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Determination of macro- and microelement contents in thyme oil and rosemary oil by ICP-OES

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Essential oils are defined as complex mixtures of natural compounds that contain aldehydes, unsaturated and saturated phenols, hydrocarbons, alcohols, and terpenes. Thyme oil, obtained by steam distillation from the perennial plant and its flowers known as *Thymus vulgaris*, has antioxidant, antiseptic, antibacterial, antifungal and calming properties. It has been used as a medicinal plant since ancient times. It is also used as a preservative in foods and cosmetics. Rosemary (*Rosmarinus officinalis* L.) is one of the most preferred natural sources of active drug substances. Rosemary oil is a natural oil obtained from the leaves and flowers of the rosemary plant. Rosemary oil has antibacterial and antioxidant properties. In addition, it is stated that rosemary is among the plants that the body supports in protecting against cancerous cell formation. The content of elements (Cu, B, P, Fe, Na, Mg, K, Ca) in commercial thyme and rosemary oils was investigated by ICP-OES after wet digestion. 0.5 g sample was weighed and HNO_3-H_2O_2 (10 ml, 2:1, v/v) was added. The oil samples were heated at 80 °C until clear solutions were obtained. The method was validated through accuracy, precision, linearity, recovery, LOD (limit of detection) and LOQ (limit of quantification) parameters. The lowest metal content in thyme oil was found for Fe (0.68 µg/g) while the highest metal content - for P (100.73 µg/g). For rosemary oil, the lowest metal content was found for Mg (0.15 µg/g) while the highest metal content - for P (220.98 µg/g).

Keywords: Thyme oil; Rosemary oil; ICP-OES, Essential oil, Wet digestion

INTRODUCTION

Salvia species are used in cosmetics, food and traditional medicine. Salvia species are rich in terms of essential oils. Salvia leaves have biological properties such as antioxidants, antibacterial, anti-inflammatory, anticancer, and antimutagenic. *Salvia* leaves and essential oils are used in heart disease treatments. [1]. The *Lamiaceae* family has the most aromatic and medicinal plants. More than 3000 aromatic plants are known to exist in Turkey 's flora. Medicinally important species of the genera *Salvia*, *Origanum*, *Mentha* and *Thymus* have been the focus of recent studies in Turkey. There are many studies in the literature with respect to *Salvia* species. Maral *et al.* investigated the essential oil components and antioxidant activities of *Salvia karamanensis* [2]. Chang *et al*. analysed the selected compounds from *Salvia* herbs by HPLC method [3]. Raskovic *et al*. studied the chemical composition of *Rosmarinus officinalis* aerial essential oil by GC/MS [4].

The leaves of *Thymus* species are used in traditional medicine for the treatment of colds. In addition, thyme plant has an important biological activity in terms of antioxidant, antimicrobial, analgesic and anti-inflammatory properties [5]. Bazylko *et al.* determined the content of luteolin in *T. vulgaris*, and its liquid and dry extracts [6]. Malankina *et al.* examined the composition of the essential oil of cultivars of *Thymus serpyllum* L. by GC-MS [7].

Essential oils are defined as complex mixtures of natural products that include phenols, aldehydes, unsaturated and saturated hydrocarbons, terpenes, and alcohols [8]. In this study, we aim to determine the element concentrations in commercial thyme and rosemary oils by ICP-OES after wet digestion.

EXPERIMENTAL

Instrumentation

An inductively coupled plasma–optical emission spectrometry (ICP-OES) (Spectro/ Spectroblue) was used the measurements. The nebulizer argon gas, plasma-Ar, and auxiliary gas flow rate were 1.0 L min^{-1}, 12 L min^{-1}, and 1.0 L min^{-1}, respectively. Rf power was 1.4 kW.

Reagents and standards

Nitric acid (65%) (Sigma-Aldrich, Steinheim, Germany) and hydrogen peroxide (35%) were used for digestion procedure. Concentration ranges of standard solutions prepared by dilution from 1000 µg/mL stock solution: 1.25 - 25 µg/mL for Na, 5-100 µg/mL for Mg, 15-300 µg/mL for Ca and K, 5-100

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µg/mL for P, 2.5-50 µg/mL for Fe, 0.5-10 µg/mL for Cu and B.

Preparation of samples

Commercial thyme and rosemary oil samples were purchased in Turkey. The oil samples were analyzed by ICP-OES to determine element concentrations.

