

## Phytochemical Screening, Antioxidant Capacity Measurement, and Mineral Content Determination of *Thymus vulgaris L.* Extracts

**Khaled Muftah Elsherif<sup>1</sup>, Abdunaser Mabrok Ewlad-Ahmed<sup>2</sup>, Ebtessam Abdallah Ahmed Alhbad<sup>2</sup>, Abdulrhman Mohammed Iqneebir<sup>2</sup>**

<sup>1</sup>Libyan Authority for Scientific Research, Tripoli, Libya

<sup>2</sup>Chemistry Department, Faculty of Arts and Science Msallata, ElMergib University, Al-Khoms, Libya

\*Corresponding author: [elsherif27@yahoo.com](mailto:elsherif27@yahoo.com)

Received: 5 September 2023; Accepted: 3 November 2023; Published: 15 December 2023

### Abstract

The aim of this study was to evaluate the chemical composition and antioxidant activity of *Thymus vulgaris L.* extracts. *T. vulgaris L.* is a medicinal plant that has various bioactive compounds. Four different solvents (ethanol, water, ethyl acetate, and chloroform) were used to extract these compounds from the plant. The phytochemical screening of the extracts showed that the ethanolic extract had the highest diversity of compounds, including coumarins, flavonoids, alkaloids, tannins, phenols, carbohydrates, and proteins. The total phenolic and total antioxidant contents of the ethanolic extract were measured by the Folin-Ciocalteu and phosphomolybdenum methods, respectively. The ethanolic extract had a high phenolic content of 77.7 mg gallic acid equivalent/g dry weight, which indicates its potential antioxidant capacity. The mineral content of *T. vulgaris L.* was also assessed by flame photometry and atomic absorption spectrophotometry after dry digestion. The plant contained five macroelements (K, Na, Ca, Mg, P) and three microelements (Fe, Cu, Zn) in different concentrations. The highest concentration was found for potassium (15259 mg/kg), followed by calcium (5118 mg/kg) and sodium (4793 mg/kg). The lowest concentration was found for phosphorus (1400 mg/kg), which was still higher than the microelements. Among the microelements, zinc had the highest concentration (24.82 mg/kg), followed by iron (17.44 mg/kg) and copper (14.98 mg/kg). The results of this study provide useful information for the users, collectors and practitioners of medicinal plants from polluted areas, as they can evaluate the quality and safety of *T. vulgaris L.* for human consumption.

**Keywords:** *Thymus vulgaris L.*; Phytochemical screening; Total phenolic; antioxidant contents; Mineral analysis

### Introduction

Some medicinal plants, including *T. vulgaris L.*, have been recognized for their effects on the immune system (Nadi et al., 2023). These plants have been widely utilized

by people worldwide as herbal remedies to address various health concerns, making up approximately 64% of the global population (Li et al., 2024). Moreover, many synthetic drugs are derived from plant chemicals, highlighting the significant role of plants in

pharmaceutical development (Li et al., 2024). Interestingly, certain plants and their active compounds possess the ability to modulate immune responses (Patil et al., 2021). For instance, compounds such as flavonoids and hydroxylated phenols found in herbs have demonstrated antimicrobial properties and can combat infections (Patil et al., 2021). As the immune system plays a crucial role in various diseases, regulating its functions presents a potential avenue for treatment (Khazdair et al., 2021). Herbal interventions can influence the immune system by impacting the production and release of cytokines and immunoglobulins, altering the activities of immune cells, and influencing the expression of cellular receptors (Habashy et al., 2018).

*T. vulgaris L.*, a perennial herb native to central and southern Europe, North Africa, and Asia, has a rich history of traditional medicinal use (Posgay et al., 2022). It has been employed to treat gastroenteric and bronchopulmonary disorders, thanks to its anthelmintic, carminative, sedative, and diaphoretic properties (Posgay et al., 2022). The essential oil derived from *T. vulgaris* has been extensively studied, revealing a range of beneficial properties including antiworm, antispasmodic, antiseptic, antimicrobial, and antioxidant effects (Becer et al., 2023). As a result, *T. vulgaris* has emerged as a focal point for research, with investigations focused on its chemical composition and biological activities (Becer et al., 2023).

In recent years, there has been a growing interest in phytochemicals as potential natural sources of antioxidant and antimicrobial agents (Begum et al., 2022). The utilization of synthetic antioxidants in the food industry is heavily regulated, with restrictions imposed on their application and concentration (Begum et al., 2022). Concerns surrounding the safety of chemical preservatives have sparked debates, as they have been associated with potential carcinogenic and teratogenic effects, as well

as residual toxicity (Kalinda & Rioba, 2020). Plant-derived polyphenols, including those found in *T. vulgaris* and other herbs, have garnered significant attention due to their potential antioxidant and antimicrobial properties (Nadeem et al., 2022).

Phenolic compounds, in particular, exhibit notable free-radical scavenging (antioxidant) activity, attributed to their ability to donate hydrogen or electrons, the stability of resulting antioxidant-derived radicals, their reactivity with other antioxidants, and their metal chelating properties (Elsherif et al., 2023). These characteristics contribute to their potential therapeutic applications in various fields.

