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# Comparative study on the elemental composition of different parts of cultivated *Physalis alkekengi* (Solanaceae)

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*Abstract*: This study aimed at analyzing and comparing the elemental composition of different parts of cultivated *Physalis alkekengi* (ljoskavac): rhizome with roots, stem with leaves, fruit, and inflated calyx. The contents of twenty--one macro- and micro-elements were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). In addition, the patterns of the distributions of both macro- and micro-elements were subjected to AHC analysis which gave different grouping of samples in sub-clusters. Generally, potassium, calcium, iron, and aluminum were the most abundant elements, but with different distribution in examined parts. High contents of iron and aluminum were detected in a stem with leaves, followed with samples of rhizome with roots and calyx, while potassium dominates in samples of calyx and stem with leaves. Edible fruits did not contain potentially toxic metals in concentration higher than permissible limits, wherein the lowest contents of lead and aluminum were detected; cadmium was under limit of quantification. Arsenic, mercury, and thallium were below the method detection limit.

*Keywords*: ljoskavac; ICP-OES; macro- and microelements; AHC.

## INTRODUCTION

Among cultivated species frequently represented in the diet of humans (chili – *Capsicum annuum*, tomato – *Lycopersicon esculentum* and potato – *Solanum tuberosum*), the Solanaceae family contains wild growing species, such as those from the fifth largest genus within the family, *Physalis* L.[1] The genus *Physalis*, a clearly defined genus within the nightshade family, comprises about 70–95 species famous for their attractive appearance, application as foods and natural remedies, which justifies worldwide cultivation. The *Physalis* species are low to

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large annual or perennial herbaceous plants (up to 1 m tall), or shrubs with attractive papery husk calyx (lantern-like) wrapping the globose yellow to orange, smooth-skinned berry (fruit) small (4-7 mm) or large (10-20 mm) with a juicy or dry pericarp and with many small seeds. Name *physalis*, derived from the Greek word *phusa*, means bladder and corresponds to the inflated calyx which completely covers the fruit during its growing and ripening periods and protects it against conditions of biotic and abiotic stress. Members of the genus are mostly native to the Americas, with the centers of distribution in Mexico (over 70 species) with pronounced endemism; then United States and Central America, and finally South America with the least species. A few species are registered in Asia and Europe, and six in China.[2,3] Surely, the most attractive in a diet and ethnomedicine are fruits of various species, such as *P. peruviana*, *P. pubescens*, *P. alkekengi*, *P. angulata*, *etc*. Depending on the region of origin and national cuisine, the fruits are consumed raw, or in sauces, compotes, pies, jams, or relishes. According to an ethnobotanical survey of Arenas & Kamienkowski, the leaves of the species *P. angulata* are also edible and used in salads.[3] *Physalis* species are natural sources of diverse compounds, thus many ethnopharmacological properties are attributed to phytochemicals as potential bioactive principles: minerals, vitamins, carotenoids, sterols, phenols, phenolic acids, flavonoids, glycosides, tannins, alkaloids, *etc*. Due to edible fruits, numerous taxa from *Physalis*, cultivated or wild growing, present economically useful crops. Less than *P. peruviana*, but also *P. philadelphica*, *P. ixocarpa*, *P. pubescens* and *P. alkekengi* are cultivated or collected from native populations for their edible fruits and nutritional value.[4] The most famous representative of the genus is *P. peruviana*, colloquially known as golden berry or Cape gooseberry, is frequently used in the food industry due to its nutritional value, pleasant sensory characteristics and as a great source of vitamins C, A, E, B3, B6; and the elements iron, magnesium, potassium, phosphorus, and calcium.[1,3,5–10] *P. alkekengi* is also recognized and described in modern and traditional medicine and also cuisine. Mature fruit of the plant (*Fructus alkekengi*) is a strong diuretic and laxative and could be locally applied (balm) as a healing agent in gout, rheumatism, erysipelas, syphilis therapy, as an accelerator in wound healing, *etc*. Juicy, sweet fruit with a certain bitter-sour note, contains sugars, organic acids, a trace of alkaloids, and high content of vitamins and carotenoids. Unripe fruit can be mildly poisonous, so it is necessary to collect mature fruit in August and September and subsequently discard the poisonous calyx. Whether used as a food or in medicine, the fruits of *P. alkekengi* can be consumed raw or dried, as salad, aqueous extracts, syrup, compote, or jam. An adult should not eat more than 30 raw berries a day. The edibleness of the fruits is widely known in the countries of Europe and Japan. Except for mature fruit, other parts of the *P. alkekengi* are not edible.[8,11–13] According to the literature, there is no data on the elemental composition of *P.*

