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Assessment of some parameters of the chemical composition of *Thymus kotschyanus* Boiss. Growing in different ecological zones of the Republic of Armenia

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ABSTRACT

Background: *Thymus kotschyanus Boiss.*, commonly known as thyme, is a small semi-shrub valued for its aromatic and medicinal properties. Found throughout the Middle East and the Caucasus, it adapts to various ecological conditions and is rich in biologically active compounds, including essential oils, flavonoids, and phenolic acids. Its diverse chemical profile has made it a focus of pharmacological research for its antimicrobial, antioxidant, and anti-inflammatory effects, highlighting its potential therapeutic applications.

Objective: The study aimed to investigate the bioactive compounds in wild *T. kotschyanus* Boiss. plants grown under diverse ecological conditions, with an emphasis on how these compounds change in response to varying environmental contexts.

Materials and Methods: The chemical composition of *Thymus kotschyanus* Boiss. was investigated during its flowering phenophase. Aerial parts of plants were collected from the Tavush (40°880518N, 45°427958E) and Kotayk (40°17'56"N, 44°33'15"E) regions in Armenia between 2021 and 2023. The study focused on analyzing ash and moisture content, phytochemical screening, and mineral content.

Results: The ethanolic extracts of *Thymus kotschyanus Boiss* from both regions contained a diverse array of bioactive compounds, including coumarins, flavonoids, alkaloids, tannins, phenols, carbohydrates, proteins, and saponins. Plants from the Tavush region exhibited a moisture content 1.83% higher than those from the Kotayk region, reflecting a 36.1% increase. Similarly, the ash content was 1.3% higher in Tavush plants, resulting in a 17.1% increase compared to Kotayk plants. Analysis of macro- and microminerals revealed that potassium was the most abundant macromineral, followed by calcium, magnesium, phosphorus, and sodium. Among the microminerals, iron had the highest concentration, followed by zinc and copper.

Conclusion: The study demonstrated significant differences in the biologically active compounds, mineral composition, moisture, and ash content of *T. kotschyanus Boiss.* from different ecological zones in Armenia. Plants from the Tavush region exhibited higher moisture and ash content, as well as elevated levels of vitamins A and C, phenolic content, and essential minerals such as calcium, magnesium, sodium, potassium, and phosphorus, compared to those from the Kotayk region. These findings underscore the impact of environmental conditions on the quality and composition of *T. kotschyanus Boiss.*, highlighting its potential as a valuable source of biologically active compounds and essential minerals.

Keywords: aromatic plants, bioactive compounds, essential minerals, wild thyme, *T. kotschyanus Boiss*

Graphical abstract: *T. kotschyanus* Boiss, aromatic perennial herb, bioactive compounds, ethanolic extract

INTRUDUCTION

In the family Lamiaceae, the genus *Thymus* includes about 350 species of aromatic perennial herbs and compact shrubs [1]. In the Republic of Armenia, five species are found, among which *T. kotschyanus* Boiss., commonly known as thyme, is particularly noteworthy.

This herb has a rich history of medicinal use and culinary applications [2–12], making it a subject of interest for researchers aiming to understand its chemical composition in greater detail. The ethanolic extract of *T. kotschyanus* Boiss. has shown potential due to its antioxidant and health-enhancing properties [13–17].

For medicinal purposes, the vegetative part of the plant is utilized due to its sharp, pleasant, and aromatic scent. This species thrives in mountainous and foothill regions of Armenia, particularly on rocky, sun-drenched slopes, depressions, hills, steppe and alpine meadows, and in pine and juniper forests. It often forms thickets and reproduces both through seeds and vegetative propagation. The plant has oval evergreen leaves measuring 3–8 mm in length, and its fragrant flowers range from lilac to pink-purple, and sometimes white.

Wild thyme has been recognized as a medicinal plant since ancient times. It is described in the book 'Necessary for the Ignorant' authored by the prominent Armenian physician Amirdovlat Amasiatsi in the 15th century. The original manuscript, preserved in the Matenadaran, contains extensive information in five languages about the medicinal properties of various plants, including Thymus species found in the Middle East. These plants are described as functional products that contribute to overall well-being and potentially reduce the risk of diseases [18]. *T. kotschyanus* Boiss.'s aromatic flavor and health benefits make it a versatile herb for culinary use and as a calming tea [19]. Additionally, thyme products can reduce bacteria in the mouth and help prevent oral diseases such as halitosis (bad breath), gingivitis, periodontitis, and caries [20–22].

