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A Comparative Study on the Chemical Constituents of Four Libyan Herbs: *Mentha Piperita*, *Matricaria Chamomile* L, *Rosmarinus Officinalis* L, and *Thymus Vulgaris*

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A B S T R A C T

The chemical compositions of four medicinal plants grown in Libya, specifically Mentha, Chamomile, Rosmarinus, and Thymus, have been identified. The levels of ten macro-metals (Na, K, Ca, Mg), essential heavy metals (Fe, Cr, Cu, Zn), and toxic metals (Cd, Pb) were analyzed. Wet digestion was used to dissolve the samples, and the levels of macro-metals, essential metals, and toxic metals were measured with a flame photometer and flame atomic absorption spectrometry, respectively. The investigation revealed the following results (in mg/kg): Ca ranged from 2009.20 to 6184.20; Mg from 2145 to 3479; Fe from 155.36 to 370.95; Cr from 23.71 to 73.91; Cu from 6.31 to 23.71; Zn from 9.23 to 27.32; Pb from 0.14 to 0.30; Cd from 0.02 to 0.09. Cadmium and lead concentrations in Thymus and Rosmarinus were found to be lower the detection limits of flame atomic absorption spectrometry. The moisture levels varied from 9.95% in Rosmarinus to 14.43% in Menthe, while the total ash amounts are still pending. Additionally, the phytochemical properties of the plant leaves were evaluated through a screening process. The results of this research revealed the existence of phytochemicals like alkaloids, flavonoids, tannins, glycosides, and saponins.

1. Introduction

Medicinal plants are essential components in various herbal formulations used in traditional medicine (Stanojkovic-Sebic *et al.*, 2015). These plants contain a variety of beneficial compounds that can be obtained from the soil, water, or air (Tercan *et al.*, 2016; Elsherif *et al.*, 2023a). Different elements and metals are found in varying concentrations within these compounds. Important components, including micro and macro elements, are necessary for the best growth and functioning of living beings (Mandal, 2021; Najah *et al.*, 2015a).

The chamomile plant, known scientifically as *Matricaria Chamomilla* L., is a healing herb that is part of the Asteraceae / Compositae family of bloom-producing plants. Chamomile possesses a range of helpful properties such as anti-inflammatory, antioxidant, antibacterial, analgesic, antiseptic, antispasmodic, and sedative characteristics. Its ability to treat depression makes it highly effective in reducing

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anxiety. Furthermore, chamomile has been used for a very long time to cure a wide range of ailments, including gout, rheumatic pain, haemorrhoids, foot ulcers, eczema, wounds, and chickenpox. The essential oils were commonly employed in aromatherapy and cosmetics (Serban *et al.*, 2020).

Mentha species have a strong historical presence in traditional medicine. Often, they are used either brewed as tea or incorporated into various herbal products like lotions, perfumes, and soaps (Serban *et al.*, 2021; Dinu *et al.*, 2021).

Rosemary, also known as *Rosmarinus officinalis* L. in scientific terms, is a member of the Lamiaceae family of plants. Although originally from the Mediterranean region, it is now found in various geographic locations. This plant is described as a perennial herb, displaying a shrub-like structure, and growing to a maximum height of two meters. Its branches are particularly decorated with green leaves, releasing a distinctive scent. R. *officinalis* is well-known for its flexibility, being used as an ornamental plant, culinary spices, natural food preservatives, and more (de Oliveira *et al.*, 2019; Elsherif *et al.*, 2023b).

Thymus vulgaris L., the scientific name for thyme, is a perennial herb belonging to the *Lamiaceae* family. It is indigenous to southern Italy and the western Mediterranean region. *Thyme*, which is valued for its medicinal and culinary properties, is grown in over 350 different species worldwide (Abu-Darwish and Abu-Dieyeh, 2009), particularly in developing countries (Figas *et al.*, 2021; Elsherif *et al.*, 2023c).

Trace mineral elements are essential or harmful parts of plants that play a role in metabolism. The complex effects of heavy metal poisoning on plants greatly influence the bioactive substances found in medicinal plants. As a result, this results in a reduction in the excellence, security, and effectiveness of botanical products from nature (Abu-Darwish and Abu-Dieyeh, 2009; Iordache *et al.*, 2022; Yaghi *et al.*, 2022; Elsherif, and Aljaroushi, 2021). Therefore, in the midst of the numerous quality control evaluations done on medicinal plants and raw materials, the evaluation of metals, especially those that are harmful, is extremely important. Industrial pollution affecting farmland and forests has become a major environmental concern in various areas worldwide (Dghaim *et al.*, 2015a; Najah *et al.*, 2015b; Elsherif *et al.*, 2023d).

