



Journal of **Plant Sciences**

ISSN 1816-4951



Academic
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www.academicjournals.com

Research Article

Minerals Analysis of *Salvia palaestina* Leaves: Quantification and Detection

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Abstract

Background and Objective: Herbal medicine is widely practised in Palestine. In particular, *Salvia palaestina* (sage in English) is intensively used but its usage merely relied on traditional heritage rather than a scientific basis. Twenty volatile, semi-volatile and major components were identified in *Salvia palaestina* (*S. palaestina*) leaves samples. This study conducted to determine the minerals contents of *S. palaestina* leaves. **Materials and Methods:** The mineral analysis was carried out using inductively coupled plasma optical emission spectrometry (ICP-OES) to determine aluminium, calcium, sodium, potassium, magnesium content and others. The concentration of each element in the samples was calculated on a dry matter basis. **Results:** Eighteen minerals were screened in dried *S. palaestina* leaves. Found that *S. palaestina* leaves were rich in minerals particularly, potassium, but it turned out that the samples examined contained a remarkable amount of aluminium, which might affect the health due to its accumulation properties. **Conclusion:** The study revealed that *Salvia palaestina* leaves contained appreciable amounts of minerals. The amount of aluminium in the tested samples was exceeding the permissible limits. Thus, accumulation and long-term usage of the *S. palaestina* leaves may deteriorate health.

Key words: Minerals, analysis, *Salvia palaestina*, leaves, quantification, detection

Citation: Hejaz, H.A., R. Sabbobeh, N. Halayqa, A. Jahajha and S. Abu-Lafi, 2022. Minerals analysis of *Salvia palaestina* leaves: Quantification and detection. J. Plant Sci., 17: 53-61.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Throughout ages, humans have relied on plants for their availability as a source of food, clothing, flavours, fragrances, medicine, etc^{1,2}. Up to the present, herbal medicines which formed the basis of healthcare throughout the world since the earliest days of mankind are still widely practised in many developing countries, especially in the Middle East³. This originates from economic reasons and their deep belief that herbs aren't harmful⁴. However, it is nearly always going side by side with medicine⁵. Similarly, herbal medicine is considered an integral part of Palestinian culture and plays a pivotal and indispensable role in current public healthcare. There are lots of medicinal plants which is used to treat several diseases but the efficacy, safety, toxicity, dosage and usage instructions haven't ever been investigated scientifically and almost always are verbally inherited from one generation to another⁶.

Salvia species contain various secondary metabolites, particularly essential oils and to a lesser extent, compounds such as sterols, flavonoids, phenols, monoterpene, sesquiterpenoids, diterpenoids and triterpenoids. Some secondary metabolites concomitantly carry out physiological functions^{7,8}. Different factors may determine the composition and yield of the essential oils. These variables include seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant utilized, post-harvest drying and storage conditions. In our previous research, the essential oils of *S. palaestina* were analyzed and twenty major components were identified⁹⁻¹¹. One of the most popular Palestinian herbal plants is *Salvia* (Sage in English and Miramya in Arabic). It is the largest genus of the *Lamiaceae* or (*Labiatae*) family and comprises about 900 species¹². The name is derived from Latin (*salvare*) which means 'to save or to cure' referring to its curable medicinal properties¹³. Several species of *Salvia* are used in folk medicine all around the world to treat microbial infections, cancer, malaria, inflammation, etc.¹⁴ due to its biological activities, such as antimicrobial, antimalarial, antioxidant, antitumor, antidiabetic, anxiolytic, sedative and anti-inflammatory activities¹⁵⁻¹⁹. From its name, wild *S. palaestina* is native to Palestine. It was described and named by George Bentham in 1835. Palaestina is referring to its geographical distribution in the mountains between Gaza and Jerusalem. (http://en.wikipedia.org/wiki/Salvia_palaestina). It grows 1-2 ft (0.30 to 0.60 m) in tall. The green leaves vary in shape and size, with light hairs on both sides. It has glands that release secondary metabolites of essential oils when rubbed, crushed, or heated. The hairy leaves can be nearly 8-10 cm long. Its flowers

are white to whitish lilac in colour. The flowering season starts in April. In addition to Palestine, this plant is commonly found in Egypt, Syria, Lebanon, Southern Turkey, Iran, Northern Iraq and Jordan²⁰.

