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Original Research Article

Determination of trace element levels in flowers and leaves of vicia faba by ICP-MS

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GRAPHICAL ABSTRACT

ABSTRACT

As the flowers and leaves of *vicia faba* contain high levels of levodopa used in the treatment of Parkinson's disease, its use in alternative medicine is becoming more and more common. In general, the flowers and leaves of *vicia faba* are consumed as tea. Besides some trace elements, they show a significant role in human nutrition and may pose a risk to human health at high levels. However, there is not much literature on chemical analysis of flowers and leaves of *vicia faba*. Their element content has not been studied yet. Inductively coupled plasma mass spectrometry (ICP-MS) has been used to determine the trace elements contents of the *vicia faba* flowers and leaves. Mineral distribution ranging from the trace to the main elements for this plant samples were dried, weighed, digested and analyzed by ICP MS. Thirteen element contents of flowers and leaves have been analyzed. Potassium, calcium and magnesium have been found at high concentrations in flowers and leaves. Among trace metals, iron had the highest concentration, followed by Zinc, Aluminium, Manganese, Chromium, Copper, Nickel, Lead and Cadmium. The analysis showed that the toxic Cadmium element is in a low concentration and was within the limit allowed by the World Health Organization. As a result, vicia faba leaves and flowers can be used safely as medicinal plants. However, it should not be consumed in excessive amounts.

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INTRODUCTION

Vicia faba, widely known as ''Bakla'' in Turkey, is commonly grown for its nutritious seeds and vats that are eaten by millions of people worldwide. It belongs to Fabaceae and is high in protein like many legumes because of its ability to fix nitrogen from the air via a symbiotic relationship with root nodulous bacteria. Today faba bean is cultivated in the world's temperate and subtropical areas, and in the tropics

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at higher altitudes. The stalking blossoms are set on an axis without branching. The stalks are low, 1-6 blooming and are axillary between the principal stalk and the base. The flowers are aromatics, the blowjobs are white, the outer petal is marked with a central, dark brown, basal or black blotch and is papilionate. There are 10 stamens in each flower, nine of which are fused into a partial tube, with the 10 th stamen free. The ovaries are placed above the sepals, petals and stamens. The style is about 3 mm long and is abruptly turned upside down, with a tuft of hair close to the stigma[1]. **Fig. 1** demonstrates the flowers and leaves of vicia faba.

Fig. 1. Flower and Leaves of Vicia faba

Levodopa is a precursor to the synthesis of noradrenaline and dopamine in humans and animals, a classical antiparkinsonian drug. It is used for clinically treating Parkinson's disease [1]. Chemical synthesis, phytoextraction, microbial enzymatic conversion, and so on are key production methods for levodopa.

There are many methods of chemical synthesis. But seperating dextrodopa and levodopa mixture is the principal challenge. Dextrodopa has no physiologicalotation effect on the human body so in medicine the optical movement of dopa must be controlled. Microbial fermentation products contain many insoluble and soluble impurities that must be removed to separate high purity levodopa. There are various insoluble and soluble impurities in the microbial fermentation products which need separating high purity levodopa. It has numerous problems, such as complicated procedures, hard infrastructure, poor recovery and high production costs. As levodopa is in the plant, it is possible to reduce steps and expenses by extracting levodopa from plants[2]. Levodopa is currently mainly produced from cat beans, the highest levodopa content is only 6 percent[3-5]. However, the highest levodopa content of faba bean flowers is up to 11.83 percent[1]. Vicia faba flowers can therefore be used as good raw materials to extract Levodopa.

Although the content of vicia faba, levodopa used in the treatment of Parkinson's disease, has been extensively researched, there is much less analysis of trace elements of this product. Growing interest in flowers has increased research on their nutritional value, biological activity and bioactive components[6]. A wide range of phytochemicals present in flowers may prevent aging and chronic diseases associated with inflammation and oxidative stress. Studies on composition and bioactivities of flowers are available in the literature. However, further safety and use reports of flowers are required if they are to be considered functional foods or part of our diet[7]. Egebjerg has already demonstrated the lack of information about its toxicological effects[8]. They analyzed 23 known flowers, and nine contained compounds with or possible toxic consequences. Studies relating to the phytochemical composition and flowers bioactivities are needed to avoid these situations.

