as the "Ornament of the Mountains" are distributed in the Eastern Mediterranean subregion. Turkey, like many other species, is the world's most important gene center for numerous species of the genus *Origanum* L. Among the public, *O. onites* is known as "thyme, Izmir thyme, cheese thyme, stone thyme, moth thyme, Izmir mercanköşkü and ball thyme" (Güner et al., 2012).

Origanum onites L. is used in the treatment of various ailments such as gastrointestinal disorders, diabetes, high cholesterol, leukemia, bronchitis, etc. The traditional use, phytochemistry and pharmacology of *O. onites* have been investigated. Several different terpenoids, triterpene class acids, phenolic acids, hydroquinones, flavonoids, hydrocarbons, sterols, pigments, fatty acids, tocopherols and inorganic compounds have been identified mainly in the aerial parts of this plant. Pharmacological studies have revealed that extracts obtained from various solvents and individual compounds exhibit antimicrobial, antiviral, antioxidant, insecticidal, anticancer, hepatoprotective, genotoxic, antidiabetic, cholinesterase inhibitor, anti-inflammatory, analgesic activities, etc. (Tepe et al., 2016).

Due to their complex phytochemical profiles, both *Origanum* and *Thymus* species have gained considerable attention in scientific research. Studies have highlighted their biological activities including antibacterial, antifungal, antiviral, antitumoral and antioxidant effects. In addition, the chemical variability observed among species and even among populations of the same species underscores the importance of standardized quality assessment (Baser, 2008; Skendi et al., 2017; Marchese et al., 2016; Spyridopoulou et al., 2019). Also factors such as geographical origin, environmental conditions, harvesting time and processing techniques may influence the concentration of bioactive compounds.

Given this background, the present study focuses on the evaluation of physicochemical characteristics and bioactive potential of *Origanum onites* and *Thymus fallax*, two species native to different regions of Turkey. By analyzing parameters such as heavy metal content, ash values, moisture levels and total phenolic concentration, the study aims to contribute to the standardization, safety assessment and quality control of these widely used Lamiaceae species.

2. Materials and Methods

2.1. Plant materials

Thymus fallax and *Origanum onites* were identified in their natural habitat and the aerial parts of the plants were collected in June 2024 due to their flowering period. *Thymus fallax* was collected from Silopi (Şırnak Province), while *Origanum onites* was collected from Ödemiş (İzmir Province). The plant parts were completely dried in a ventilated but sunless enclosed area, then crushed, ground and powdered. Extracts

were prepared from the leaves of the powdered plants without further storage. For this purpose, herbal teas were prepared using the infusion method due to the traditional use of the powdered leaves of both plants as herbal teas. Both extracts were then desolventized in a low-pressure rotavapor (Buchi R-100) until dry. The water extracts of *Thymus fallax* and *Origanum onites* were then stored in a refrigerator at +4 °C until use.

2.2. Heavy metal analysis (WDXRF)

Atomic absorption spectroscopy (AAS), developed after 1955, is based on the principle of measuring the decrease in light intensity caused by the absorption of electromagnetic waves emitted by a radiation source by gaseous atoms. The absorbed electromagnetic rays are generally ultraviolet and visible. To analyze an element using atomic absorption spectroscopy, the element must first become neutral, then vaporized and then scattered into the path of an electromagnetic beam coming from a source. This process is accomplished either by spraying a solution containing the element in compound form into a high-temperature flame in mist form, or by placing a sample (solution or solid) containing the element in compound form in a carbon sample container and igniting the container with an electric arc (Skoog et al., 2014).

Atomic absorption spectrometry consists of a beam source that emits the spectrum of the element under study, an atomizer in which the sample is separated into atoms, a monochromator (wavelength selector) that separates the wavelength under study from other wavelengths and a detector section where the beam intensity is measured.

Heavy metal concentrations of both species were determined using the Wavelength Dispersive X-ray Fluorescence (WDXRF) technique. This method allowed rapid, nondestructive quantification of elemental composition. Results were evaluated in accordance with European Pharmacopoeia 8.0 limits (EDQM, 2013).