Wet digestion procedure

0.5 g samples were weighed and HNO_3 -H_2O_2 (10 ml, 2:1, v/v) was added. The oil samples were heated at 80 °C until clear solutions were obtained. The samples were dissolved in 5 ml of 0.2 M HNO_3, filtered and made up to 10 ml with ultrapure water. ICP-OES was used for determination of metal contents. Analyses were repeated 3 times.

RESULTS AND DISCUSSION

Vegetable oils are the oldest known medicinal products. People frequently use plants in the treatment of various ailments and plants are also used today in oil and food production. Prevention and detection of contamination is important for human health [9].

In this work, an ICP-OES method was developed for the determination of Na, Ca, K, B, P, Cu, Fe, Mg in commercial thyme and rosemary oils. For this purpose, the samples were digested using a mixture of nitric acid and hydrogen peroxide.

Limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for method validation. LOD and LOQ values are given in Table 1. Calibration standards were used for the determination of LOD and LOQ values. LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively. σ is the standard deviation of the intercept of the regression equation and S is the slope of the calibration curve. All experiments were performed in triplicate and the results expressed as mean. The concentrations (µg/g) of Na, Ca, Mg, K, B, Fe, Cu, and P in thyme and rosemary samples are presented in Figures 1-8. Relative standard deviations (RSD) were found below 2%.

Table 1. LOD and LOQ values

	P	Mg	Cu	Na	K	Fe	Ca	B
LOD, ng/mL	0.01	0.0004	1.7	0.0004	0.032	6.1	0.04	2.4
LOQ, ng/mL	0.04	0.0013	5.6	0.0014	0.107	20.4	0.14	7.9