The essential oil (EO) of *T. kotschyanus* Boiss. contains significant amounts of thymol (18.8%), carvacrol (17.4%), o-cymene (15.4%), and geraniol (10.7%). Non-volatile compounds include phenolic acids (such as rosmarinic, salvianolic, and caffeic acids) and flavonoids (glucosides of luteolin, apigenin, and their derivatives). These bioactive compounds contribute to the plant's antioxidant, antimicrobial, antitumor, and cytotoxic properties [23–26]. *Thymus* species thrive in diverse environments, such as dry, sandy soils in steppe zones, and are resilient enough to establish themselves in coniferous and deciduous forests, meadows, and rocky terrains.

Functional foods, which offer health benefits beyond basic nutrition, are of significant research interest, particularly in the fields of health enhancement and food technology [27–31].

The core principle behind functional foods is their inclusion of bioactive compounds. These compounds can be sourced from plants, fungi, or animal products. By systematically identifying the bioactive components in these foods, evaluating their bioavailability, determining appropriate dosages, and pinpointing relevant biomarkers, we can gain a clearer insight into the specific roles these compounds play within functional foods [32].

Geoclimatic factors and soil types are known to influence the chemical composition of thyme EO, leading to the development of different chemotypes with distinct sensory properties and biological activities [33–37].

This study aims to investigate the bioactive compounds in wild *T. kotschyanus* Boiss. plants growing under varying ecological conditions. By focusing on different environmental contexts, we seek to understand how these compounds vary across diverse habitats.

MATERIALS AND METHODS

We investigated the chemical composition of *Thymus kotschyanus* Boiss. during its flowering phase. Aerial parts were collected from the Tavush and Kotayk regions of Armenia between

2021 and 2023. Specifically, the plants were gathered at the beginning of the flowering period from Verin Karmiraghbyur village, Tavush Province (40°88'518"N, 45°42'7958"E), and Nor Artamet village, Kotayk Province (40°17'56"N, 44°33'15"E).

The extraction methods used in this study followed established procedures described in the literature [38]. To determine the moisture content, 3.00 grams of the powdered sample were accurately weighed and placed in a dry, clean crucible. The crucible, along with the sample, was then dried in an oven at 100°C for 3 hours. Moisture content was calculated using the formula:

$$\text{Moisture Content } \% = \frac{\text{Initial Weight-dry weight}}{\text{Initial Weight}} \times 100$$

To determine the ash content, approximately 3.00 grams of the sample were accurately weighed and transferred to a clean, dry crucible. The crucible was then heated in a muffle furnace at 500–600°C until all organic matter had evaporated. The remaining inorganic ash was weighed, and ash content was expressed as a percentage using the formula:

$$\text{Ash Content } \% = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

hytochemical screening was conducted according to established protocols from the relevant literature [39]. Phenolic content was assessed using the Folin-Ciocalteu assay [40], and essential metal analysis was performed using X-ray fluorescence (XRF) [41]. The iodine titration method was employed to determine the vitamin C content in *T. kotschyanus* Boiss., while the total vitamin A and vitamin E contents in the samples were measured using the chemical method described by Idris et al. [42].

Data from three separate experiments were combined and reported as mean values. The treatment means were evaluated using the standard error (*SE*) of the mean. Significant differences between means were determined using a *student's t-test,* with a significance threshold set at P < 0.05.

RESULTS

A diverse array of biologically active compounds was identified in the alcoholic extracts of plants collected from both ecological zones. The analysis confirmed the presence of coumarins, alkaloids, flavonoids, phenols, carbohydrates, tannins, proteins, and saponins in these extracts. Additionally, Table 1 displays the moisture and ash content of *T. kotschyanus* Boiss from different agroecological zones. Notably, the moisture content was higher in the Tavush population (6.9%) compared to the Kotayk population (5.07%).

Table 1. Moisture and Ash Contents of T. kotschyanus Boiss from Different Agro-Ecological Zones

Location of plants population	Moisture Content (%) ± SE	Ash Content (%) ± SE
Verin Karmiraghbyur (Tavush)	6.9 ± 0.03	8.9 ± 0.1
Nor Artamet (Kotayk)	5.07 ± 0.03	7.6 ± 0.2

mean ± SE, n = 3

The difference in moisture content between the Tavush and Kotayk regions was 1.83% (6.9% - 5.07%). The percentage increase in moisture content for plants from Tavush compared to those from Kotayk was approximately 36.1% [(1.83 / 5.07) × 100]. This indicates that *T. kotschyanus* Boiss plants from the Tavush region had a higher moisture content than those from the Kotayk region. The difference in ash content between the two regions was 1.3% (8.9% - 7.6%). The percentage increase in ash content for plants from Tavush compared to those from Kotayk was approximately 17.1% [(1.3 / 7.6) × 100], suggesting that plants from Tavush also exhibited higher ash content.