2.3. Determination of loss on drying

One of the most basic and important analytical procedures performed on food products is the analysis of water content. The dry matter remaining after water removal is generally referred to as total dry matter. Moisture analysis is required in foods for the following reasons (Karagözlü and Akbulut, 2012):

- To control compliance with standards
- To determine the commercial value of food
- To determine the stability or storage life of food
- To determine the sensory quality characteristics of food
- To determine the suitability of food for packaging and transportation

Loss on drying (LOD) was measured following procedures described in the European Pharmacopoeia 8.0. Approximately 2–3 g of powdered plant material was subjected to controlled incineration and drying steps to determine the corresponding percentage values (EDQM, 2013).

2.4. Determination of ash content

Ash from a food is the inorganic residue remaining after the combustion of organic matter. When foods are heated at 500-6000°C, water and volatile compounds evaporate, while organic compounds burn. Mineral compounds remain in the residue as oxides, sulfates, phosphates, silicates or chlorides, depending on the combustion conditions and the composition of the burned food. Ash contains high amounts of sodium, potassium, calcium and magnesium, while iron, copper, zinc, manganese, aluminum, iodine, arsenic, fluorine and other trace elements are present in small amounts (Yetim, 2001). Ash determination is based on the principle of drying the weighed sample and incinerating it at a temperature of approximately 400-700°C. The remaining ash is then weighed and its amount determined. Ash calculations are based on dry matter (Cemeroglu, 2019).

Total ash content was measured following procedures described in the European Pharmacopoeia 8.0. Approximately 2–3 g of powdered plant material was subjected to controlled incineration and drying steps to determine the corresponding percentage values (EDQM, 2013).

2.5. Determination of total phenolic content

Total phenolic content analysis was carried out according to the Folin-Ciocalteu method applied by Singleton and Rossi (1965), which is a common method. According to this method, 2.8 ml of deionized water was added to 100 μ l (0.1 ml) of the extract solution diluted in appropriate ratios, then 2 ml of 2% Na_2CO_3 was added and finally 0.1 ml of 50% Folin-Ciocalteu reagent was added and mixed. After standing in the dark for 30 min at 25 °C, the absorbance against water was measured at 750 nm. Total phenol content was calculated as gallic acid equivalent (GAE μ g/mL) using the gallic acid calibration curve. Experiments were carried out in triplicate and the results were averaged (Singleton and Rossi, 1965).

2.5. Statistical analysis

All experiments were performed in triplicate, and results are expressed as mean $\pm$ SD. GraphPad Prism software was used for statistical analysis.

3. Results

3.1 Heavy metal content

The WDXRF analysis demonstrated that the heavy metal contents of both *O. onites* and *T. fallax* were within the acceptable limits established by pharmacopoeial standards and no toxic metal accumulation was detected in either extract. The concentrations of all metals identified during the analysis are presented in Table 1, providing a comprehensive profile of the elemental composition of the samples.

Table 1. Heavy metal contents of water extracts of *Thymus fallax* and *Origanum onites* plants.

Elements	<i>Origanum onites</i> (%)	<i>Thymus fallax</i> (%)
Na	0.9041	1.2067
Mg	1.7974	2.3571
Al	0.0380	0.0810
Si	0.0749	0.1369
P	0.7286	0.4272
S	0.6247	0.6984
Cl	0.1473	0.9382
K	3.9409	6.4317
Ca	1.7779	4.2056
Cr	0.0033	-
Mn	0.0048	0.0162
Fe	0.0149	0.0192
Ni	0.0028	-
Cu	0.0024	0.0020
Zn	0.0036	0.0047
Rb	0.0035	-
Sr	0.0015	0.0075
Br	-	0.0031
H2O	89.9295	83.4646
Total	100	100 *

* The results of heavy metal contents of *Thymus fallax* and *Origanum onites* plant extracts are given as % mass.

3.2. Ash content and moisture levels

According to the moisture content determination method described in the European Pharmacopoeia, the plant extracts exhibited the following results:

- *Thymus fallax* showed a moisture content of 16%.
- *Origanum onites* exhibited a moisture content of 14%.

Similarly, the ash content of the extracts was assessed in accordance with the ash determination procedure outlined in the European Pharmacopoeia. The analyses revealed that:

- *Thymus fallax* contained 14% total ash.
- *Origanum onites* contained 7% total ash.

These values are clearly presented in Table 2, providing a comparative overview of the physicochemical characteristics of the studied extracts.

Table 2. Total phenolic substance amounts, ash and moisture contents of *Thymus fallax* and *Origanum onites* extracts.