This study aimed to conduct a qualitative phytochemical screening of four *T. vulgaris L.* extracts (water, ethanol, ethyl acetate, and chloroform) to identify the bioactive compounds present. Furthermore, the investigation aimed to assess the moisture and ash contents of the plant, as well as determine the levels of total phenols and antioxidants. Additionally, the concentrations of potassium, sodium, calcium, magnesium, phosphorus, copper, iron, and zinc in the herbal plant sample were measured using flame photometry and flame atomic absorption spectrometry.

## Methods

### *Harvesting and processing plant material*

The aerial parts of *T. vulgaris L.*, were collected from the Msallata region of Libya between January and March 2022. A plant expert from the Botany Department in the Faculty of Science at Misurata University verified the plant samples morphologically. The voucher specimens were kept in the Laboratory of Plant Science in the same department. The collected plant samples were cleaned and shaded at room temperature (24-25°C) for 15 days on laboratory benches. Subsequently, the dried plant material was powdered using a blender

and stored in an air-tight container for future use.

#### *Extraction procedures*

In this study, the plant powder underwent maceration using 200 mL of one of four solvents: distilled water, ethanol, chloroform, or ethyl acetate. The ratio of plant powder to solvent was 20 g to 200 mL, following the methods described in literature (Najah & Elsherif, 2016). After maceration, the mixture was left to stand at room temperature for 72 hours before being filtered with Whatman No.1 filter paper. The resulting filtrate was then concentrated using a water bath and dried in an oven at 40°C. This process resulted in the production of a brownish-black semi-solid extract. The crude plant extracts were collected and stored in airtight glass bottles in the refrigerator for future use. These extracts were then utilized for phytochemical screening and evaluating the total phenols and total antioxidant activity of the plants.

#### *Chemicals, reagents, and instruments*

The study extracted plant samples using solvents such as ethanol, chloroform, and ethyl acetate and reference materials and reagents (ascorbic acid, gallic acid, sodium phosphate, ammonium molybdate, and Folin-Ciocalteu reagent), which were all obtained from the Sigma-Aldrich distributor. All the chemicals, reagents, and solvents used in the study were of analytical grade, unless otherwise specified. The study employed the following equipment for analysis: a rotary evaporator (Hei-VAP, Heidolph), a UV-Vis spectrophotometer (6300, Jenway), an Atomic Absorption System (AAS) (Varian 220 FS), and a Flame Photometer (PFP7, Jenway).

#### *Phytochemical Screening*

The current investigation involved the identification of various compounds, such as carbohydrates, proteins, phenols, alkaloids, flavonoids, tannins, saponins, steroids,

glycosides, coumarins, and terpenes, within the four plant extracts (aqueous, ethanolic, ethyl acetate, and chloroform). The identification process followed established procedures described in relevant literature sources (Elsherif & Aljaroushi, 2021; Najah et al., 2015).

#### *Determination Yield, Moisture, and ash*

The determination of the extraction yield involved measuring the quantity of dry extract obtained per 100 g of fresh plant sample. The calculation of the extraction yield was carried out by evaporating the solvent-extracted plant in a water bath set at 40°C, followed by drying it for 24 hours in an air oven at the same temperature. The extraction yield was then calculated by considering the final dry weight of the extract, utilizing the following equation Elsherif & Aljaroushi, 2021:

$$\% \text{ Yield} = \frac{\text{Wt of dry extract (g)}}{\text{Wt of fresh sample (g)}} \times 100 \quad (1)$$

To determine the moisture content of the plant sample powder, a dry and clean crucible was utilized to weigh 3.00 g of the sample powder. This crucible, containing the sample, was then placed in a drying oven at 100°C for 3 hours. After completing the drying process, the crucible was left to cool in a desiccator for 15 minutes before being reweighed. This process was repeated until a consistent weight was achieved. To calculate the moisture content of the sample, the equation below (Yaghi et al., 2022) was used, where  $w_1$  represents the weight of the sample before drying and  $w_2$  represents the weight of the sample after drying:

$$\% \text{ Moisture} = \frac{w_1 - w_2}{w_1} \times 100 \quad (2)$$

To assess the ash content of the plant sample, a clean and dry porcelain crucible was used to weigh 3.00 g of the sample. The crucible, with the sample inside, was then placed in a muffle furnace and subjected to a temperature of 550°C for a duration of 3 hours. After completion of the heating

process, the crucible was allowed to cool in a desiccator for 15 minutes before being reweighed. The ash content was determined by employing the following equation (Yaghi et al., 2022):

$$\% \text{ Ash} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100 \quad (3)$$

#### *Determination of total phenols*

The determination of the total phenolic content in the plant extracts was conducted using a slightly modified version of the Folin-Ciocalteu reagent method (Akbari et al., 2023). In this method, phenols are converted to phosphomolybdate-phosphotungstic acid in an alkaline medium, resulting in a blue-colored solution whose absorption at 760 nm is measured.

To perform the assay, 0.25 ml of the ethanolic extract was combined with 1.0 ml of diluted (1:10) Folin-Ciocalteu reagent in a test tube. The mixture was then diluted to 10 ml with distilled water. After 5 minutes in darkness, 0.8 ml of a 7.5% Na<sub>2</sub>CO<sub>3</sub> solution was added to each tube, and thorough mixing was performed manually. Subsequently, the test tubes were kept in a dark location for 60 minutes before measuring the absorbance of the samples using a UV spectrophotometer at a fixed wavelength of 760 nm.