*alkekengi*, except the study on the rhizome by Xu *et al*.[14] Aware of the many benefits from the *Physalis* taxa, and the importance of novel research, this study aimed at determining and comparing the contents on macro- and micro-elements in different parts of cultivated *Physalis alkekengi* – rhizome with roots (hereafter rhizome-roots), stem with leaves (hereafter stem-leaves), mature fruits and inflated calyx (hereafter calyx). Furthermore, these would be the first results (comparative study) on the elemental composition of different parts of *P. alkekengi*.

## EXPERIMENTAL

### *Plant material*

The plant material was planted in March and collected in September 2020. Cultivation of *P. alkekengi* individuals was performed from seeds in sets of plastic containers of uniform diameter under the same light conditions depending on the seasonal variation of the photoperiod, on the standardized substrate Floradur® (mixed with sand in the mass ratio of 4:1), and watering twice a week with an equal amount of water. Voucher specimens were deposited in the Herbarium of the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš (HMN; Voucher No. 14543). All the examined plant parts of cultivated *P. alkekengi* are summarized in Fig. 1.

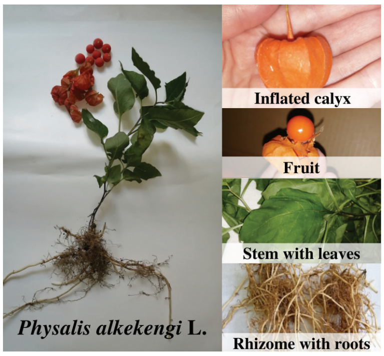


Fig 1. Cultivated *P. alkekengi* L.; plant material used to determine the elemental composition.

### *Sample preparation and parameters of ICP-OES analysis*

For the experiment, fifteen plant individuals were collected. The plant material was divided and organized into four plant parts: rhizome with roots, stem with leaves, fruits, and calices. Prior a digestion, the rhizomes with roots were washed with deionized water and drained. All samples were chopped into small pieces with stainless-steel scissors, dried in a drying oven (at 60 °C) to a constant mass, and finally, the samples were weighed: rhizome-roots 1.0134 g, stem-leaves 1.0515 g, fruit 1.2661 g, and calyx 1.0169 g of average dry mass, performed in triplicate, $n = 3$. Digestion of samples was realized according to slightly modified procedure of Mosetlha *et al.*[15] Each sample was mineralized in an Erlenmeyer flask with 20 mL of concentrated $HNO_3$. The samples were covered with a watch glass and left overnight. The following day, the digests were evaporated, and then diluted with 0.5 % $HNO_3$ (in ultra-pure deionized water, 0.05 µs cm$^{-1}$) up to the volume of 25 mL, followed by filtration (grade 589/3 blue ribbon). The analysis was performed using an iCAP 6000 inductively coupled plasma optical emission spectrometer (Thermo Scientific, Cambridge, UK), which uses the Echelle optical design and a charge injection device solid state detector. The nebulizer was glass concentric. iTEVA software from Thermo Scientific (Cambridge, UK) was used to collect and analyze the data.[16] Multi-element standard solution IV of the microelements Al, As, Ba, Be, B, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se, Tl, V and Zn, standard solution III of the macroelements Ca, K, Mg and Na, as well as individual standard solutions of Si, P and Hg (TraceCERT, Fluka Analytical, Switzerland) were used for calibration. Linearity in checked intervals was satisfied with a coefficient of determination above 0.9994. All measurements were performed in triplicate. Parameters of conducted ICP-OES analysis based on a calibration curve: wavelength of selected emission lines, linearity of the calibration curves, coefficient of determination ($R^2$), limit of detection (*LOD*) and limit of quantification (*LOQ*) of the calibration for each element determination are given in Table S-I of the Supplementary material. The *LOD* and *LOQ* values were calculated using the $3\sigma$ and $10\sigma$ criterion, respectively.[17]

*Statistical processing data*

Statistical data processing was performed by Statistica 8 software (Statsoft, Inc., Tulsa, OK, USA). Two statistical matrices included the content of macro- and microelements as original variables. Agglomerative Hierarchical Clustering (AHC, using the Ward's method and Euclidean distance) was conducted to visualize how the contents of macro- and microelements affect differentiation among studied samples (plant parts).