Plants	Total phenolic substance amounts (GAE µg/mL)	Ash contents (%)	Moisture levels (%)
<i>Thymus fallax</i> *	41.009 ± 2.059 *	14	16
<i>Origanum onites</i>	28.152 ± 0.389	7	14

* The obtained values (total phenolic contents) were expressed as gallic acid equivalents (µg/mL); ash content and moisture levels of the extracts were expressed as % mass.

3.3 Total phenolic content

Using the calibration curve equation generated with gallic acid in different concentrations (Figure 1), the antioxidant activities of the plant extracts were quantitatively determined. The obtained values were expressed as gallic acid equivalents (µg/mL), providing a standardized basis for comparison among the samples. As presented in Table 2, notable variations were observed in the antioxidant capacities of the extracts, reflecting differences in their phenolic compositions. Overall, the results demonstrate that the gallic-acid-based calibration approach offers a reliable and reproducible means of evaluating the antioxidant potential of the studied plant materials.

Total phenolic concentration of aqueous extracts was quantified as:

Origanum onites: 28.152 ± 0.389 µg GAE/mL

Thymus fallax: 41.009 ± 2.059 µg GAE/mL

These data indicate that *T. fallax* exhibits a higher phenolic content compared to *O. onites*.

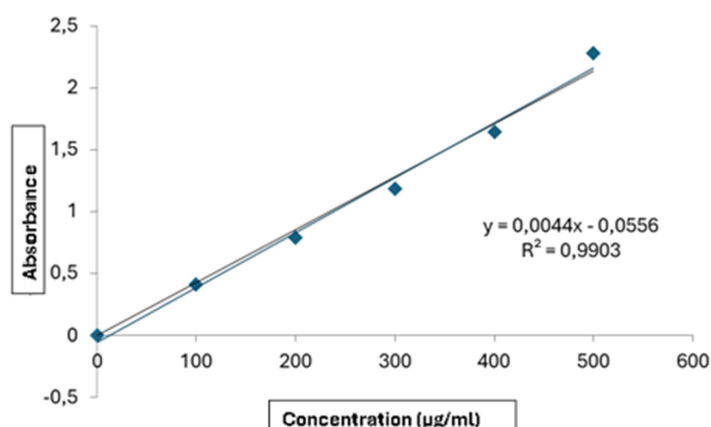


Figure 1. The calibration curve equation generated with gallic acid in different concentrations.

4. Discussion

The present study provides a comprehensive quality evaluation of *Origanum onites* and *Thymus fallax* aqueous extracts based on elemental composition, physicochemical parameters and phenolic profiles. The detection of essential macro- and microelements such as potassium, calcium, magnesium, manganese, zinc and copper in both species confirms their contribution to the mineral composition of herbal teas prepared from these plants. Minerals such as potassium and magnesium are known to play pivotal roles in plant physiology—including protein synthesis, photosynthesis, enzymatic functions and water regulation—and are also essential for maintaining electrolyte balance and metabolic stability in humans. The notably high potassium levels detected in both extracts are therefore consistent with the physiological relevance of this element and may contribute positively to the nutritional value of the herbal preparations. Similarly, the presence of calcium and magnesium in relatively higher proportions highlights their functional role in cellular processes and potential dietary contribution when consumed as herbal infusions.

The WDXRF results showed that the concentrations of toxic elements, including lead, cadmium and mercury, were below detectable limits, indicating that the raw materials were collected from non-contaminated environments or handled appropriately during processing. This is an important safety outcome, as environmental contamination from industrial activity, agricultural practices or atmospheric deposition can introduce toxic metals into medicinal plants. Compliance with pharmacopoeial standards strengthens the reliability of both species for traditional medicinal use and supports their safe incorporation into herbal formulations.

Physicochemical analyses further contributed to the quality assessment. Moisture content values for both species fell within acceptable limits, ensuring stability during storage and reducing the risk of microbial or enzymatic degradation. Ash value determinations also provided insight into the total mineral and inorganic matter content of the samples. The relatively higher ash value of *T. fallax* suggests a greater accumulation of inorganic constituents, which resonates with the mineral findings obtained through WDXRF analysis. Such parameters are essential benchmarks for authentication, purity evaluation and detection of possible adulteration in herbal materials.