The concentration of phenolic compounds in the extracts was determined using a gallic acid calibration curve. Gallic acid standards were prepared at concentrations of 10, 20, 40, 60, 80, and 100 mg/L to establish the calibration curve. The amount of phenolic compounds present in the extracts was expressed as gallic acid equivalence (mg gallic acid/g dry sample).

#### *Antioxidant activity*

The determination of the total antioxidant capacity (TAC) in the ethanolic extract of *Thymus vulgaris* was conducted using the phosphomolybdenum assay, as

outlined in the literature (Divya et al., 2023). This assay involves the reduction of Mo(VI) to Mo(V) by the antioxidant compounds present in the plant extract, resulting in the formation of a green phosphate/Mo(V) complex. The absorbance of this complex is measured spectrophotometrically at 695 nm, and the TAC is expressed in milligrams of ascorbic acid equivalents (mg ascorbic acid/g dry sample).

To perform the assay, 0.3 mL of each ethanolic extract solution was mixed with 3.0 mL of phosphomolybdenum reagent (composed of 28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulfuric acid) in capped test tubes. The samples were then incubated in a water bath at 95°C for 60 minutes. After cooling to room temperature, the absorbance of the solutions was measured against a blank using a UV-visible spectrophotometer at 695 nm (0.3 mL ethanol without plant extract).

To calculate the TAC of the extracts, an ascorbic acid calibration curve was employed. Ascorbic acid standards were prepared at concentrations of 20, 40, 60, 80, 100, and 120 mg/L. The TAC values were expressed as milligrams of ascorbic acid equivalents (mg ascorbic acid/g dry sample).

#### *Determination of essential metals*

The concentrations of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P), iron (Fe), and zinc (Zn) in the powdered samples were determined using a Jenway flame photometer and a VARIAN 220 atomic absorption spectrometer (Elbagermi et al., 2020). The dry digestion method was employed to prepare the samples for analysis (Alkheraz et al., 2019). To prepare the sample, 1.0 g of the material was placed in a porcelain crucible. The temperature of the furnace was gradually increased from room temperature to 550°C over the course of one hour. The sample was then ashed for approximately 4 hours until a

white or grey ash residue was obtained. The residue was dissolved by mixing it with 2.0 ml of concentrated HNO<sub>3</sub> and heating it slowly as required. The resulting solution was transferred to a 100-ml volumetric flask and brought up to volume. A blank control was prepared using only the solvent in the same manner. The samples were stored in polyethylene containers in a refrigerator until analysis.

Before analysis, all glassware was cleaned by soaking it in a 10% nitric acid solution overnight and rinsing it three times with distilled water. It is important to acknowledge that determining the mineral and heavy metal content in samples is crucial for evaluating their nutritional and toxicological properties. However, the accuracy of the results can be influenced by

various factors, including the sample type, the analytical method employed, and the sample preparation procedure.

## Result and Discussion

### Phytochemical screening

The phytochemical screening experiments conducted on different plant extracts (ethanol, aqueous, chloroform, and ethyl acetate) of thyme revealed the presence of diverse bioactive compounds. These phytochemical compounds have well-established medicinal significance. The findings of the phytochemical screening are presented in Table 1.

**Table 1.** Phytochemical Analysis of Four Extracts from *T. vulgaris L.*

No.	Phytochemicals	Solvents			
		Ethanol	Water	Ethyl acetate	Chloroform
1	Steroids	-	-	-	-
2	Coumarins	+	+	+	+
3	Flavonoids	+	+	+	+
4	Alkaloids	+	+	+	-
7	Tannins	+	+	-	-
8	Phenols	+	+	-	-
11	Carbohydrates	+	+	+	+
12	Terpenes	-	-	-	-
13	Saponins	-	-	-	-
14	Glycosides	-	-	-	-
15	Proteins	+	+	+	+

• (+): present; (-): absent

The investigation of both ethanolic and aqueous extracts, as shown in Table 1, indicated the presence of coumarins, flavonoids, alkaloids, tannins, phenols, carbohydrates, and proteins. However, glycosides, saponins, steroids, and terpenes were not detected in any of the plant extracts. The observation reveals that the ethyl acetate extract contains coumarins, alkaloids, flavonoids, carbohydrates, and proteins, while it lacks tannins, phenols, steroids,

terpenes, saponins, and glycosides. Based on the findings presented in Table 1, it was observed that the chloroform extract exhibited the presence of coumarins, flavonoids, carbohydrates, and proteins. Conversely, glycosides, saponins, phenols, tannins, alkaloids, steroids, and terpenes were not detected in the chloroform extract.

The study conducted by Dahiya & Purkayastha (Dahiya & Purkayastha, 2012) demonstrated that the ethanolic extract of

*Thymus* contained tannins and steroids, which aligns with our findings. However, their results differed from ours regarding the presence of glycosides, saponins, flavonoids, and terpenes. Conversely, the study by Nema et al. (Nema et al., 2015) indicated that the ethanolic extract exhibited positive results for alkaloids, carbohydrates, flavonoids, and tannins, which is consistent with our results. However, their findings diverged from ours in terms of glycosides, saponins, and steroids. Benbelaïd et al. (Benbelaïd et al., 2013) reported the presence of flavonoids, tannins, terpenes, saponins, steroids, and coumarins in thymus extract, which is consistent with current research. However, they did not detect any alkaloids in their sample.