Plants are essential sources of micronutrients and minerals for human beings and monitoring their levels is highly required for both dietary and medicinal purposes. Metallic and non-metallic elements are very important for the human body, they are required in a certain amount to maintain healthy growth²¹. Herbs contain a certain amount of metals, therefore using them in treating illness might affect the general health and even more, it can be a factor of disease generation in certain cases²². Besides, herbs might contain metals as contaminants, which hurt health. The determination of metals in plants can be considered as a marker of safety and purity^{23,24}. To the best of our knowledge, no studies are reporting about the metal type and amount in *Salvia* species at large including Palestine. Heavy metals in medicinal herbs have been studied in many countries as an indicator of pollution and mainly to evaluate the impact of traffic on metal concentration in herbs growing on nearby roads. One of these researches was conducted in Montenegro on *S. Officinalis* leaves and the outcome of this research was that the concentration of heavy metals decreases at 50-100 m from the edge of major roads. Therefore, the impact of road traffic through the pollution of aromatic herbs was noted^{22,25-27}. Moreover, research showed that the ability of different medicinal plants to accumulate minerals depends on the plant species, the geographical location and environmental conditions and thus it was advised to examine the availability of certain minerals in plants before using them²⁸. Thus, this study determines the minerals contents of *S. palaestina* leaves to assess the nutritive, medicinal values and safety. An intensive literature survey indicated that there is no single study on the *S. palaestina* minerals in Palestine.

MATERIALS AND METHODS

Study area: This study was conducted from 15 March, 2015 to 31 June, 2016 in Hebron University.

Reagents: Milli-Q ultra-pure water (resistivity>18 megaohms (MΩ·cm), high purity nitric acid 3% optima grade was purchased from fisher chemicals and fluke multi element standard solution 5 for ICP (Trace cert.) Lot # BCBH5213V.

Instruments: Perkin Elmer ICP-OES (DV7300), muffle furnace 6000 (USA).

Sample preparation: Two samples of dried leaves of *S. palaestina* (1g and 0.5 g) were transferred into a silica crucible and kept into a muffle furnace for ashing at 450°C for 7 hours and then 2 mL of 3% HNO₃ was added to crucible. Care was taken to ensure that all ash was in contact with the acid. Then, the crucible containing the acid solution was kept on a hot plate and digested to obtain a clean solution. The final residue was dissolved in 3% HNO₃ solution and made up to 25 mL. Working standard solutions were prepared by diluting the stock solution with 3% HNO₃.

Blank and standard solution preparation: To prepare the blank and the standard solutions, a 3 % HNO₃ solution was used as a diluent (42 mL of high purity HNO₃ Acid 70% diluted into 1000 mL volumetric flask (V.F.) with Milli-Q water used as blank and as a diluent in standards preparation)

All standards were prepared from stock multi elements standard solution 5 for ICP.

Standard No. 1: Dilute 0.05 mL from stock multi elements standard solution 5 into 100 mL (V.F.) using diluent 3% HNO₃ and complete to mark

Standard No. 2: Dilute 0.5 mL from stock multi elements standard solution 5 into 100 mL (V.F.) using diluent 3% HNO₃ and complete to mark

Standard No. 3: Dilute 1.0 mL from stock multi elements standard solution 5 into 100 mL (V.F.) using diluent 3% HNO₃ and complete to mark

Standard No. 4: Dilute 2.5 mL from stock multi elements standard solution 5 into 100 mL (V.F.) using diluent 3% HNO₃ and complete to mark

Standard No. 5: Dilute 5.0 mL from stock multi elements standard solution 5 into 25 mL (V.F.) using diluent 3% HNO₃ and complete to mark