All these types of plant-based pharmaceuticals are included in the nutritional supplement category which are not subject to strict regulation. While medicinal plants are regarded as safe by ordinary people, examining in more depth the possibility of toxic metal build-up and control or unwanted elements, it is reasonable and desirable to evaluate material products as a requirement for their use[9,10]. If you harvest the medicinal plants from the flora spontaneous, because of where they were collected, they may be contaminated by heavy metals[11,12]. In this way, contamination with known or unknown contaminants raises the possibility of medicinal plant crop production, in pollution-protected areas, an important additional argument besides the economic issues. The basic raw plant material composition should be a critical issue with regard to the quality and safety of herbal products. In this way, basic nutrients (K, Na, Mg, Fe, K, Mn, P and Zn), micro and trace elements (Co, Cr, Fr, Ni, Se and V) and unwanted, potentially toxic elements (Al, Ba, As, Sn, Co, Cd, Cr, Hg, Ni, Pb and Sb) are typically monitored.

The composition of trace elements in medicinal plants is closely linked to soil nature, temperature, plant capacity to absorb nutrients and environmental pollution[13,14]. Some of the trace elements (e.g. chromium, iron, copper, manganese, zinc and selenium) play a significant role in nutrition for humans. Such elements may however pose a risk to human health, including low absorption of oxygen in high blood, kidney and liver diseases[15,16]. Even low levels of trace elements not essential such as aluminum, cadmium, arsenic, barium, nickel and lead can cause toxicity that include cancer, neurological, skin and gastrointestinal disorders[15-17].

While mineral content studies in medicinal herbs have been conducted worldwide, to our knowledge, few literature studies have been recorded on levels of trace elements in the vicia faba flowers. The main goals of this analysis were therefore to examine the levels of therteen trace elements (Na, K, Ca, Mg, Fe, Zn, Al, Mn, Cr, Cu, Pb, Cd and Ni) in vicia faba flowers, and the levels of trace elements have been quantified by ICP-MS.

EXPERIMENTAL

Reagents and chemicals

All chemical products were purchased from Merck and were of the highest quality. The standard stock solutions (1000 mg L^{-1}) were obtained from Merck for the ICP-MS measurements. De-ionised water (18.2 MOhm-cm) was obtained from a Milli-Q water purification system. Glassware was cleaned with a 10 percent (v v^{-1}) solution of HNO_3 and rinsed a few times with deionized water.

Microwave Assisted Digestion.

Microwave-assisted digestion of the sample was conducted prior to analysis to minimize the effects of the organic plant matrix. In this method, 1 g dried ground plant sample was weighed into teflon digestion vessels and 8 mL of $HN0_3$ solution (65 percent, v v^{-1}) and 4 mL of H_2O_2 solution (30 percent, v v^{-1}) were added. Teflon vessel cover was closed and the mixture was mineralised by applying the following heating method in a closed microwave system (Multiwave 3000, Germany). Heating systems for the Microwave Oven: The system of combustion began at 500 W, was ramped for one minute, and kept for four minutes. The second step began at 1000 W, ramped for five minutes and then held for five minutes. The third step began with a power of 1400 W, ramped for five minutes, then kept for ten minutes and then allowed cooling of burned samples. Upon cooling, sample solutions were transferred quantitatively into volumetric flasks of 100 mL and made with de-ionized water up to the volume.

Apparatus

Determining multiple elements was conducted by an Agilent 7700 ICP-MS device equipped with the technology to reduce collision/reaction cell interference. The continuous sampling system consisted of an autosampler, a Scott double-pass spray chamber, a glass concentric MicroMist nebuliser, a quartz torch and nickel cones. Elemental concentrations have been quantified using the MassHunter Work Station Software. The ICP-MS parameters, the daily instrumental optimization the conditions and the chosen isotopes for determination are summarized in **Table 1**.

