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# CONTRIBUTION TO THE KNOWLEDGE OF THE ANTIOXIDANT POWER, PHENOLIC AND MINERAL COMPOSITION OF *SANGUISORBA MINOR* SCOP.

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**Abstract:**In this study, extracts of the aerial partsof *Sanguisorba minor*Scop. wereanalysed for antioxidant activity, quantification of the total phenolic and flavonoid and bioaccumulation potential of heavy metals.The total phenols amounts was the highest at acetone´sextracts of root (117.27 mg GAE /g dry weight), stem and leaf (133.61 mg GAE /g dry weight ) and flower (116.77 mg GAE /g dry weight) and total flavonoids acetone´sextractsof flower (50.69 mg RU/g). The highest DPPH-scavenging capacity had flower methanol extract 7.08 ($IC_{50}$ μg/ml). The plant accumulates large amounts of Zn, Ni and Cu in the root, stem and leaf and flower. Otherwise Mn, Cr and Pb are represented in whole plant.

**Key words:***Sanguisorba minor*, antioxidant potential, total phenolics, metals

## Introduction

Medicinal plants have a long tradition in all nations, plant-based treatment has been used in natural medicine since ancient times, and experiences and knowledge are passed down from generation to generation. What was previously used as a form of alternative treatment, today becomes a supplement to conventional treatment methods, as a result of this trend there is a great interest in medicinal and aromatic plants.

In this paper, the medicinal properties will be examined of the plant species *Sanguisorba minor*Scop. specie belong to the *Rosaceae*family. Plant species of this family exert a wide range of biological activities such as antioxidant (Barral-Martinez*et al*. 2021, Šola *et al*. 2022).

***Sanguisorba minor***Scop.,garden burnet(Grlić, 1990), small burnet (Mišić, Lakušić, 1990.), is an edible perennial herbaceous plant. Specie belonging to

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the *Rosaceae* family.It has composite leaves with dentate leafleat, one of them, terminal. It is widespread in central and southern Europe, in southwest Asia and northwest Africa(Mišić, Lakušić, 1990.),.

*Sanguisorba minor*, plant specie has not been sufficiently studied in terms of its antioxidant effect. Certain investigations of *Sanguisorba* species indicate they are rich sources of bioactive compounds and shows significant beneficial effects (Grbz, 2005.Zhao*et al.* 2017, Ayoub, 2003).Scientific studies indicate that *S. minor* has numerous positive effects that are related to the content of bioactive compounds (Ayoub, 2003, Arıhana*et al.* 2015).

The present study aimed to examine the phenolic composition and potential antioxidative properties of *S. minor*. The plant sample was taken from a location is polluted. Considering the greater pollution of the environment and the presence of various metals and other toxic side products of various branches of industry and traffic in the soil, due to the ability of plants to adopt these ingredients, it is necessary to determine their content so that the plants, in addition to being useful, do not have a negative effect. For each element, the maximum limit concentration that a plant can contain without having a negative effect is prescribed and defined (WHO, 1994). In this study, we aimed to determine the bioaccumulation potential of ten heavy metals.

## Matherials and methods

Plant material from Sanguisorba minor was collected from the tailings in Kosovska Mitrovica. The plant material, consisting of both underground and surface parts of the plant in the flowering phase, was air-dried and prepared for analysis. From each sample of dried plant material S. minor, part of material was finely grinded, then used for analysis.

## Determination of total phenol content of the extracts

The total phenolic content of the extract was determined by the Folin–Ciocalteu method as described by Peter *et al.*, 2011. The test exemplars were prepared in triplicate for each analysis. The absorbance was determined using spectrophotometer, $\lambda$ max = 725 nm. The level of phenolics was expressed in terms of gallic acid equivalent per gram of plant extract (mg GA·$g^{-1}$).

**Determination of total flavonoid content of the extracts**

The concentration of flavonoids in plant extracts was determined by the Quettier-Deleu*et al*. 2000. The absorbance was determined using spectrophotometer at $\lambda$ max = 430 nm. Rutin (mg/ml) was used to prepare the standard curve, the level of flavonoid was expressed in terms of rutin equivalent per gram of plant extract (mg $RU \cdot g^{-1}$).