RESULTS AND DISCUSSION

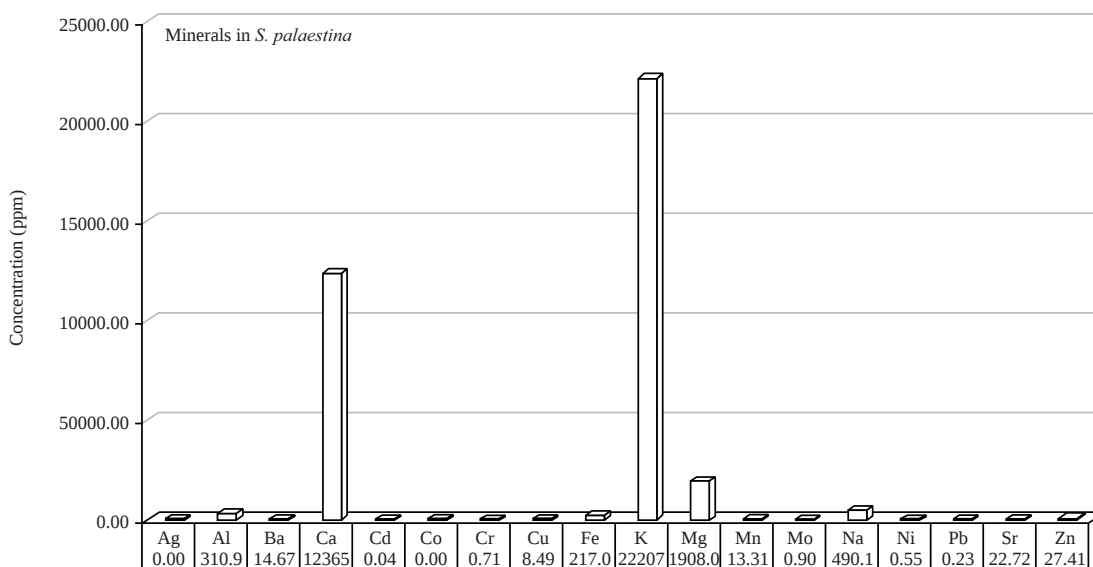
S. palaestina leaves are found to be rich in minerals particularly, potassium, but it turned out that the samples examined contained a remarkable amount of aluminium, which might affect the health due to its accumulation properties. Thus, the general belief those medicinal plants are safe and devoid of toxicity could be misconstrued. Due to the increasing use of herbal medicines worldwide, the safety, efficacy and quality of medicinal plants have become a major concern. Several works have been reported in many developed countries on the minerals content of medicinal plants, unfortunately, there is no single report regarding

mineral contents in these herbs in Palestine. Present work was carried out to screen the availability of minerals in *S. palaestina* leaves by using inductively coupled plasma optical emission spectrometry (ICP-OES) which is a trustable tool for the determination of various elements in liquid and solid samples. Eighteen minerals were screened that, silver (Ag), aluminium (Al), calcium (Ca), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), potassium (K), sodium (Na), barium (Ba), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), strontium (Sr) and zinc (Zn).

Five different concentrations of standard solution for each mineral were prepared as described in the methodology section, then standard calibration curves were established and correlation coefficients were calculated. There was clear linear relation between concentrations and emissions for the studied element, the limit of detection (LOD) and the limit of quantitation (LOQ) were determined for each element as in Table 1. Ag and Co were not detected in *S. palaestina* leaves. While it was found that the concentrations of other minerals were as follows: Al (310.9 ppm), Ba (14.665 ppm), Ca (12365 ppm), Cd (0.0413 ppm), Cr (0.7078 ppm), Cu (8.49 ppm), Fe (217.0375 ppm), K (22207.5 ppm), Mg (1908.5 ppm), Mn (13.3088 ppm), Mo (0.8969 ppm), Na (490.125 ppm), Ni (0.5543 ppm), Pb (0.2267 ppm), Sr (22.7188 ppm) and Zn (27.41 ppm) as represented in Table 1.