## RESULTS AND DISCUSSION

The results obtained on the elemental composition of different parts of cultivated *P. alkekengi* are presented in Table I – macroelements: Ca, K, Mg, P, Na; and Table II – microelements: Al, B, Ba, Be, Co, Cr, Cu, Cd, Fe, Mn, Ni, Pb, Se, Si, V, Zn.

TABLE I. The content ($c \pm SD^a$ / mg g$^{-1}$; mean values of element content (all measurements were performed in triplicate, $n = 3$); *SD* – standard deviation) on macroelements in the studied samples of cultivated *P. alkekengi*; samples: R – rhizome-roots, SL – stem-leaves, C – calyx, F – fruit

| Element | Sample | | | |
|---|---|---|---|---|
| | R | SL | F | C |
| Ca | 3.28±0.02 | **14.51**±0.05 | 0.503±0.002 | 4.20±0.04 |
| K | 13.48±0.08 | 34.81±0.04 | 12.66±0.03 | **38.5**±0.3 |
| Mg | 1.59±0.09 | **3.11**±0.05 | 1.361±0.004 | 1.568±0.003 |
| P | 1.65±0.010 | 2.98±0.02 | 3.32±0.02 | **4.15**±0.02 |
| Na | 0.0018±0.0001 | 0.0018±0.0001 | 0.0015±0.0001 | **0.0114**±0.0001 |

Among all measured macroelements, K was the most abundant in all samples (Table I): C (calyx) > SL (stem-leaves) >> R (rhizome-roots) ≈ F (fruit). Previously, Aguilar-Carpio *et al.*[18] determined the growth dynamics and yield of Cape gooseberry cultivation by varying the concentrations of the nutrient solution under greenhouse conditions. They claimed $K^+$ could be absorbed to the greatest extent by plants, thus, adequate nutrition enriched with this element is associated with increases in fruit yield and quality. Right after K, Ca was the next abundantly present element in the studied samples, but with the highest content in the SL samples. Considering other macroelements, Mg dominates in the SL samples, while P in the C samples. The conducted statistical (AHC) analysis segregates plant parts according to the content of macroelements (Fig. 2a). In the cluster, two branches are distinguished, R and F samples on one side, and SL and C on the other side. Regarding the microelements, Al and Fe are the two elements that obviously dominate in the group, with similar contents among the studied samples (Table I): SL >> R > C >> F. The AHC singled out three entities: 1) SL; 2) F; 3) R and C (Fig. 2b).

TABLE II. The content ($c \pm SD^a$ / mg g$^{-1}$; mean values of element content (all measurements were performed in triplicate, $n = 3$); *SD* – standard deviation)on microelements in the studied samples of cultivated *P. alkekengi*; samples: R – rhizome-roots, SL – stem-leaves, C – calyx, F – fruit; *SD* – standard deviation; ND – not detected; *LOQ* – the limit of quantification

| Element | Sample | | | |
|---|---|---|---|---|
| | R | SL | F | C |
| Al | 424±4 | **899**±11 | 7.60±0.04 | 255±1 |
| B | 12.7±0.2 | 23.8±0.2 | 11.4±0.1 | **32.6**±0.4 |
| Ba | 8.54±0.07 | **17.8**±0.2 | 0.948±0.002 | 6.57±0.04 |
| Be | 0.0148±0.0000 | **0.0357**±0.0000 | ND[b] | 0.0049±0.0000 |
| Co | 0.301±0.007 | **0.51**±0.01 | 0.079±0.002 | 0.202±0.003 |
| Cr | **11.12**±0.06 | 10.73±0.01 | 7.94±0.01 | 10.93±0.08 |
| Cu | 4.56±0.06 | 8.01±0.09 | 8.7±0.1 | **19.7**±0.2 |
| Cd | 0.046±0.002 | **0.058**±0.002 | <*LOQ*[c] | 0.027±0.000 |
| Fe | 406±3 | **741**±6 | 10.36±0.06 | 240±1 |
| Mn | 21.3±0.2 | **42.7**±0.3 | 11.54±0.03 | 18.2±0.1 |
| Ni | **7.66**±0.08 | 6.89±0.07 | 5.99±0.03 | 6.98±0.03 |
| Pb | **1.37**±0.02 | 1.31±0.05 | 0.99±0.02 | 1.19±0.04 |
| Se | **1.72**±0.08 | 1.37±0.02 | 1.16±0.03 | 1.36±0.05 |
| Si | 17±1 | 26±2 | 24±1 | **34**±1 |
| V | 4.82±0.05 | **12.3**±0.1 | 3.26±0.03 | 4.58±0.02 |
| Zn | 19.39±0.09 | 20.73±0.06 | 17.62±0.04 | **25.4**±0.1 |