According to a study conducted by Roby et al. (Roby et al., 2013) on *Thymus* extract, tannins were found in the ethyl acetate extract, which is consistent with our findings. However, their results differed from ours in terms of alkaloids, steroids, flavonoids, and saponins. Additionally, the research conducted by Labiad et al. (Labiad et al., 2017) revealed the presence of alkaloids, flavonoids, and saponins in the ethyl acetate extract, which aligns with our results. However, their findings diverged from ours

regarding steroids, proteins, and coumarins. According to the study conducted by Roby et al. (Roby et al., 2013) on *Thymus* extract, it was reported that the chloroform extract contained alkaloids, steroids, saponins, and tannins, which is consistent with our findings. However, their results differed from ours in terms of flavonoids.

#### *Yield, moisture, and ash levels*

The yields were determined by calculating the percentage of the extract weight relative to the original weight of the dried sample used. As shown in Table 2, the findings indicated that the extraction percentages were influenced by the choice of solvent, with aqueous extracts displaying the highest yield values. The results of the *Thymus* extracts ranged from 3.75% to 29.33%. Notably, the highest percentage was obtained with ethyl acetate, followed by water (13.5%), chloroform (4.5%), and finally, the lowest yield was observed with ethanol. These outcomes were comparatively higher than those reported in the investigation conducted by Labiad et al. (Labiad et al., 2017).

**Table 2.** Yield, Moisture, and Ash Contents of *T. Vulgaris L.*

%	Ethanolic extract	Aqueous extract	Ethyl acetate extract	Chloroform
<b>Yield</b>	3.75±0.20	13.50±0.68	29.33±1.47	4.50±0.23
<b>Moisture</b>	13.80±0.69			
<b>Ash</b>	8.00±0.40			

- Mean ± standard deviation of three replicates

Also, Table 2 presents the moisture value of the plant examined in this study. In comparison to the findings reported by Ifesan et al. (Ifesan et al., 2006), our study revealed relatively higher moisture contents, which can be attributed to the utilization of fresh plant material. Furthermore, the ash content result obtained in this study was found to be higher compared to those reported by

Sadowska et al. (Sadowska et al., 2017) and Ifesan et al. (Ifesan et al., 2006). The ash contents of the plants analyzed in this study are presented in Table 2.

#### *Total Phenols contents*

Phenolic compounds are plant metabolites that have antioxidant activity due

to their redox properties. They can scavenge free radicals by donating hydrogen atoms from their hydroxyl groups. The solubility of phenolic compounds depends on the polarity of the solvent and the number and position of the hydroxyl groups. Ethanol was chosen as the extraction solvent because it can dissolve phenolic compounds with different hydroxyl configurations. The phenolic content of the ethanolic extract was determined by using the Folin-Ciocalteu method, which measures the reduction of a phosphomolybdic-phosphotungstic acid complex by phenolic compounds. A standard curve of gallic acid (10–80mg/L) was used to calculate the phenolic content of the extract, expressed as Gallic Acid Equivalents (GAE) per gram of dry extract weight (Figure 1). The equation of the standard curve was  $y = 0.006 x$ , with a coefficient of determination ( $R^2$ ) of 0.9995.

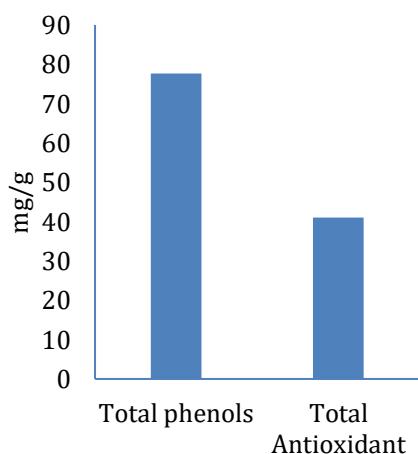


Figure 1. Total phenolic and antioxidant contents in studied plants *T. vulgaris L.*

Our findings demonstrated similar total phenolic contents when compared to the results obtained by Amamra et al. (Amamra et al., 2018), who determined the total phenols in six solvent extracts of *T. vulgaris*, ranging from 77.27 mg/g to 270.9 mg/g. Additionally, a recent investigation conducted by Labiad et al. (Labiad et al., 2017), which involved several *T. vulgaris* extracts, revealed comparable levels of total phenolics. In

contrast, Habashya et al. (Habashya et al., 2018) reported lower total phenolic levels than our present study [3.00 - 44.16 mg/g]. The values of phenolic content in our study showed slight variations compared to those reported in the literature. These differences may be attributed to varying levels of sugars, carotenoids, or ascorbic acid, as well as factors such as duration, geographical variation, or methods of extraction, which can influence the quantities of phenols present.

Figure 2 demonstrates a positive correlation between the total phenolic content and extract concentration, indicating that higher concentrations of the extract correspond to higher phenolic content. This relationship confirms the efficacy and suitability of the method employed in the estimation process.