All of the samples were corrected blank form and analyzed in triplicate, with $_{45}$Sc, $_{72}$Ge as internal standards. Calibration curves were developed by testing standard fresh solutions immediately before analysis of the samples. Everywhere, Linear responses with zero intercept were obtained, coefficients of correlation greater than 0.9995 and relative standard deviation less than 4 percent.

Table 1. Working conditions (ICP-MS)

Parameter	Conditions
Generator frequency	27 MHz
RF Power	1 550 W
Argon flow rate	15 L min^{-1}
Auxiliary gas flow rate	0.9 L min^{-1}
Helium flow rate	5 and 10 mL min^{-1} for high energy mode
Micro-mist nebulizer gas flow rate	1.1 L min^{-1}
Spray chamber	Quartz, Double pass spray chamber
Sample uptake	0.1 rps
Number of replicates	3
Integration time	0.3-1.0 s
Internal standards	$_{45}$Sc and $_{72}$Ge,
Analytical Isotopes	$_{23}$Na, $_{39}$K, $_{40}$Ca, $_{24}$Mg, $_{52}$Cr, $_{56}$Fe, $_{66}$Zn, $_{27}$Al, $_{63}$Cu, $_{206}$Pb, $_{111}$Cd, $_{55}$Mn, $_{60}$Ni, $_{45}$Sc and $_{72}$Ge
Concentration range	1 and 1.000 µg L^{-1}
ISIS-DS parameters	
Loop volume	150 µL
Uptake time	40 s
Acquisition delay	40 s
Rinse time	During data acquisition

Quality Control Analysis and Statistical

The sample analysis was carried out in triplicate experiments, and the same sample protocol was followed by blank experiments. Analytical methods have been checked in accordance with the ICH Guideline (ICH Harmonization Tripartite Guideline) [18]. LOD was estimated as the concentration corresponding to three times the standard deviation (3σ) of the independent blank corrected results obtained from a set of reagent blanks (n = 10), LOQ was estimated as the concentration corresponding to ten times the standard deviation (10σ) of independent blank corrected results obtained from a set of reagent blanks (n = 10). Linearity curves (for analytical standards) were determined by the correlation coefficient and obtained values were $R^2 > 0.9995$. Exactness was tested using accredited reference materials *Peach* leaves (SRM 1547) and *Camellia sinensis* leaves (SRM 3254) (Merck, Turkey) and spiked experiments. The recoveries vary from 85 to 112 percent and from 87 to 110 percent were verified for certified reference materials and spiked experiments, respectively. Precision was assessed taking into account the coefficient of variation (CV, in percentage) for 16 sample replicates. Using XLSTAT software, statistical one way variance analysis (ANOVA) and Tukey testing were carried out. The components were essentially analyzed using Pirouette tools.

RESULTS AND DISCUSSION

The basic compositions of the leaves and flowers of Vicia Faba were determined using ICP MS. A total of 13 elements (Na, Ca, K, Mg, Fe, Al, Zn, Mn, Cr, Pb, Cu, Ni and Cd) were analyzed in the flowers and leaves of this plant. The results of the analyses weare given in **Table 2**. Each result is at least three independent tests on average. These elements in the flowers and leaves of the plant plays a crucial role in forming secondary metabolites, which are responsible for the pharmacological effects of the plant. In this study, Vicia Faba leaves exhibited higher concentrations of K, Na, Ca, Cr, Mg, Fe, Pb, Al, Cu, Cd, Ni and Mn compared to their flowers.

Calcium vanquish high blood pressure issues, heart attack, premenstrual syndrome, colon cancer and keeps bones healthy and reduces the risk of osteoporosis in older age[19,20]. The average calcium for leaves in this syudy was 36 193 mg kg^{-1} and was 6 053 mg kg^{-1} for flowers.

Magnesium increases the sensitivity to insulin, helps prevent diabetes and its complications and reduces blood pressure [21]. Additionally, magnesium is involved in the enzymatic reactions of food metabolism and cell part synthesis, nerve impulse transmission, body temperature control, detoxification, energy generation and healthy bones and teeth formation [22]. The average content of magnesium for leaves was 131.07 mg kg^{-1}, and for flowers was 20.04 mg kg^{-1}.