Sixteen elements were detected and quantified in dried *S. palaestina* leaves. Detected elements were arranged in decreasing order as follows: K, Ca, Mg, Na, Al, Fe, Zn, Sr, Ba, Mn, Cu, Mo, Cr, Ni, Pb, Cd as shown in Table 1. The concentration of both silver and cobalt was below the detection limit. Fig. 1 represents the availability of minerals in *S. palaestina* leaves. The abundant mineral was potassium in the tested leaves and high levels of it are consistent with its important role in the biosynthesis of primary and secondary metabolic products in plants²⁹.

Minerals are elements that originate in the soil and can't be created by living creatures. But all creatures need minerals in certain amounts to maintain proper health. Plants absorb minerals from the soil then animals get their minerals from the plants or other animals they eat. Human beings obtain minerals either directly from plants or indirectly from animal sources. Although several works have been reported on the minerals content of medicinal plants in many developed countries, however, this work was the first to be conducted in Palestine. The efficacy, safety and quality of herbs are of important concern for both consumers and health authorities. In *S. palaestina* leaves the abundant mineral was potassium (22207.5 mg kg⁻¹) which is the major ion inside every living

Fig. 1: Minerals in *S. palaestina* leavesTable 1: The detected mineral, related wavelength, LOD, LOQ and the concentration in *S. palaestina* leaves

Analyte names	Wavelength (nm)	LOD (ppb)	LOQ (ppb)	Analyte concentration (ppm)
Ag	328.065	1.3893	4.6311	ND
Al	394.395	5.2188	17.3960	310.9
Ba	455.386	0.1401	0.4670	14.665
Ca	315.885	25.9416	86.4719	12365
Cd	228.797	2.2294	7.4313	0.0413
Co	238.885	2.2912	7.6373	ND
Cr	267.704	2.6253	8.7510	0.7078
Cu	324.752	1.5875	5.2918	8.49
Fe	259.933	1.8094	6.0313	217.0375
K	766.46	20.3046	67.6820	22207.5
Mg	285.211	5.2790	17.5968	1908.5
Mn	257.608	0.4265	1.4218	13.3088
Mo	202.03	4.4794	14.9314	0.8969
Na	589.571	3.1390	10.4634	490.125
Ni	231.599	4.3459	14.4862	0.5543
Pb	216.999	28.9402	96.4674	0.2267
Sr	421.525	0.0333	0.1110	22.7188
Zn	213.856	1.61035	5.3678	27.41

plant and animal cell. It is involved in nerve impulses, muscle contractions, including the heart muscle and in influencing osmotic balance³⁰⁻³³. The ion pump (Na⁺/K⁺ATPase) uses ATP to pump sodium ions out of the cell and potassium ions into the cell³⁴. The optimal daily required intake (DRI) of potassium was estimated by the Institute of Medicine in 2004 as 4,700 mg of potassium. Several research groups related stroke with low potassium intake^{30,35-37}. In practice, banana is well known as a rich source of potassium, 100 g of banana contains about 358 mg of potassium this means that each gram of banana contains 3.58 mg while *S. palaestina* leaves contain about 22.2075 mg g⁻¹. In other words, one weight of *S. palaestina* leaves contains potassium approximately seven times more

than that of the same weight of banana³⁸. If assumed that about 2 g of leaves are needed to make one cup of *S. palaestina* tea, then quantities of minerals in one cup are illustrated as in Table 2. It was found that the concentrations of the minerals in one cup of *S. palaestina* tea were as follows: Al (0.6218 mg), Ba (0.0293 mg), Ca (24.73 mg), Cd (0.0001 mg), Cr (0.0014 mg), Cu (0.0170 mg), Fe (0.4341 mg), K (44.415 mg), Mg (3.817 mg), Mn (0.0018 mg), Mo (0.0018 mg), Na (0.9803 mg), Ni (0.0011 mg), Pb (0.0005 mg), Sr (0.0454 mg) and Zn (0.0548 mg) as shown in Table 1.