The aim of this study was to evaluate the concentrations of necessary elements (potassium, magnesium, sodium, calcium, iron, and zinc) and toxic heavy metals (chromium, cadmium, and lead) in *Mentha Piperita*, *Matricaria Chamomilla* L, *Rosmarinus Officinalis* L, and *Thymus Vulgaris* vegetation. Samples of several plants were gathered from various markets in Misurata City, Libya. Moreover, the research sought to discover a variety of bioactive compounds present in the powdered plant samples under investigation.

2. MATERIALS AND METHODS

2.1 Sample collection

The plants used in this research, specifically *Mentha Piperita*, *Matricaria Chamomilla* L, *Rosmarinus Officinalis* L, and *Thymus Vulgaris*, were obtained from nearby markets in Misurata City. Each plant was obtained three to four samples in total.

2.2 Sample preparation

The specimens were collected from nearby markets, washed with tap and distilled water, and then allowed to dry naturally. Afterwards, the dried plants were ground with an electric grinder, and the resulting powder was placed in sealed glass bottles for future use.

Water and ethanol were used as solvents to extract the plant components. The dehydrated powdered plant substance was soaked in the solvent (30 g/120 ml) for a duration of 24 hours. After that, the mixture was passed through Whatman No. 4 paper, and this filtration procedure was done thrice. The volume that was taken out was then condensed with a rotary evaporator and stored for later uses (Rebiai *et al.*, 2013; Sulaiman *et al.*, 2021).

2.3 Phytochemical screening

Phytochemical screening was used to identify the various bioactive components present in the plants under investigation, following the procedures outlined in previous research (Iqbal *et al*., 2015; Najah and Elsherif, 2016). The dried-ground samples were found to include alkaloids, flavonoids, tannins, saponins, steroids, and glycosides, as shown in Table 1. The qualitative results were represented by the symbols (+) for phytochemical presence and (-) for phytochemical lack.

Table 1. Phytochemical screening tests for plant extracts

Components	Test	Procedure	Results
Phenols	Folin–Ciocalteu	1 mL Extract + 0.5 mL Folin–Ciocalteu reagent + drops NaOH	Formation of gray or black color
Flavonoids	Ferric Chloride	2 mL Extract + drops 10% $FeCl_3$	Formation of dark brown or blackish red color
Tannins	Ferric Chloride	1 mL Extract + drops 5% $FeCl_3$	Formation of greenish brown, blue green or blue-black color
Alkaloids	Wagner	2 mL Extract + 1 mL 1% HCL + 0.5 mL Wagner's reagent	Cream, reddish brown precipitate
Saponins	Froth Test	1 mL Extract + 5 mL water	Formation of copious lather
Steroids	Salkowki	1 mL Extract + 1 mL chloroform + drops conc. H_2SO_4	Formation of brown ring
Glycosides	Keller-Kiliani	1 mL Extract + 2 mL acetic acid + 5% $FeCl_3$ + 2-3 drops conc. H_2SO_4	Formation of reddish brown ring

2.4 Moisture and Ash contents

In order to calculate the moisture content of the plants, water had to be removed from the plant material at a specific temperature. The moisture content was then calculated based on the weight loss that occurred. Each specimen was given about 5 g of plant material, which was then incubated for 7 hours at 105 °C in metal cups. The specimens were then weighed again while at room temperature in order to determine the amount of moisture present (Tercan *et al*., 2016; Alkherraz *et al*., 2019).

A part of each specimen was burned and turned into ash in order to determine the total ash content in plant samples. 3.0 g of plant material were extracted from each specimen and put into porcelain crucibles. Prior to usage, the crucibles' weight was measured. After that, the crucibles were put in an ash furnace and heated to between 550 and 600 °C, which is when the specimens turned grey. The sample' weight was then measured once more at room temperature in order to ascertain the ash content (Tercan *et al*., 2016; elsherif and Kuss, 2012).