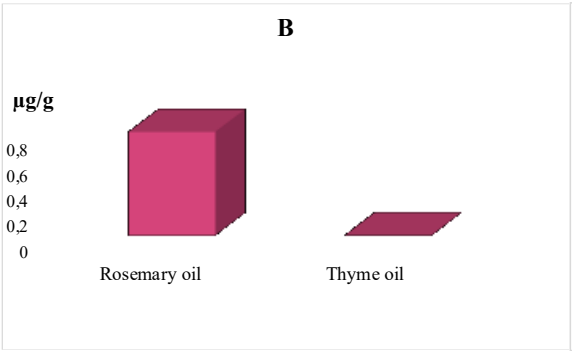

Fig. 1. B element concentrations of oil samples

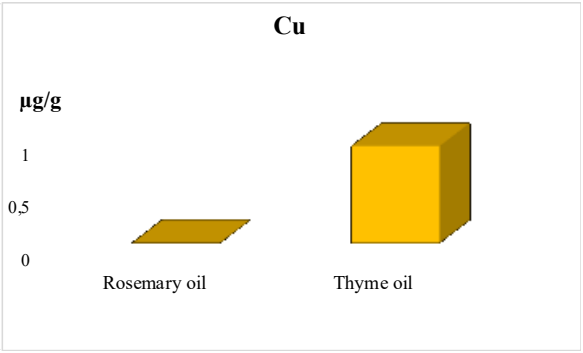

Fig. 2. Cu element concentrations of oil samples

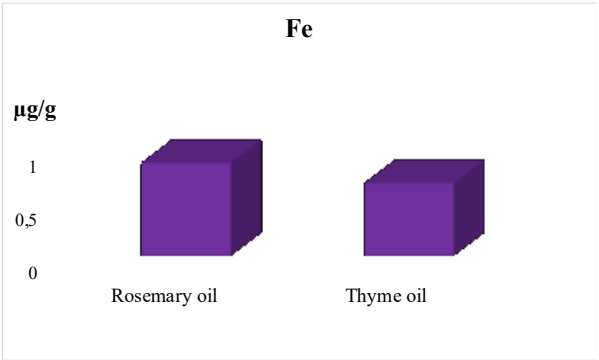

Fig. 3. Fe element concentrations of oil samples

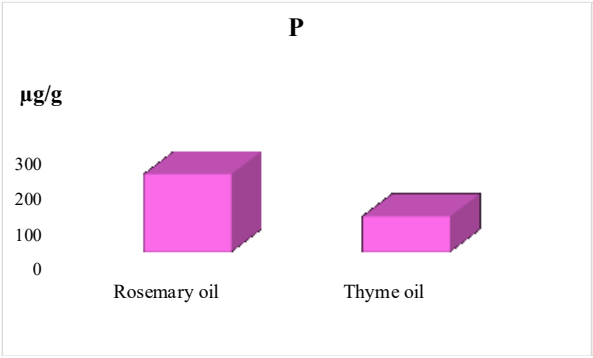

Fig. 4. P element concentrations of oil samples

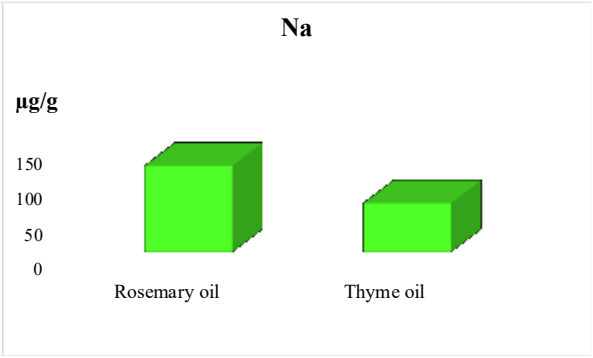

Fig. 5. Na element concentrations of oil samples

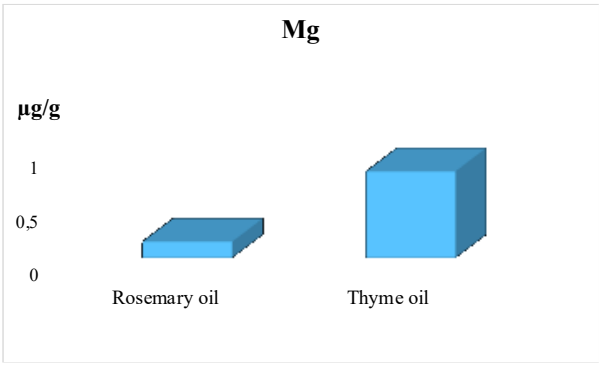

Fig. 6. Mg element concentrations of oil samples

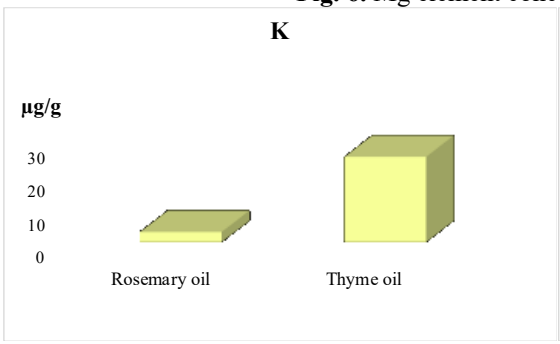

Fig. 7. K element concentrations of oil samples

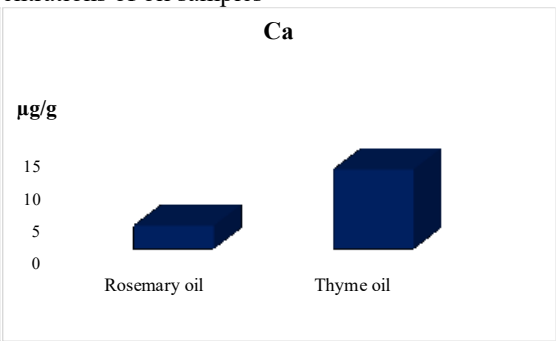

Fig. 8. Ca element concentrations of oil samples

The element concentrations after wet digestion were determined as 122.45 µg/g for Na, 3.44 µg/g for Ca, 0.15 µg/g for Mg, 3.04 µg/g for K, 0.8 µg/g for B, 0.87 µg/g for Fe and 220.98 µg/g for P in rosemary oil samples. The order of element concentrations in rosemary oil samples is P|>Na>Ca>K>Fe>B>Mg>Cu. The lowest metal concentrations in rosemary oil samples were found for Mg and Cu. The highest metal concentrations were found for P.