As shown in Figure 1, *T. kotschyanus* Boiss exhibited notable variations in vitamin content across different regions. Specifically, samples from the Kotayk region contained 5.10 mg/100 g of vitamin A, while those from Tavush had a higher concentration of 7.20 mg/100 g. Vitamin C content showed differences as well, with Kotayk samples containing 45.80 mg/100 g and Tavush samples having 55.90 mg/100 g. Additionally, thyme samples from Kotayk had 14.60 mg/100 g of vitamin E, whereas those from Tavush had slightly less, at 13.90 mg/100 g.

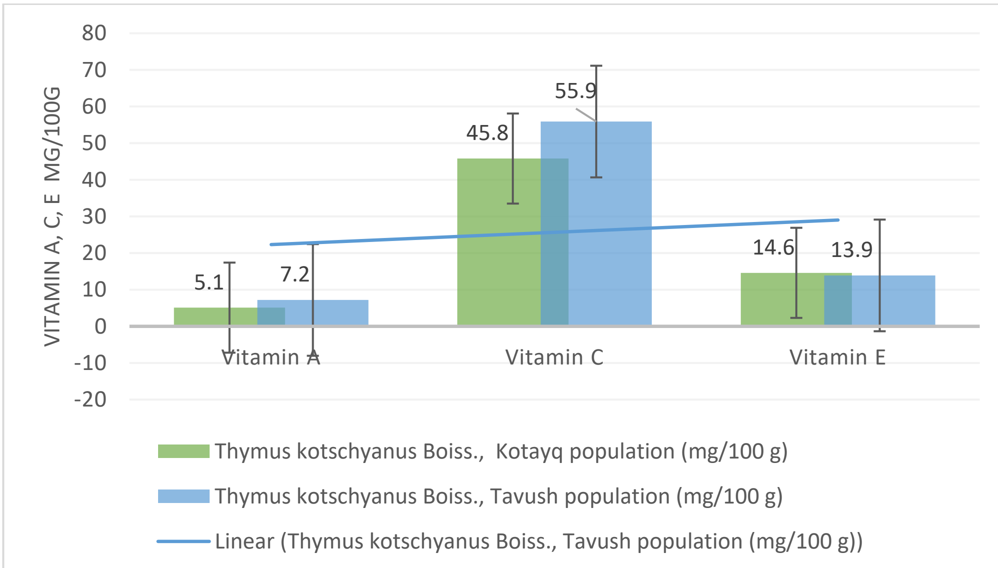

Figure 1: Vitamin C, A, and E Contents in *T. kotschyanus Boiss.* Across Different Ecological Zones

These regional variations highlight how local environmental factors can influence the nutritional profile of *T. kotschyanus* Boiss.

In the Tavush Ecological Zone, the phenol content in *T. kotschyanus* Boiss was measured at 98.6 mg/g, whereas in the Kotayk Ecological Zone, it was 72.7 mg/g (Fig. 2). This variation illustrates the impact of regional environmental conditions on phenolic compounds in the plant.

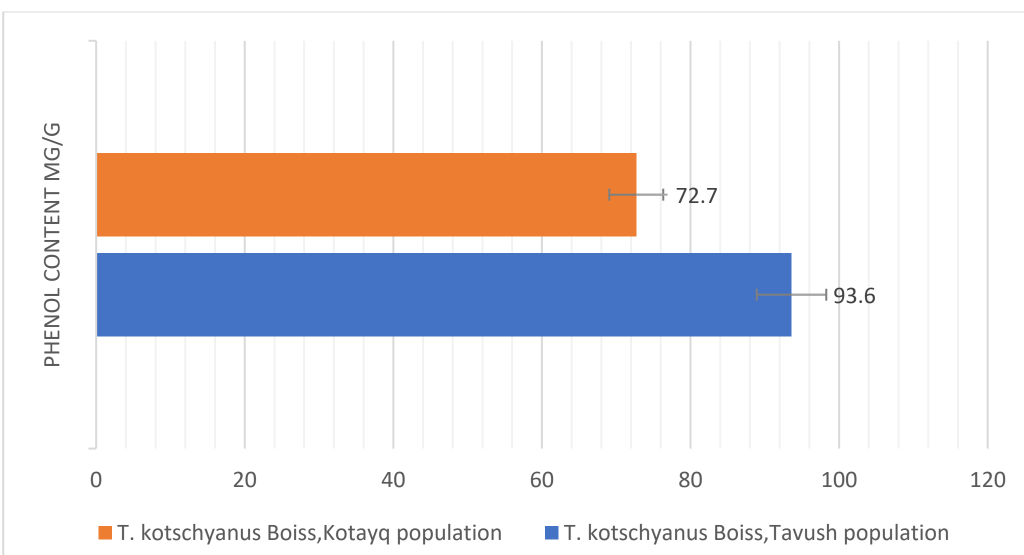

Figure 2: The phenol content in *T. kotschyanus* Boiss**.** across different regions