2.5 Metal Analysis

Analysing powdered samples allowed for the determination of the elemental composition of plant leaves. 0.5 g of the powdered plant samples were subjected to mineralisation (wet digestion) at room temperature for 24 hrs using a mixture of ultrapure HNO_3 and H_2O_2 at a ratio of 10:3 (v/v) in order to eliminate any organic materials. The resultant mixture was then diluted with ultrapure water to the desired level and passed through a 45-µm filter paper into a 25 mL volumetric flask. The Flame Photometer Model PFP7 (Jenway) and the Flame Atomic Absorption Spectrophotometer Model AA-240FS (Varian) were used to analyse the macro-metals (Na, K, Mg, Ca) and heavy metals (Fe, Cu, Zn, Cr, Cd, Pb) in the plant samples, respectively (Serban *et al*., 2020; Soylak *et al*., 2004; Elsherif *et al*., 2017; Elbagermi *et al*., 2020; Yaghi *et al*., 2021).

3. Results and discussion

3.1 Characterization of phytochemical composition of plant extracts

Table 2 presents an overview of the results obtained from the phytochemical screening of the aqueous and ethanolic extracts derived from the plants investigated in this study. The information displayed in the table shows that significant amounts of alkaloids, flavonoids, tannins, glycosides, and saponins were present in the leaves and their corresponding aqueous and ethanolic extracts. It is noteworthy to emphasise that the ethanolic extract showed higher amounts of these secondary metabolites than the aqueous extract, with the exception of saponins. It is crucial to recognise that the specific plant part being studied (such as the stem, leaves, flowers, etc.), the extraction conditions (such as the time, solvents, extraction method, etc.), the processing methods, and the environmental conditions in which the plants were grown can all affect the phytochemical composition (Zeroual *et al*., 2021). It's also noteworthy to note that there were no measurable amounts of steroids in either the ethanolic or aqueous extracts.

Table 2. Phytochemicals screening results in aqueous extracts

Plant extract		Alkaloids	Flavonoids	Tannins	Saponins	Glycosides	Steroids
Mentha	AE	+	+	++	++	++	-
	EE	++	+	+++	+	++	-
Chamomile	AE	++	++	+++	+	+	-
	EE	++	+++	+++	+	+++	-
Rosmarinus	AE	+	+	+	+++	+	-
	EE	++	+	+++	+	+++	-
Thymus	AE	+	+++	+++	+++	+	-
	EE	++	+++	+++	++	++	-

AE: aqueous extract, EE: ethanolic extract, (-): No change (absence), (+): weak observation, (++): moderate observation, (+++): strong observation

3.2 Moisture and ash contents

According to Figure 1, *Mentha* has the highest moisture content (14.43%), followed by *Thymus* (12.95%), *Chamomile* (11.96%), and *Rosmarinus* (9.95%). At 14.11%, *chamomile* has the greatest total ash concentration, followed by *Mentha* (12.33%) and *Thymus* (12.82%). It was discovered that the moisture contents exceeded those stated in Mlitan's research (Mlitan *et al*., 2013). Furthermore, compared to earlier observations, the overall ash content was much higher (Helal *et al*., 2021).

The herbal plants moisture is one of the main factors that determines their quality. The Turkish Food Codex states that tea samples, including those containing rosemary, should not have more than 10% moisture content (Tercan *et al*., 2016). The investigation found that the samples of herbal plants had moisture contents higher than 10%.

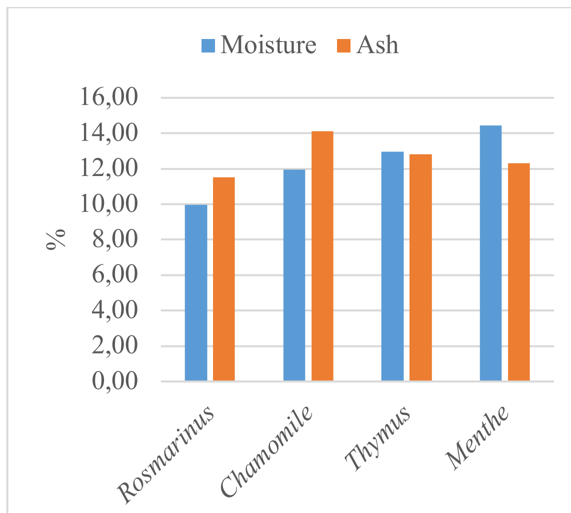

Figure 1. Moisture and ash contents of studied plants

3.3 Metal contents

To ensure that humans are getting enough nutrients, some minerals are necessary. Body functioning can be disrupted by an excess or lack of certain minerals (Iordache *et al*., 2022). Because they play important roles in metabolism, bone growth and formation, and nervous system function, calcium (Ca), potassium (K), magnesium (Mg), iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) are all thought to be essential for human health (Gupta U. and Gupta S., 2014; Mezzaroba *et al*., 2019).