When talking about potassium always comes to mind sodium which is the main cation in the outer cells in all creatures. It regulates blood volume, pressure and pH. Also, it

Table 2: Approximate minerals concentration in one cup of *S. palaestina* tea

Analyte names	Analyte concentration (ppm)	Concentration mg/2g (cup)
Ag	ND	ND
Al	310.9	0.6218
Ba	14.665	0.0293
Ca	12365	24.73
Cd	0.0413	0.0001
Co	ND	ND
Cr	0.7078	0.0014
Cu	8.49	0.0170
Fe	217.0375	0.4341
K	22207.5	44.415
Mg	1908.5	3.817
Mn	13.3088	0.0266
Mo	0.8969	0.0018
Na	490.125	0.9803
Ni	0.5543	0.0011
Pb	0.2267	0.0005
Sr	22.7188	0.0454
Zn	27.41	0.0548

is important in neuron function and osmoregulation between cells and the extracellular fluids^{39,40}. The minimum requirement of sodium is about 500 mg per day. *S. palaestina* leaves contain a relatively high concentration of potassium and a low concentration of sodium which is optimal to maintain good health^{41,42}. The second abundant mineral in the leaves was calcium which is very important for life. It plays an important role in building stronger, denser bones early in life and keeping bones strong and healthy later in life. Also, it has other significant roles such as in neurotransmitter release and muscle contraction. Long-term calcium deficiency can lead to rickets and osteoporosis⁴³. Some individuals are allergic to dairy products, others (vegetarians) avoid dairy products for ethical and health reasons. In these categories, plants could be the main source of calcium which is required daily intake for adults is nearly about 1000 mg. *S. palaestina* leaves contain about 12365 ppm, while it contains about 1908.5 ppm of magnesium for which the daily recommended value is about 300 mg for men and 270 mg for women and ratio between both minerals is required to maintain good health. Magnesium is vital since it works as a coenzyme and as a catalyst^{41,42,44-46}. Other minerals are needed in relatively very small amounts, while some of them such as Cd, Co, Pb and Cr might deteriorate human health and their availability in herbal medicines are controlled in several countries as recommended by the WHO guidelines for herbal assessing quality^{21,47}. Current results are similar and consent with the studies that determine the minerals too.

Table 3 was adopted from the WHO guidelines and illustrated the proposed national limits for toxic metals in various types of herbal products. The most common heavy metals implicated in human toxicity include lead, mercury,

arsenic and cadmium, although aluminium and cobalt may also cause toxicity⁴⁸. The accumulation of these metals in herbs is highly dependent on their availability in the soil. Moreover, cross-contamination might occur also during the drying and processing of the herb⁴⁹⁻⁵².

In Palestinian tested *S. palaestina*, all results for these metals were within allowable limits except for aluminium, which is not used in any biochemical process in living systems. It accumulates in the brain and, to a lesser extent, in the bones^{49,53}. This accumulation can persist for a very long time in various organs and tissues. High levels of aluminium may produce DNA damage, which might cause carcinogenicity⁵⁴. Aluminium has shown neurotoxicity and it was suggested that aluminium is implicated in the etiology of Alzheimer's disease and associated with other neurodegenerative diseases in humans⁵⁵⁻⁵⁷. Even more, *in vivo* studies indicated that there was a relation between aluminium and dysfunction of the male reproductive system⁵⁸. Besides, clinical studies conducted on maternal exposure had shown that aluminium might cause embryo toxicity and affect the developing nervous system in the fetus⁵⁹. The Environmental Protection Agency (EPA) has set the maximum acceptable level of aluminium in freshwater at 750 $\mu\text{g L}^{-1}$ (Kg). In *S. palaestina* leaves, the result of aluminium was 310.9 mg kg^{-1} , which means that one cup prepared by 2 g of the leaves will contain about 621.8 μg of aluminium and two cups of this tea will exceed the maximum acceptable limit (1243.6 μg^{-2} cups). Nevertheless, due to the cumulative nature of aluminium, it was suggested that a tolerable weekly intake (TWI) for aluminium rather than a tolerable daily intake (TDI) is strongly recommended. Aluminium concentration in medicinal plants depends on geographical location, soil, air and water