**Determination of the antioxidant activity of the extracts**

The free radical scavenging activity of the three extracts (methanol, acetone and ethyl acetate) of *Sanguisorbaminor*root and herba was analyzed using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) as described by the Takao *et al*., 1994. The absorbance was measured at $\lambda$ max =517 nm. Antioxidant activity was expressed as the 50% inhibitory concentration ($IC_{50}$ values at µg/mL).

**Analysis of heavy metals in samples of plant**

The concentration and content of heavy metals in the aerial parts and roots were determined by atomic absorption spectrophotometry. Heavy metals, including manganese (Mn), iron (Fe), lead (Pb), nickel (Ni), chromium (Cr), copper (Cu), cadmium (Cd), zinc (Zn), calcium (Ca), and magnesium (Mg), were measured in both underground and surface parts of the plant. The analysis of heavy metals was conducted at the Laboratory for Analytical Chemistry at the Faculty of Science, University of Kragujevac. Five samples were prepared for each analysis, and the atomic spectrophotometer (Perkin Elmer 3300) was used. The mean, standard deviation, bioaccumulation factor (BCF), and translocation factor (TF) were determined according to Kabata-Pendias (2001).

***Data analysis***

Statistical analyses of data are obtained by using, SPSS for Windows, version 21. The values of the correlation coefficient (r) were determined using the Pearson correlation coefficient. Statistically significant difference was defined as $p< 0.05$.

## Results and discussion

### Determination of total phenolic, total flavonoid contentand antioxidant activity assay

Total phenolic (TPC), flavonoid content (TFC) and antioxidant capacity of extracts, evaluated by three different solvent as well as are presented in Table 1. The values obtained for the concentration of total phenolic are presented in first part of Table 1. The results of the total phenol content in the tested parts of plant are expressed as gallic acid equivalent (the standard curve equation:y=76.735x+0.069, $r^2 = 0.994$); the values are expressed as mg GAE/g of extract. Results of total amount of phenols in root, stem and leaf and flower as the average value and standard deviation calculated based on measurments in triplicate. The highest concentration of phenols in root were extracted with acetone (117.27±1.41 mg GAE/g). The highest concentration of phenols in stem and leaf were extracted with acetone (133.61±0.85 mg GAE/g). The highest concentration of phenols in flower was extracted with methanol (152.33±1.01 mg GAE/g).Differences between this two solvents were significant in terms of phenols content in different parts of the herb ($p<0.05$). Our study shows a higher phenol content in extracts extracted with acetone. In other studies, the extracts were extracted with acetone as solvent with the significant amounts of total phenolics(Do *et al*. 2014, Aline*et al.* 2014).

**Table 1.**Total phenolic (TPC) and flavonoid content (TFC) and antioxidant capacity of *Sanguisorba minor*

| Part of plant | Solvent | TPC (mg GAE $g^{-1}$) | TFC, (mg RU $g^{-1}$) | $IC_{50}$ (µg/mL) |
|---|---|---|---|---|
| Root | **Methanol** | 81.72±1.73 | 14.42±0.42 | 46.12±1.14 |
| | **Acetone** | 117.27±1.41 | 13.09±1.13 | 40.9±0.5 |
| | **Ethyl-acetate** | 64.44±0.85 | 11.02±0.61 | 88.72±1.03 |
| Stem and leaf | **Methanol** | 120.5±1.92 | 15.66±0.54 | 47.27±0.01 |
| | **Acetone** | 133.61±0.85 | 22.38±0.54 | 9.4±±0.01 |
| | **Ethyl-acetate** | 115.22±3.34 | 8.78±0.07 | 19.1±0.53 |
| Flower | **Methanol** | 152.33±1.01 | 33.57±1.85 | 7.08±0.15 |
| | **Acetone** | 116.77±1.71 | 50.69±1.64 | 21.33±0.57 |
| | **Ethyl-acetate** | 95.88±1.57 | 32.92±1.25 | 10.45±1.05 |