As above mentioned, there is no data on the elemental composition of *P. alkekengi*, except for the study on rhizome by Xu *et al*, but with different aims.[14] Concerned by the fact that the continuous growth of the plant is strongly endangered by changing the soil elemental composition, leading to the succession cropping obstacle, they tried to find answers in the variation of elemental content between plant rhizome and the surrounding soil. By conducting ICP-MS analysis on the annual, biennial, and triennial *P. alkekengi* (healthy and rotten) rhizomes, they observed variation in trace elements with the time and state of rhizomes: reduction of Mg, K, Ca, and enrichment of V, Fe, Co, Se and Pb. Surely, most studies have focused on the study of the most famous species *P. peruviana*, especially studies on the fruit. Erkaya *et al.* considered Cape gooseberry as a good natural source of nutritive ingredients in ice cream production.[19]

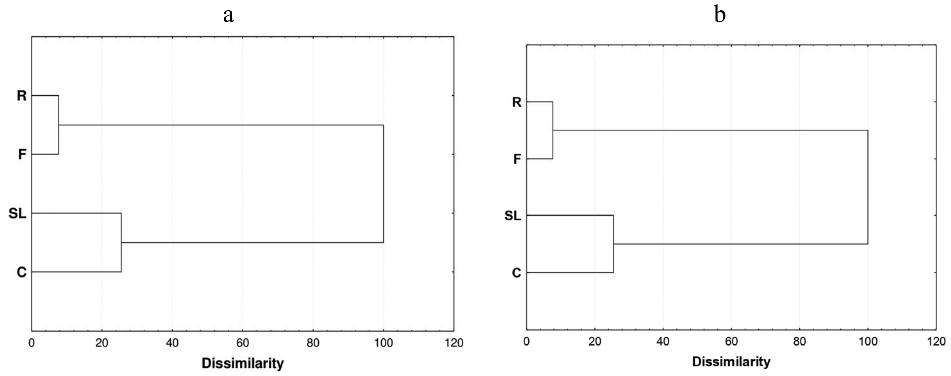


Fig 2. Results of AHC analysis conducted on two patterns: a) macroelements contents and b) microelemens contents of studied *P. alkekengi* samples (R – rhizome-roots, SL – stem-leaves, C – calyx, and F – fruit).