The Folin–Ciocalteu assay demonstrated that *T. fallax* possesses a higher total phenolic content than *O. onites*, indicating a potentially greater antioxidant capacity. This result is consistent with existing literature that identifies *Thymus* species as rich sources of flavonoids, phenolic acids and other antioxidant phytochemicals (Kulisic et al., 2005; Dapkevicius et al., 1998). Phenolic compounds are strongly associated with free-radical scavenging, anti-inflammatory and cytoprotective activities and thus the elevated phenolic content of *T. fallax* may translate into enhanced biological activity. Although antioxidant assays were not comprehensively explored in the present study, the phenolic composition findings lay the groundwork for more detailed bioactivity investigations.

Overall, the integration of elemental, physicochemical, and phytochemical analyses highlights the importance of adopting a multidimensional approach to the quality control of medicinal plants. Factors such as harvesting season, ecological conditions, processing

techniques and extraction methods have profound effects on the chemical profile and efficacy of herbal products. The data obtained in this study underscore the need for standardized preparation methods and accurate quality assessment protocols to ensure the safety, reproducibility and therapeutic potential of herbal materials. Moreover, the comparative evaluation of *O. onites* and *T. fallax* provides valuable insights into species-specific differences that may guide their future utilization in phytotherapy and functional herbal products.

5. Conclusions

This study presents a comprehensive preliminary quality assessment of *Origanum onites* and *Thymus fallax* by combining elemental composition, physicochemical parameters and total phenolic content analyses of their aqueous extracts. The WDXRF analysis confirmed that both species comply with international pharmacopoeial limits for heavy metals and no detectable levels of toxic elements such as lead, cadmium or mercury were observed. These findings are consistent with WHO recommendations regarding the safety evaluation of herbal materials and indicate that the investigated plants are environmentally safe and suitable for medicinal and dietary use, particularly in aqueous preparations such as herbal infusions (World Health Organization, 2007).

The physicochemical quality parameters, including moisture and ash values, were found to be within the acceptable ranges defined by the European Pharmacopoeia, supporting the appropriate handling and processing of the plant materials. The relatively higher ash content observed in *T. fallax* suggests a richer inorganic composition, which aligns with the elemental analysis results and supports previous reports indicating species-dependent mineral accumulation in medicinal and aromatic plants (Özcan and Akbulut, 2008). Such variations may be attributed to ecological factors, geographical origin and intrinsic physiological characteristics, highlighting the necessity of species-specific quality control approaches.

In terms of phytochemical composition, *T. fallax* exhibited a significantly higher total phenolic content than *O. onites*, suggesting a stronger antioxidant potential. This finding is in agreement with earlier studies reporting that *Thymus* species are particularly rich in phenolic compounds, including flavonoids and phenolic acids, which are closely associated with antioxidant, anti-inflammatory and cytoprotective activities (Boros et al., 2010; Dai and Mumper, 2010). The well-established relationship between phenolic content and antioxidant capacity further supports the pharmacological relevance of *T. fallax* and its traditional therapeutic use (Rice-Evans et al., 1997).

Moreover, the presence of physiologically important minerals such as potassium, magnesium and calcium in both species underlines their potential nutritional and functional value when consumed as herbal teas. Studies investigating mineral and trace element concentrations in commonly consumed medicinal herbs and their infusions have also found that such macroelements are present in considerable amounts in many herbal products, supporting the idea that regular consumption can contribute to daily mineral intake and overall nutritional value (e.g., Ca, Mg and K were all quantified in various herbal samples) (Margui et al., 2022).

Overall, the findings underline the critical role of proper plant sourcing, standardized processing conditions and validated analytical methodologies in ensuring the safety, quality and consistency of herbal products. Moreover, this study represents, to the best of our knowledge, the first comparative pharmacognostic assessment of aqueous extracts from *O. onites* and *T. fallax*, providing original data that enrich the current literature. In conclusion, both species emerge as promising candidates for safe and effective herbal preparations, with their mineral profiles, physicochemical quality and phenolic content collectively supporting their traditional uses and justifying further in-depth biochemical, antioxidant and pharmacological investigations.

Conflicts of Interests

Authors declare that there is no conflict of interests

Financial Disclosure

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Statement contribution of the authors

P.T. and A.A. collected samples, performed the laboratory analyses. Both authors wrote the draft and approved the final version.

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