Zinc is a component of a wide range of enzymes, including polymerases from ribonucleus, dehydrogenase alcohol, carbonic anhydrase dioxide, and alkyne phosphate. Animal research shows that Zinc absence can lead to developmental disturbances in the offspring during pregnancy. Zinc is an important component of living sperm, particularly human sperm. It is required for cell growth and proliferation [23]. Lack of zinc can lead to hair loss and retardering growth, delayed wound healing, and emotional discomfort [24].

Table 2. Elemental contents (mg kg^{-1}) of flowers and leaves of vicia faba.

Element	Flowers (mg kg^{-1})	Leaves (mg kg^{-1})
Sodium	2 312 ± 0.026	4 722 ± 0.042
Potassium	23 421 ± 0.88	23 074 ± 0.076
Calcium	6 053 ± 0.065	36 193 ± 0.032
Magnesium	1 751 ± 0.012	5 131 ± 0.027
Chromium	2.21 ± 0.001	9.02 ± 0.002
Iron	559 ± 0.002	1 040 ± 0.003
Zinc	48.12 ± 0.001	23.10 ± < 0.001
Aluminium	22.20 ± 0.003	35.07 ± 0.002
Copper	2.06 ± 0.001	6.03 ± 0.002
Lead	0.31 ± 0.001	0.82 ± 0.001
Cadmium	0.11 ± 0.001	0.21 ± 0.001
Manganese	20.04 ± 0.001	131.07 ± 0.002
Nickel	2.42 ± 0.001	6.04 ± 0.001

The average Zinc concentration for leaves was 23.10 mg kg^{-1}, and for flowers was 48.10 mg kg^{-1}.

The human body needs Iron for the oxygen formation which carries protein haemoglobin and myoglobin. It is an essential mineral for the prevention of enzyme-converting inhibitors associated with anemia and cough[25]. Furthermore, Iron is an important cofactor in neurotransinitor synthesis such as dopamine or epinephrine and serotonin[26]. The average content of Iron for leaves was 1040 mg kg^{-1} and for flowers was 559 mg kg^{-1}.

Manganese can help the body metabolize protein, carbohydrate metabolization and diabetes treatment[27]. In this study, the average Manganese content was 131.7 mg kg^{-1} for leaves, and 20.04 mg kg^{-1} for flowers. In this analysis, the average Manganese content was 0.130 mg kg^{-1} for leaves, and 0.020 mg kg^{-1} for flowers.

Lead is a dangerous metal and harmful product for the human body because it causes high blood pressure, kidney damage, low and fine abortion, brain damage, decreased men's fertility due to sperm injury, reduced children's learning skills and damaged nervous systems[28,29]. The average Lead content was 0.82 mg kg^{-1} for leaves and 0.31 mg kg^{-1} for flowers in our study.

For herbal medicines and products, the maximum limit for Cadmium is 0.3 mg kg^{-1} while the limit for dietary intake is recommended as 10.3 mg kg^{-1} by the World Health Organization (World Health Organization. Quality control methods for medicinal plant materials). Chromium was determined at a ratio of 9.02 mg kg^{-1} in the leaves, while its content in the flowers is 2.21 mg kg^{-1}. Toxic elements were found below the prescribed limits İn our study. Overconsumption can lead to human body toxicity. Lead *and* Cadmium *lead to acute and chronic toxicity,* adverse effects on the kidney, liver, cardiovascular and immune system, brain damage, fine abortion, men's sperm damage and loss of fertility, *children's cognitive ability* and nervous systems deterioration[30,31].

CONCLUSION

Various factors such as the quality of the soil, climate, and agricultural practices influence the basic elements a plant receives. Additionally, environmental factors such as atmosphere and waste, sampling season, plant age and condition of the soil where the plant grows may affect the content of the elements. The contents of the elements are not distributed evenly all over the plant. In this study, the different content of the elements varies depending on these factors. This research will contribute to the synthesis of novel modern drugs with different combinations of plant parts that can be used ethnomedically in the treatment of many modern diseases. The various concentrations of elements in the various regions of Vicia Faba lead to the conclusion that the plant has various properties for treating various diseases. More detailed analysis is however required for the chemical composition of this important medicinal plant's various parts.

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