In our study, we analyzed the mineral content of *T. kotschyanus* Boiss across various agro-ecological regions to understand how different environmental conditions influence the mineral composition of this plant. Table 2 presents the levels of key minerals in *T. kotschyanus* Boiss samples collected from these different regions.

Table 2. Mineral Levels in *T. kotschyanus* Boiss Grown in Different Agro-Ecological Regions

Mineral (mg/kg)	Concentration (mg/kg) in Tavush Population (Verin Karmiraghbyur) Mean ± SE	Concentration (mg/kg) in Kotayk Population (Nor Artamet) Mean ± SE
Potassium (K)	14,959 ± 298	13,052 ± 302
Calcium (Ca)	12,138.9 ± 159	11,120.4 ± 125
Magnesium (Mg)	3,410.2 ± 37	2,984.0 ± 45
Phosphorus (P)	1,665 ± 65	1,365 ± 58
Sodium (Na)	330.5 ± 55	268.06 ± 41
Iron (Fe)	125.45 ± 1.7	103.51 ± 2.5
Copper (Cu)	5.95 ± 0.5	6.76 ± 0.4
Zinc (Zn)	20.80 ± 0.7	24.55 ± 0.6

The analysis of mineral content in *T. kotschyanus* Boiss from the Tavush (Verin Karmiraghbyur) and Kotayk (Nor Artamet) populations revealed significant differences in the concentrations of various essential minerals. The Tavush population exhibited a mean calcium concentration of 12,138.9 mg/kg (±159), significantly higher than the Kotayk population's mean of 11,120.4 mg/kg (±125), reflecting an approximate 9.1% increase in calcium content. Tavush plants also showed elevated magnesium levels, with a mean concentration of 3,410.2 mg/kg (±37) compared to 2,984.0 mg/kg (±45) in Kotayk plants, corresponding to a 14.3% increase in magnesium content. Sodium levels were higher in Tavush plants (330.5 mg/kg ±55) compared to Kotayk plants (268.06 mg/kg ±41), indicating a 23.3% increase. The mean potassium concentration in Tavush plants was 14,959 mg/kg (±298), significantly higher than the 13,052 mg/kg (±302) found in Kotayk plants, representing a 14.6% increase in potassium levels. Similarly, Tavush plants had a higher mean phosphorus concentration of 1,665 mg/kg (±65) compared to 1,365 mg/kg (±58) in Kotayk plants, marking a 22.0% increase in phosphorus content. In terms of iron, Tavush plants had a concentration of 125.45 mg/kg (±1.7), higher than the 103.51 mg/kg (±2.5) observed in Kotayk plants, reflecting a 21.2% increase. Conversely, the mean copper concentration was lower in Tavush plants (5.95 mg/kg ±0.5) compared to Kotayk plants (6.76 mg/kg ±0.4), indicating an 11.9% decrease. Similarly, Tavush plants had a lower mean zinc concentration of 20.80 mg/kg (±0.7) compared to 24.55 mg/kg (±0.6) in Kotayk plants, reflecting a 15.27% decrease.

DISCUSSION

The analysis of *Thymus kotschyanus* Boiss extracts from Verin Karmiraghbyur (Tavush region) and Nor Artamet (Kotayk region) revealed significant variations in bioactive compounds, including coumarins, alkaloids, flavonoids, phenols, carbohydrates, tannins, and proteins. These compounds, known for their medicinal properties, underscore the therapeutic potential of *Thymus kotschyanus* Boiss from these distinct geographical regions.

Notable differences were observed in moisture and ash content between the two regions. The moisture content of dried plant material from Tavush was 6.9%,

compared to 5.07% from Kotayk, reflecting a 1.83% difference, or a 36.1 % increase. This higher moisture content in Tavush plants may be attributed to regional variations in climate, soil composition, or other environ- mental factors affecting water retention. Such differences are significant, as moisture content influences the plant's overall quality, nutritional value, and chemical profile.