Regarding the safe quantities of hazardous metals present in culinary or medicinal plants, there are no particular international rules in force. However, we have found a number of FAO/WHO recommendations that suggest limits of 0.30 mg/kg for lead (Pb) and 0.30 mg/kg for cadmium (Cd). Additionally, according to Iordache *et al*. (2022) the WHO recommends limits of 0.2 mg/kg and 0.3 mg/kg for Pb and Cd, respectively. While iron, zinc, chromium, copper, and manganese are categorised as trace metals by the WHO, calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) are categorised as minerals. It is noteworthy that even highly significant minerals and trace metals might have negative influences on human health (Prkić *et al*., 2018).

Table 3. Concentrations of macro-metals and heavy metals (mg/kg) in studied plants

Metal	**Concentration (mg/kg)**				
	Mentha	*Chamomile*	*Rosmarinus*	*Thymus*	*Mean*
Na	1090.00	1051.00	818.00	1012.00	992.75
Mg	3464.00	3021.00	2145.00	3479.00	3027.25
K	1145.00	1280.30	1085.24	1027.51	1134.51
Ca	2009.20	6184.20	4462.20	4022.20	4169.45
Cr	23.71	30.47	73.91	27.35	38.86
Fe	370.95	316.45	155.36	182.15	256.23
Cu	10.72	23.71	6.31	21.92	15.67
Zn	9.23	13.93	27.32	11.52	15.50
Cd	0.07	0.02	0.09	un	0.06
Pb	0.30	0.14	un	0.15	0.20

un: undetected (under limits of detection)

Table 3 displays the mineral composition of the leaves of the plants under investigation. For all studied plants, the samples under examination showed that calcium (Ca) and magnesium (Mg) had the highest average values, followed by potassium (K) and sodium (Na). These substances are essential to both plants and human health. In addition to transmitting nerve impulses, potassium helps the body maintain proper fluid and electrolyte balance. The most prevalent element in our bodies, calcium, aids in the formation and upkeep of strong bones. Magnesium is essential for many enzymes and is involved in the metabolism of proteins, lipids, and carbohydrates, among other cellular processes. However, sodium is in charge of maintaining water balance in both intracellular and intercellular contexts as well as depolarising cellular membranes. Similar concentrations of these elements were found when comparing the macro-element data with those published by other authors (Mandal, 2021; Prkić *et al*., 2018; Iordache *et al*., 2022). Figure 2 shows the concentrations of important macrominerals.

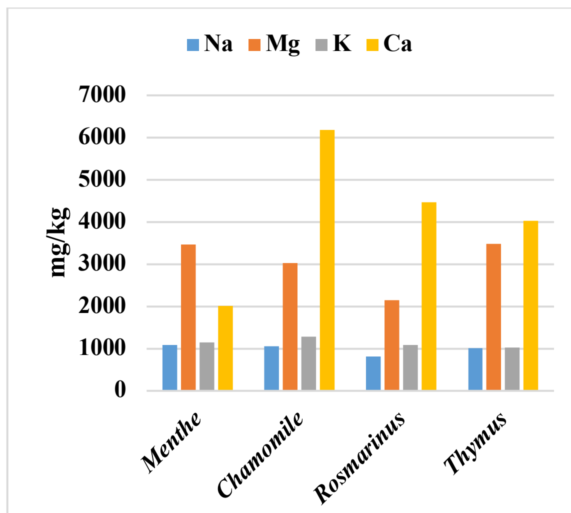

Figure 2. Levels of macro-metals in studied plants

In the following order, the plants under investigation had the highest quantities of trace essential elements: Zn (9.23-27.32 mg/kg), Cu (6.31-23.71 mg/kg), Cr (23.71-73.91 mg/kg), and Fe (155.36-370.95 mg/kg). Soil and rock materials are the main sources of the increased Fe concentration seen in herbs (Mandal, 2021). These necessary micro-elements are crucial for the growth and development of plants as well as for a number of biological and chemical processes in the human body. Additionally, they function as structural elements of protein and carbohydrate complexes that are a part of bodily physiological reactions (Bukva *et al*., 2019). Enzymes, zinc, and iron are required for the immune system to be healthy and function properly. A shortage of one metal indicates a shortage of the other. Moreover, Fe is essential for living cells and its lack is linked to anaemia, whereas zinc is required for the action of several enzymes (Yener, 2019). The concentrations of these micro-essential elements are shown in Figure 3. Other writers have previously determined and confirmed the values of all the aforementioned factors (Mandal, 2021; Prkić *et al*., 2018; Stanojkovic-Sebic *et al*., 2015; Tercan *et al*., 2016).