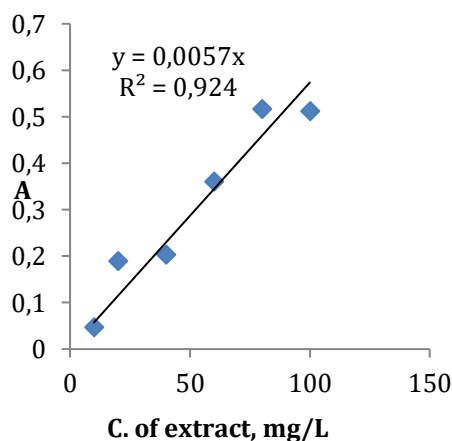


Figure 2. Variation of phenolic content with extract concentration of *T. vulgaris L.*

#### Antioxidant activity

The total antioxidant capacity reflects the cumulative effect of phenolics, flavonoids, and other reducing compounds in the plant extracts. It is measured by the phosphomolybdenum method, which involves the reduction of Mo (VI) to Mo (V) by the antioxidants and the formation of a green complex with phosphate that absorbs at 695nm. The total antioxidant capacity of the

extracts is expressed as ascorbic acid equivalents (AAE) per gram of dry extract weight, based on a standard curve of ascorbic acid (2.0 – 20.0mg/L) with the equation  $y = 0.0242 x$  and a coefficient of determination ( $R^2$ ) of 0.9986.

The total antioxidant capacity of *Thyme* was  $41.08 \pm 2.05$  mg AAE/g as shown in Figure 1. This value was much lower than those reported by Labiad et al. (Labiad et al., 2017), Habashya et al. (Habashya et al., 2018), and Albayrak et al. (Albayrak et al., 2013), who found ranges of 1.27 – 275.71, 22.77 – 166.18, and  $228.91 \pm 0.3$  mg AAE/g, respectively, for different extracts of *Thyme*. However, the antioxidant value of thyme in the current study was higher than that reported by Kaska et al. (Kaska et al., 2018), who found  $30.45 \pm 1.42$  mg/g for *T. zygioides*. The results of the current study were comparable to those of Amamra et al. (Amamra et al., 2018), who found a range of 26.87 - 94.61 mg AAE/g for various extracts of *Thyme*.

A significant linear correlation was observed between the concentration values of the extracts and their antioxidant activity, as depicted in Figures 3. The results indicate that higher concentrations of phenolic compounds in the extracts are positively associated with increased antioxidant activity.

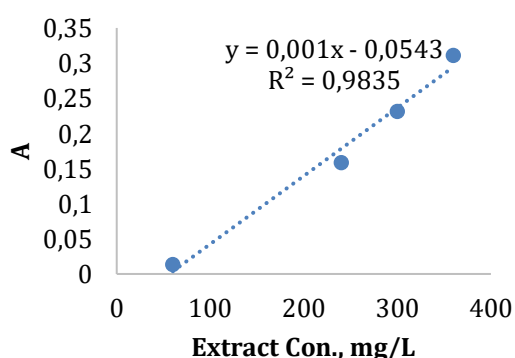


Figure 3. Antioxidant activity contents of various extract concentrations of *T. vulgaris*

### Essential metals contents

The analysis of metals in medicinal plants is an important aspect of quality control, as it can reveal the purity, safety and efficacy of the plants. The presence of metals in plants can be influenced by human activities, such as industry and agriculture, that release trace metals into the environment. Table 2 shows the total concentrations of essential metals in *T. vulgaris L.*, expressed as mg of metal per kg of plant. The concentrations of the analyzed metals varied widely. The highest concentrations were found for macroelements (Na, K, Ca, Mg, P), which are required by plants in large amounts. Among the heavy metals, Zn had the highest concentration (24.82 mg/kg), while Cu had the lowest (14.98 mg/kg). Heavy metals are usually needed by plants in small amounts, but can be toxic at high levels.

**Table 3.** Major and Minor Metal Levels in *T. Vulgaris L.*

Metal	Content (mg/kg)
Ca	5118±55
Mg	2124±36
Na	4793±42
K	15259±305
P	1400±56
Fe	17.44±0.88
Cu	14.98±0.60
Zn	24.82±0.75

Potassium had the highest metal concentration in the tested plant, with 15259 mg/kg. Potassium is a major mineral that is mainly found inside the body cells, unlike sodium, which is mainly found outside. Calcium had the second highest metal



concentration, with 5118 mg/kg. These values were lower than those reported by Edeoga et al. (Edeoga et al., 2006) and Daniel et al. (Daniel et al., 2011), who found 24600 mg/kg and 17460 mg/kg of calcium, respectively, in different medicinal plants. Calcium is an essential mineral that can help prevent degenerative and inflammatory diseases, such as heart diseases, skin infections, arthritis, gout, and respiratory tract infections.