Then the element composition, antioxidant property, and polyphenol content of *Salvia officinalis* leaves were analyzed. Ca, S, and K were determined in macerated and infused (20 % alcoholic water) extracts (3726-4600, 28071-19010 and 38500-47983 µg/100 mL, respectively). As the alcohol content of the tincture increased, the mineral content of the extract decreased. K and Ca were higher than our results [10]. Hebatallah *et al.* investigated the effects of foliar application of riboflavin at different concentrations (0, 25, 50, and 100 mg/L) on rosemary plants during two cutting seasons. In addition, rosemary leaves's element concentrations were determined by FAAS and ICP-MS [11].

The element concentrations in thyme oil samples after wet digestion were found to be 69.36 µg/g for Na, 11.9 µg/g for Ca, 0.81 µg/g for Mg, 25.25 µg/g for K, 0.92 µg/g for Cu, 0.68 µg/g for Fe and 100.73 µg/g for P. The order of element concentrations in thyme oil samples is P>Na>K>Ca>Cu>Mg>Fe>B. The lowest metal concentrations in thyme oil samples were observed for B and Fe. The highest metal concentrations were found for P.

The antioxidant - antibacterial activity and chemical composition of *Thymus praecox* was investigated by Sener *et al.* [12]. Experimental results showed that thyme extracts have antibacterial activities against some bacteria. Cu, Fe, Na, Mg and Ca concentrations were 0.97 µg/g; 93 µg/g; 100.944

µg/g; 2300 µg/g; 40000 µg/g, respectively. Fe, Na, Mg, Ca concentrations determined in thyme oil in our study were lower than those of Sener *et al*. Cu concentrations were similar to our results [12]. Drioiche *et al.* investigated the chemical composition and antimicrobial activities of *Thymus vulgaris*, *Thymus satureioides* and *Thymus zygis* essential oils. Fe concentrations in our study were similar to their study [5].

Arsenijevic *et al.* analysed the volatile fraction of the leaves of *Thymus pannonicus* by headspace extraction. Cu, Ni, Cr, Na, Mn, Co, Zn, Fe, Ca, Mg, and K were determined using FAAS, GFAAS and ICP-OES. Their assay results were higher than our results [13]. Adequate dietary iron intake is crucial to reduce the incidence of anaemia. High doses of iron can lead, however, to tissue damage. Cu is an important element for human health, but high doses can cause damage to the liver and kidneys. Copper deficiency in children can lead to hypochromic anaemia, leukopenia and osteoporosis [14]. Fe and Cu have many positive effects on human health. Inadequate intake of these metals can lead to undesirable pathological problems. It has been observed that possible contamination with Cu and Fe may originate from oil processing equipment and Cu contamination in oils may be due to environmental pollution [9]. Potassium is an important electrolyte which ensures the conductivity of the nerves and the active transport of the mineral apatite in the bones. Excess sodium in the body can cause heart disease. Ca is important for patients with kidney failure. In a study, Ca levels in hardaliye were reported as 0.310-711.1 mg/kg [15]. Recommended dietary intakes (RDI) for Na and Ca have been established by the Food and Nutrition Board, Institute of Medicine, National Academies, Washington, DC, USA. Potassium is important with regard to human health. A diet with a low level of potassium can lead to heart problems [16]. Potassium is important in the regulation of intracellular osmolarity, cell membrane transport and activation of enzymes including glycolysis and respiration. The necessary potassium requirement is 782 mg per day [17]. Playing an important role in human metabolism, phosphorus is an essential nutrient. The required daily amount of phosphorus is approximately 800-1200 mg. Magnesium is an important element in the body as it is a component and activator of many enzymes and the daily requirement of magnesium is between 300 and 500 mg. Table 2 shows the recommended daily allowance (RDA) data [18].

Table 2. Reference values for recommended daily allowance [16]

Mineral nutrients and trace elements	Recommended daily allowance (RDA)
Calcium	1000 mg
Sodium	2400 mg
Magnesium	350 mg
Iron	15 mg
Boron	< 20 mg
Potassium	3500 mg
Manganese	5 mg
Phosphorus	1000 mg
Zinc	15 mg
Copper	2 mg

CONCLUSION

The concentrations of Ca, K, P, B, Mg, Cu, Fe, and Na in commercial thyme and rosemary oil samples were determined. This study was the first to investigate the oils of thyme and rosemary by means of wet digestion-ICP-OES. The HNO_3-H_2O_2 digestion method was used for determination of the multi-element contents. The element concentrations were below the RDA levels, so the analysis results indicate that as a contribution to dietary intake, thyme and rosemary oils are not a good source of essential elements.

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