Toxic substances like heavy metals may be present when preparing, harvesting, or storing medicinal plants. Furthermore, a variety of heavy metal contamination might arise during plant culture as a result of fertiliser application, nutritional content, and soil composition. Additionally, contamination may occur when herbal materials are processed and packaged (Dghaim *et al*., 2015b).

Non-essential and hazardous elements, such as lead and cadmium, were detected in almost comparable levels in all of the samples that were tested. Due to their potential for both acute and long-term environmental pollution, these metals offer serious dangers. Interestingly, lead was not found in the *Rosmarinus* samples, but cadmium was not found in the *Thymus* samples. It is significant to note that the World Health Organization's maximum allowable values for lead and cadmium (10 mg/kg for lead and 0.3 mg/kg for cadmium) were not exceeded by the levels of lead and cadmium in the analysed samples (Figure 4) (Iordache *et al*., 2022).

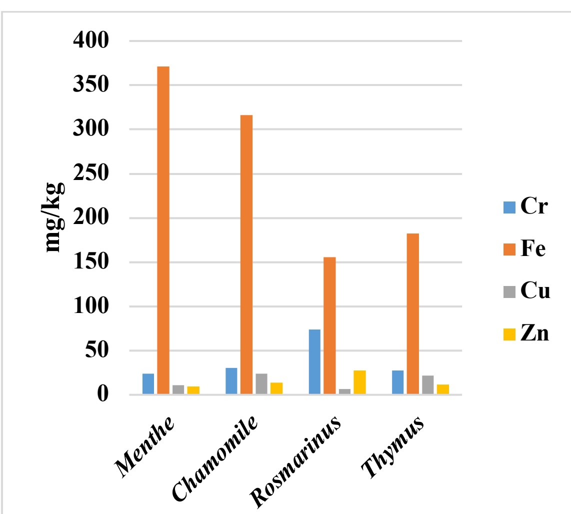

Figure 3. Levels of heavy essential metals in studied plants

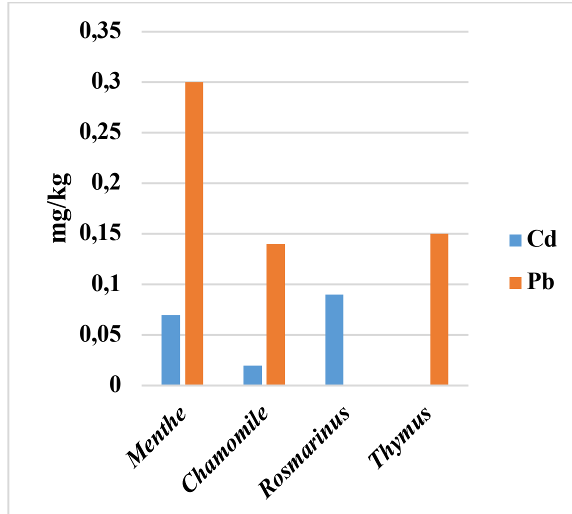

Figure 4. Levels of toxic metals in studied plants

4. Conclusion

For a very long time, people have been using aromatic and medicinal herbs as medicine. Alternative medicine is becoming more and more popular, and this trend is only getting stronger every day. The abundance of different minerals found in plants including *Mentha*, *Chamomile*, *Rosemary*, and *Thymus* is revealed by our research findings. Furthermore, we have seen similar trends in the concentrations of the main phytochemicals. The hierarchy of heavy metal and vital mineral concentrations for all species is as follows: iron, chromium, copper, zinc, lead, cadmium, calcium, magnesium, potassium, sodium, and sodium. It is noteworthy that the samples from Thymus showed quantities of cadmium below the detection threshold, whereas the ones from Rosmarinus showed concentrations of lead below the detection threshold.

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