In our investigation of the tested medicinal plants, we observed variations in the levels of sodium, magnesium, and phosphorus. Specifically, at a dry weight basis, the levels were found to be 4793 mg/kg, 2124 mg/kg, and 1400 mg/kg, respectively (refer to Table 3). Notably, the highest content was observed for sodium. Our study's results were higher than the findings reported by Daniel et al. (Daniel et al., 2011), who recorded 289 mg/kg. However, our results were lower than the records of Edeoga et al. (Edeoga et al., 2006), who reported a content of 6000 mg/kg. Phosphorus is a fundamental component of many enzymes, serving as a crucial element in phosphoproteins. Phospholipids, on the other hand, play a vital role in nerve conduction. Additionally, phosphate acts as the primary ion in both extra and intracellular fluids. It facilitates the absorption of dietary constituents, helps maintain a slightly

alkaline blood pH, regulates enzyme activity, and participates in the transmission of nerve impulses. The notable levels of minerals present in these medicinal plants indicate that their leaves could serve as an alternative source of essential macrominerals in the diet. The minerals found in these medicinal plants may play a significant role in human nutrition. The maximum concentration of the essential micro mineral iron was measured at 17.44 mg/kg in *Thymus*. This value was lower than the level reported in the study conducted by Al-aubadi (281.29 mg/kg) (Al-aubadi, 2011). Adequate levels of iron are crucial for the well-being of both plants and the provision of nutrients to humans and animals. Iron is considered an essential micronutrient for almost all organisms. Its importance stems from its ability to exist in two redox states ( $Fe^{2+}/Fe^{3+}$ ), which enables it to serve as a catalyst in numerous biochemical reactions. The levels of zinc and copper in *Thymus* were determined to be 24.82 mg/kg and 14.98 mg/kg, respectively. The zinc content observed in this study was lower than that reported by Al-aubadi (47.27 mg/kg) (Al-aubadi, 2011). Zinc and copper are recognized as essential elements for the growth of humans, animals, and plants, and they play vital roles in various metabolic processes. Figure 4 depicts the levels of macro and micro elements.

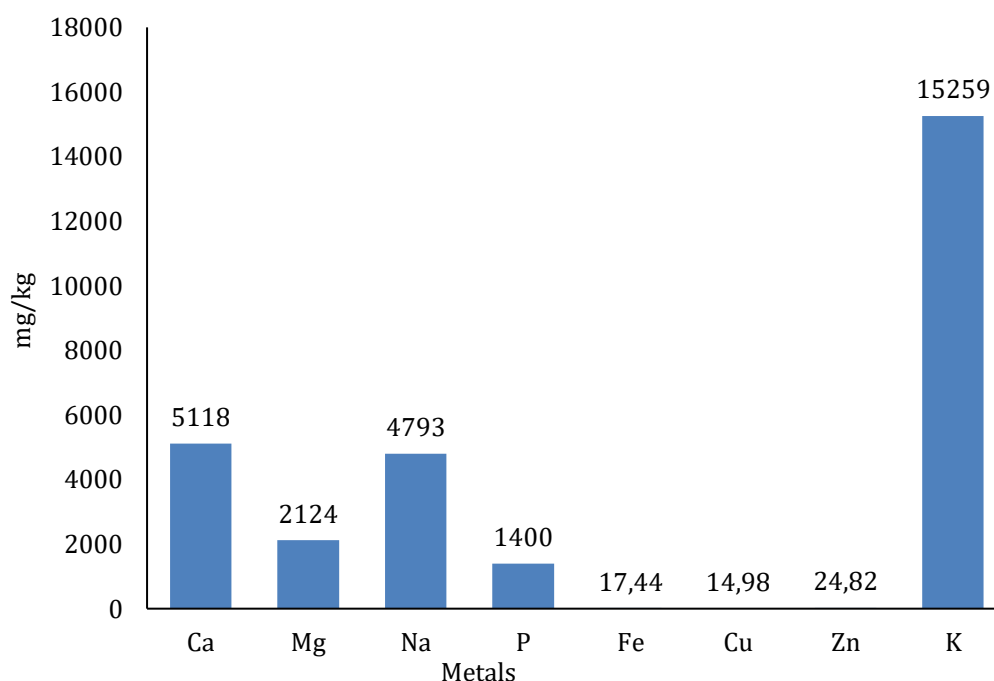


Figure 4. Levels of Metals in *T. Vulgaris L.*

## Conclusion

In conclusion, this study examined the phytochemical screening, antioxidant capacity, and mineral content of *Thymus vulgaris L.* extracts. The phytochemical analysis revealed the presence of various bioactive compounds such as coumarins, flavonoids, alkaloids, tannins, phenols, carbohydrates, and proteins in the ethanolic extract of *T. vulgaris L.*

Analysis of macro and micro minerals demonstrated varying concentrations within the *Thymus vulgaris L.* extracts. Potassium was found to be the most abundant macro mineral, followed by calcium and sodium, while phosphorus exhibited the lowest concentration. Among the micro minerals, zinc exhibited the highest concentration, followed by iron and copper.

The yields of the *Thymus* extracts ranged from 3.75% to 29.33%, with the highest percentage obtained from the ethyl acetate extract. The moisture content was determined to be 13.8%, and the ash content was found to be 8%. Furthermore, the

phenolic content of the Thyme extract was measured at 77.7 mg gallic acid equivalent per gram.

These findings provide valuable insights for users, collectors, and practitioners of medicinal plants, particularly those sourced from polluted areas, regarding the chemical composition and mineral content of *Thymus vulgaris L.* Moreover, the high antioxidant capacity of Thyme, with a value of  $41.08 \pm 2.05$  mg AAE/g, suggests its potential health benefits and supports its utilization in various applications.

## Acknowledgement

We would like to thank the Chemistry Department of Elmergib University's Faculty of Arts and Science for supplying all of the equipment needed to perform this research.