Table 3: Examples of national limits for toxic metals in herbal medicines and products

For herbal medicines	Arsenic (AS)	Lead (Pb)	Cadmium (cd)	Chromium (Cr)	Mercury (Hg)	Copper (Cu)	Total toxic metals as lead
Canada							
Raw herbal materials	5 ppm	10 ppm	0.3 ppm	2 ppm	0.2 ppm		
Finished herbal products	0.01 mg/day	0.02 mg/day	0.006 mg/day	0.02 mg/day	0.02 mg/day		
China							
Herbal materials	2 ppm	10 ppm	1 ppm		0.5 ppm		20 ppm
Malaysia							
Finished herbal products	5 mg Kg ⁻¹	10 mg Kg ⁻¹			0.5 mg Kg ⁻¹		
Republic of Korea							
Herbal materials							30 ppm
Singapore							
Finished herbal products	5 ppm	20 ppm			0.5 ppm	150 ppm	
Thailand							
Herbal materials, finished herbal products	4 ppm	10 ppm	0.3 ppm				
Who recommendations		10 mg Kg ⁻¹	30 mg Kg ⁻¹				
For other herbal products							
Nation sanitation foundation draft proposal (raw dietary supplement) ^a	5 ppm	10 ppm	0.3 ppm	2 ppm			
Nation sanitation foundation draft proposal (finished dietary supplement) ^a	0.01 mg/day	0.02 mg/day	0.006 mg/day	0.02 mg/day	0.02 mg/day		

contamination. In general, the uptake of metals by plants is influenced by several factors including metal concentrations in soils, cation-exchange capacity, soil pH, organic matter content, types and varieties of plants and plant age. However, the most important factor is the concentration of the metal in the soil and the existing environmental conditions^{28,49,60-62}.

The presence of minerals in medicinal plants including *S. palaestina* could cause drugs food interactions. Therefore, special recommendations for consumers might be necessary to prevent *S. palaestina* minerals from binding with drugs such as tetracycline or ciprofloxacin and affecting their absorption (take the drug 1 hr before or 2 hrs after this plant). Finally, the general belief that medicinal plants are safe and devoid of toxicity could be misapprehended and therefore, WHO recommended that medicinal plants should be checked for the presence of certain metals that might deteriorate the general health⁴⁷. *S. palaestina* sample was obtained from a very famous herbal shop in Ramallah and further work is needed to screen the availability of the minerals in other samples from other Palestinian governorates. Since the safety and efficacy of herbal medicine are closely related to the quality of the source, we recommend conducting a deepen discussion with the Palestinian regulatory bodies to warrant safer consumption of these herbs.

CONCLUSION

Screening of native *S. palaestina* leaves minerals revealed that it is rich in potassium. One gram of leaves contains seven

times more potassium than that of the same weight as a banana. Besides, these leaves were rich in calcium and magnesium. The general belief that medicinal plants are safe and devoid of toxicity could be misconstrued. Hence, the amount of aluminium in the tested sample was exceeding the permissible limits and due to the accumulation properties of this element, long-term usage may deteriorate health.

SIGNIFICANCE STATEMENT

This study discovered that *S. palaestina* leaves are rich in minerals especially, potassium, but it turned out that they contain a high amount of aluminium that can be beneficial/harmful for health. This study will help the researchers, consumers and regulatory bodies to avoid drugs food interactions, toxicity and to uncover the critical areas of the safety of medicinal plants that many researchers were not able to explore. Thus a new theory on medicinal plants' safety and toxicity may be arrived at.

ACKNOWLEDGMENT

We would like to thank the Central Public Health Laboratory (CPHL) staff, Ministry of Health in Ramallah for providing the instrument for the analysis. Special thanks to Mr. Ibrahim Salem for facilitating this research at the Ministry of Health in Ramallah.

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