Similarly, ash content was higher in Tavush plants (8.9%) compared to Kotayk plants (7.6%), showing a 1.3% difference, which represents a 17.1% increase. This suggests a greater concentration of mineral elements in Tavush plants, potentially due to variations in soil mineral composition. Ash content is a crucial indicator of the plant's mineral composition and overall nutritional value.

Dauqan and Abdullah [40] highlighted thyme oil as a valuable source of vitamins, including B-complex, folic acid, beta-carotene, and vitamins A, K, E, and C [43]. Our study also assessed vitamin content, revealing that Tavush plants contained 2.1 mg/100 g more vitamin A and 10.1 mg/100 g more vitamin C compared to Kotayk plants, representing increases of approximately 41.18% and 22.03%, respectively. Higher levels of vitamin C, an essential nutrient with various health benefits [44-46], were found in Tavush plants. However, the vitamin E content was 0.70 mg/100 g lower in Tavush plants, indicating a 4.79% decrease. Vitamin E, a critical antioxidant, helps protect cells from oxidative damage [47-48]. The lower vitamin E content in Tavush plants might suggest a trade-off between different vitamins based on regional growing conditions.

The Tavush population of *Thymus kotschyanus* Boiss exhibited a significantly higher phenolic content (93.6 mg/g) compared to the Kotayk population (72.7 mg/g). Phenols are known for their antioxidant and health-promoting properties [49-50]. The phenolic content in Tavush plants aligns with previously reported values, such as those by Shahar et al. (2023), who found a phenol content of 86.6 mg/g in creeping thyme [51].

Mineral concentrations varied significantly between regions. Tavush plants exhibited higher levels of essential macroelements: calcium (9.16% higher), magnesium (14.3% higher), sodium (23.3% higher), potassium (14.6% higher), and phosphorus (22.0% higher). These elements are vital for various physiological functions, including cell wall structure, photosynthesis, and nutrient transport [52-57]. Higher mineral levels in Tavush plants may reflect differences in soil composition and environmental conditions.

Among microminerals, iron concentration was 21.2% higher in Tavush plants compared to those from Kotayk. Iron is essential for chlorophyll synthesis and electron transport, and deficiency can lead to leaf yellowing (iron chlorosis). However, excess iron can cause oxidative stress and other detrimental effects [58]. Conversely, Tavush plants had lower concentrations of copper (11.9% lower) and zinc (15.27% lower) compared to Kotayk plants. Copper and zinc are crucial for enzyme functions and growth regulation, and imbalances in these minerals can impact plant health and productivity [59-60].

These findings underscore the influence of geographical and environmental factors on the biochemical properties of *Thymus kotschyanus* Boiss. They highlight the importance of considering regional variations when evaluating the quality and composition of medicinal plants. Future research should explore a broader range of environmental conditions and geographical locations to gain a comprehensive understanding of these effects. Longitudinal studies could provide deeper insights into seasonal variations and long-term adaptability. Additionally, investigating different extraction methods could optimize the production of high-quality extracts. Collaboration with agricultural scientists to develop sustainable cultivation practices could further enhance the desired chemical properties of *Thymus kotschyanus* Boiss.

CONCLUSION

Our study revealed significant regional variations in *Thymus kotschyanus Boiss.*, with plants from Tavush exhibiting higher moisture, ash, vitamin A, vitamin C, phenolic content, and essential minerals compared to those from Kotayk. A diverse array of bioactive compounds, including coumarins, flavonoids, alkaloids, tannins, phenols, carbohydrates, proteins, and saponins, was identified in the ethanolic extracts from both regions. Tavush plants had elevated levels of calcium, magnesium, sodium, potassium, phosphorus, and iron, while copper and zinc concentrations were lower compared to Kotayk plants. These differences highlight the impact of environmental conditions on the plant's quality and nutritional value. The findings suggest that *Thymus kotschyanus Boiss.* from Tavush may offer enhanced bioactive properties and nutritional benefits. Future research should explore the effects of seasonal and geographical variations in greater detail to optimize the use of this plant in various applications.

Abbreviations: ANAU: Armenian National Agrarian University; mg/kg: milligrams per kilogram; EO: essential oil; Ca: calcium; Mg: magnesium; Na: sodium; K: potassium; P: phosphorus; Fe: iron; Cu: copper; Zn: zinc; SE: Standard Error.

Conflicts of interest*:* The authors declare that they have no conflict of interest.

Author Contributions: MH, GM, AS, and YuM, MH drafted the experimental design. MH, GM, KK, and YuM, GA conducted the experiments. GM, AS, GA and MH were involved in data collection, analysis, and drafting the initial manuscript. All authors reviewed and approved the final version of the manuscript.

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