## Reference

Akbari, M., Razavi, S. H., Khodaiyan, F., Blesa, J., & Esteve, M. J. (2023). Fermented

- corn bran: A by-product with improved total phenolic content and antioxidant activity. *LWT - Food Sci. Technol.*, 184, 115090.  
<https://doi.org/10.1016/j.lwt.2023.115090>
- Al-aubadi I.M.K. (2011). The Nutritional and chemical content of basil leaves *Ocimum basilicum* L. *J. Biotech Res. Center*, 5(2), 67-74.
- Albayrak, S., Aksoy, A., Albayrak, S., & Sağdıç, O. (2013). In vitro antioxidant and antimicrobial activity of some Lamiaceae species. *Iran. J. Sci. Technol. Trans. A Sci.*, 37(A1), 1-9.
- Alkherraz, A., Hashad, O., Elsherif, K. (2019). Heavy metals contents in some commercially available coffee, tea, and cocoa samples in Misurata City-Libya. *Prog. Chem. Biochem. Res.*, 2(3), 99-107.
- Amamra, S., Cartea, M.E., Belhaddad, O.E., Soengas, P., Baghiani, A., Kaabi, I., Arrar, L. (2018). Determination of total phenolics contents, antioxidant capacity of *Thymus vulgaris* extracts using electrochemical and spectrophotometric methods. *Int. J. Electrochem. Sci.*, 13, 7882-7893.  
<https://doi.org/10.20964/2018:08:57>
- Becer, E., Altundağ, E. M., Güran, M., Vatansever, H. S., Ustürk, S., Hanoğlu, D. Y., Başer, K. H. C. (2023). Composition and antibacterial, anti-inflammatory, antioxidant, and anticancer activities of *Rosmarinus officinalis* L. essential oil. *S. Afr. J. Bot.*, 160, 437-445.  
<https://doi.org/10.1016/j.sajb.2023.07.028>
- Begum, T., Follett, P. A., Mahmud, J., Moskovchenko, L., Salmieri, S., Allahdad, Z., Lacroix, M. (2022). Silver nanoparticles-essential oils combined treatments to enhance the antibacterial and antifungal properties against foodborne pathogens and spoilage microorganisms. *Microb. Pathog.*, 164, 105411.  
<https://doi.org/10.1016/j.micpath.2022.105411>
- Benbelaïd, F., Khadir, A., Abdoune, M. A., Bendahou, M. (2013). Phytochemical screening and in vitro antimicrobial activity of *Thymus lanceolatus* Desf. from Algeria. *Asian Pac. J. Trop. Dis.*, 3(6), 454-459.  
[https://doi.org/10.1016/S2222-1808\(13\)60100-0](https://doi.org/10.1016/S2222-1808(13)60100-0)
- Dahiya, P., Purkayastha, S. (2012). Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates, *Indian J. Pharm. Sci.*, 74(5), 443-450.  
<https://doi.org/10.4103/0250-474X.108420>
- Daniel, V. N., Daniang, I. E., Nimyel, N. D. (2011). Phytochemical analysis and mineral elements composition of *Ocimum basilicum* obtained in jos metropolis, plateau state. Nigeria, *IJET-IJENS*, 11(6), 161-165.
- Divya, M., Shanti, G., Amalraj, S., Amiri-Ardekani, E., Gurav, S., & Ayyanar, M. (2023). Evaluation of in vitro enzyme inhibitory, anti-inflammatory, antioxidant, and antibacterial activities of *Oldenlandia corymbosa* L. and *Oldenlandia umbellata* L. whole plant extracts. *Pharmacol. Res. Mod. Chin. Med.*, 8, 100286.  
<https://doi.org/10.1016/j.prmcm.2023.100286>
- Edeoga, H. O., Omosun, G., Uche, L. C. (2006). Chemical composition of *Hyptis suaveolens* and *Ocimum gratissimum* hybrids from Nigeria. *Afr. J. Biotechnol.*, 5(10), 892-895.
- Elbagermi, M. A., Antisar, A. B., Elsherif, K. M. (2020). Evaluation of essential and heavy metal levels in pasteurized and