They determined content of selected macro- and micro-elements in the fruit Ca, K, Mg, P, S, Na, Fe, Zn, Mn, and Ni. If comparing that elemental composition of Cape gooseberry[16] with the results of this study, the fruit of *P. alkekengi* contained higher amounts of K, P, Mg, Ni, Mn, while the contents of Ca, Fe and Zn were lower than published. In the study of Karasakal different microwave acid digestion procedures were tested to determine concentrations of Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, Zn, P in many tropical fruits.[20] In case of the *P. peruviana* fruit, most of tested systems for digestion gave the following order for the contents were K >> Mg > S > Na > Ca > Zn > Mn > Fe, which largely agrees with the present results obtained for the *P. alkekengi* fruit, as follows: K >> P > > Na > Mg > Ca >>> Zn > Mn > Fe. Furthermore, the results of El Sheikha *et al.*[21] indicate the highest content of K, followed by P in the fruit juice of *P. pubescens*. A Study of *P. angulata*, grown under normal conditions and treated by Al in the nutrient solution, showed different patterns of elemental composition caused by stress.[22] Obviously, the root of *P. angulata* accumulates the highest levels of Al. Moreover, the certain condition of stress (0.16 M of Al) increased the content of P in the stems and roots; K, Cu, and Mo in all parts of the plants; and reduced the content of Ca, Mg, Fe, and Zn in the tested *P. angulata* plants. Since the contents of Al are high in SL, R, and C samples, as follows, the question is whether it affects the content of other macro- and micro-elements. Surely, by varying the cultivation conditions, the correlation between Al content with other elements could be determined. Karasakal also found a high content of Al in the fruit of Cape gooseberry.[20] High doses of toxic Al could cause damage to the human nervous system and the advised limit value for Al intake is 24 μg $g^{-1}$ 60 kg body weight.[20] If these parameters are considered, an adult person could eat approx. 3 g (dry matter) of *P. alkekengi* fruits, which suggests that concentrations of Al are a kind of acceptable level for human health. On the contrary, other parts of the plant contain Al in a very high concentration. Overall, considering the allowed intake of dry fruit (calculated *via* the Al content), other nutritional macro- and microelements concentrations are below the recommended daily allowance levels. The most common toxic heavy metals include As, Pb, Cd and Hg. The FAO/WHO (2007)[23] prescribes limits of these toxic heavy metals in raw herbs. The permissible limit for Pb in herbs is 10 and 0.3 mg $kg^{-1}$ for Cd. The content of Pb and Cd is quite lower, especially in the fruit sample where the content of Cd falls under the limit of quantification. In all studied samples As, Hg, and Tl were below the limit of detection.

## CONCLUSIONS

According to data obtained, there are differences in the elemental composition of the studied parts of the cultivated *Physalis alkekengi*. K, Ca, Fe and Al were the most abundant elements, but with different distribution in the studied samples. The conducted statistical analysis showed different grouping of samples depending on the contents of macro- and micro-elements. Respecting similarities from AHC analysis on the macro-elements pattern, the rhizome-roots sample corresponds to the fruit sample, while the stem-leaves to the calyx. On the other hand, the distribution pattern of microelements led to segregation of the rhizome-roots sample and calyx sample. Respecting the nutritional aspect, the edible fruits did not contain potentially toxic elements (Pb, Cd, Al) in concentration higher than the permissible limits, while the content of useful elements is not negligible. Furthermore, the contents of Pb and Al were the lowest in the fruit sample, while Cd was present at a value under the *LOQ*. In all studied samples, As, Hg, and Tl were below the limit of the method detection. By improving the growing conditions, primarily to reduce the Al content, the content of the macro- and micro-elements could be influenced. Consequently, by increasing the daily intake of the fruits, concentrations of useful elements could reach up to the recommended daily allowance levels. Thus, *P. alkekengi* presents a useful crop and should be studied from multiple aspects.

## SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/10947, or from the corresponding author on request.

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ИЗВОД

УПОРЕДНА ЕЛЕМЕНТНА АНАЛИЗА РАЗЛИЧИТИХ ДЕЛОВА ГАЈЕНЕ БИЉНЕ ВРСТЕ *Physalis alkekengi* (SOLANACEAE)

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У овом раду први пут је испитиван елементни састав различитих делова гајене биљне врсте *Physalis alkekengi* (љоскавац): ризома са кореном, стабла са лишћем, плода и каликса. Садржај двадесет и једног макро- и микроелемента одређен је методом оптичке емисионе спектрометрије са индуктивно куплованом плазмом (ICP-OES). На матрицама са садржајима испитиваних елемената спроведена је кластер анализа, на основу које је утврђено различито груписање узорака према садржају микро- и макроелемената. Генерално, K, Ca, Fe и Al јесу најзаступљенији елементи, али са различитим обрасцем дистрибуције у испитиваним узорцима. Висок садржај Fe и Al нађен је у узорку стабла са лишћем, затим у узорку ризома са кореном и, напослетку, у узоку каликса. Највећи садржај K нађен је у узорку каликса, потом у узорку стабла са лишћем. Потенцијално токсични метали нису детектовани у плоду у концентрацији већој од прописане, већ је нађен најнижи садржај Pb и Al, док је садржај Cd испод квантификационог лимита методе. У свим испитиваним узорцима садржај за As, Hg и Tl је испод лимита детекције за дату методу.

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