- long-life cow milk. *Int. J. Adv. Pharm.*, 8(1), 6-14.
- Elsherif, K.M., & Aljaroushi, A.M. (2021). Biochemical Properties Evaluation of some Libyan dates. *Chem. Rev. Lett.*, 4(4), 213-220. <https://doi.org/10.22034/CRL:2021:252938:1057>
- Elsherif, K.M.M., Ewlad-Ahmed, A.M., Alhlbad, E.A.A. (2023). Evaluation of some Chemical and Biochemical Constituents in Ocimum Basilicum Available in Msallata City- Libya. *Adv. J. Chem. Sect. B: Nat. Prod. Med. Chem.*, 5(2), 197-212. <https://doi.org/10.22034/ajcb:2023:385782:1155>
- Habashy, N. H., Abu Serieb, M. M., Attiaa, W. E., & Abdelgaleilc, S. A. M. (2018). Chemical characterization, antioxidant and anti-inflammatory properties of Greek Thymus vulgaris extracts and their possible synergism with Egyptian Chlorella vulgaris. *J. Funct. Foods*, 40, 317-328. <https://doi.org/10.1016/j.jff.2017.11.022>
- Habashy, N. H., Abu Serieb, M. M., Attiaa, W. E., Abdelgaleilc, S. A. M. (2018). Chemical characterization, antioxidant and anti-inflammatory properties of Greek Thymus vulgaris extracts and their possible synergism with Egyptian Chlorella vulgaris. *J. Funct. Foods*, 40, 317-328. <https://doi.org/10.1016/j.jff.2017.11.022>
- Ifesan, B.O.T., Ijarotimi, O.S., Osundahunsi, O.F. (2006). Evaluation of the Antioxidant Activity of Ocimum sp. *J. Food Technol.*, 4, 318-321.
- Kalinda, R.S., Rioba, N.B. (2020). Phytochemical Analysis and Efficacy of Rosemary (Rosmarinus Officinalis) and Mint (Mentha Spicata) Extracts Against Fall Armyworm (Spodoptera Frugiperda) on Baby Corn (Zea Mays). *Plant. Acta Chem. Malays.*, 4(2), 66-71.
- Kaska, A., Çiçek, M., Deniz, N., & Mammadov, R. (2018). Investigation of Phenolic Content, Antioxidant Capacities, Anthelmintic and Cytotoxic Activities of Thymus zygoides Griseb. *J. Pharm. Res. Int.*, 21(1), 1-13. <https://doi.org/10.9734/IPRI/2018/39688>
- Khazdair, M. R., Gholamnezhad, Z., Rezaee, R., & Boskabady, M. H. (2021). Immunomodulatory and anti-inflammatory effects of Thymus vulgaris, Zataria multiflora, and Portulaca oleracea and their constituents. *Pharmacol. Res. Mod. Chin. Med.*, 1(1), 100010. <https://doi.org/10.1016/j.prmcm.2021.100010>
- Labiad, M.H., Harhar, H., Ghanimi, A., Tabyaoui, M. (2017). Phytochemical screening and antioxidant activity of Moroccan Thymus satureioides extracts. *J. Mater. Environ. Sci.*, 8(6), 2132-2139.
- Li, K., Xia, T., Jiang, Y., Wang, N., Lai, L., Xu, S., Yue, X., Xin, H. (2024). A review on ethnopharmacology, phytochemistry, pharmacology and potential uses of Portulaca oleracea L. *J. Ethnopharmacol.*, 319(2), 117211. <https://doi.org/10.1016/j.jep.2023.117211>
- Nadeem, H.R., Akhtar, S., Sestili, P., Ismail, T., Neugart, S., Qamar, M., Esatbeyoglu, T., (2022), Toxicity, Antioxidant Activity, and Phytochemicals of Basil (Ocimum basilicum L.) Leaves Cultivated in Southern Punjab, Pakistan, *Food*, 11(9), 1239. <https://doi.org/10.3390/foods11091239>

- Nadi, A., Shiravi, A. A., Mohammadi, Z., Aslani, A., Zeinalian, M. (2023). Thymus vulgaris, a natural pharmacy against COVID-19: A molecular review. *J. Herb. Med.*, 38, 100635. <https://doi.org/10.1016/j.hermed.2023.100635>
- Najah, Z., Elsherif, K.M. (2016). Analytical and Phytochemical Studies on Zizphus Lotus. *Eur. J. Biomed. Pharm. Sci.*, 3(7), 574-577.
- Najah, Z., Elsherif, K.M., Kawan, E., Farah, N. (2015). Phytochemical Screening and Heavy Metals Contents of Nicotiana Glauca Plant. *Int. J. Pharm. Pharm. Res.*, 4(3), 82-91.
- Nema, S. S., Shaban, T. M. A., El-Banna, H. A., Radi, A. M., El-Gendy, A. A., Owis, A. I. (2015). Phytochemical and pharmacological studies of ethanolic extract of Thymus vulgaris. *World J. Pharm. Pharm. Sci.*, 4(10), 1988-2001.
- Patil, S. M., Ramu, R., Shirahatti, P. S., Shivamallu, C., Amachawadi, R. G. (2021). A systematic review on ethnopharmacology, phytochemistry and pharmacological aspects of Thymus vulgaris Linn. *Heliyon*, 7(5), e07054. <https://doi.org/10.1016/j.heliyon.2021.e07054>
- Posgay, M., Greff, B., Kapcsandi, V., Lakatos, E. (2022). Effect of Thymus vulgaris L. essential oil and thymol on the microbiological properties of meat and meat products: A review. *Heliyon*, 8(1), e10812. <https://doi.org/10.1016/j.heliyon.2022.e10812>
- Roby, M.H.H., Sarhan, M.A., Selim, K.A.H., Khalel, I.K. (2013). Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (Thymus vulgaris L.), sage (Salvia officinalis L.), and marjoram (Origanum majorana L.) extracts. *Ind. Crops Prod.*, 43, 827-831. <http://dx.doi.org/10.1016/j.indcrop.2012.08.029>
- Sadowska, U., Kopeć, A., Kourimska, L., Zarubova, L., Kloucek, P. (2017). The effect of drying methods on the concentration of compounds in sage and thyme. *J. Food Process. Preserv.*, 41(6), 1-11. <https://doi.org/10.1111/jfpp.13286>
- Yaghi, M.M., Elsherif, K.M., El Ziani, N.O. (2022). Heavy Metals Analysis of Baby Cereal Available in the Libyan Markets. *Libyan J. Basic Sci.*, 19(3), 1-12.