The results of the total flavonoids in the tested parts of plant are expressed as rutin equivalent, (the standard curve equation:y=14.78x+0.027, $r^2 = 0.995$); the values are expressed as mg RU/g of extract.Measurmentswerenin triplicate. The highest concentration of flavonoids in root were extracted with methanol (14.42±0.42 mg RU/g9.The highest concentration of flavonoids in stem and leaf were extracted with non-polar acetone (22.38±0.54 mg RU/g).The highest concentration of flavonoids in flower were extracted with acetone (50.69±1.64 mg RU/g).Previous studies reported that different solvents significantly affected flavonoids especially acetone(Dailey and Vuong, 2015, Do Q.D.,2014).

The antioxidant activity is expressed in terms of $IC_{50}$ (μg/ml) values (Table 1). The obtained values of antioxidant activity examined by DPPH radical scavenging activity range from 7.08 ± 0.15 to 21.33 ± 0.57 μg/ml. The largest capacity to neutralized DPPH radicals was measured in ethyl-acetate extract from root (88.72 ± 1.03 mg/ml), methanol extract from stem and leaf (47.27 ± 0.01 mg/ml) and acetone extract from flower (21.33 ± 0.57 mg/ml). Our results compared to (Pereira *al.*,2011) whose $IC_{50}$ value (30 ± 0.0 mg/ml). This result suggest that the flowers of *Sanguisorba minor* are likely to have high antioxidant capacity, more than root, stem and leaf.

Based on the mean values of the metal concentration in the soil, we can compare them in the following order: Fe> Mg>Ca>Mn>Pb> Cr> Ni> Zn> Cu. The values of the concentration of heavy metals were much higher in comparison with Table 3, except for Cu (113.88 mg $kg^{-1}$). Based on the concentrations (Soriano *et al.*, 2010) the tested soil is moderately polluted. The study (Hasanović*et al.*, 2022) coincide with our results. In that study *Sanguisorba minor*grows on soil with high concentrations of heavy metals. Our results (Table 2) showed that in the soil on which the tested specie grew had a higher value than the maximum allowed concentration of metals we tested and a high content of Fe (7807mg $kg^{-1}$). If iron is accumulates in plants to high levels than is toxic, it can act catalytically via the Fenton reaction to generate hydroxyl radicals, which can damage lipids, proteins and DNA (Connolly, Guerinot, 2002).

Table 2. The content of investigated metals (mg $kg^{-1}$) in soil and specie *Sanguisorba minor*

| **Metal** | **Soil (mg/kg)** | **Root (mg/kg)** | **Stem and leaf herb (mg/kg)** | **Flower (mg/kg)** |
|---|---|---|---|---|
| Mn | 2685.58±16.06 | 458.78±0.94 | 162.58±0.81 | 475.08±27.51 |
| Ni | 199±0.97 | 21.26±0.32 | 1.25±0.02 | 26.2±0.37 |
| Ca | 3649.76±31.20 | 21277±55.20 | 17253.8±83.59 | 27694.38±66.29 |

| | | | | |
|---|---|---|---|---|
| Mg | 4540.54±23.4 | 8829.64±78.07 | 7209.8±68.34 | 11089.12±28.21 |
| Fe | 77363.08±682.37 | 7807.36±83.25 | 723.32±1.72 | 7276.08±38.16 |
| Zn | 176.24±0.78 | 92.74±0.53 | 36.84±0.49 | 77.76±0.74 |
| Cr | 453.36±1.36 | 72.32±0.81 | 5.84±0.05 | 70.32±0.51 |
| Pb | 873.66±2.057 | 121.74±0.77 | 22.32±0.43 | 87.86±0.75 |
| Cu | 113.88±0.8 | 19.32±0.39 | 7.47±0.05 | 18.38±0.42 |

Table 3. Classification of contaminated soil according to heavy metal content (mg $kg^{-1}$) (Soriano *et al*., 2010; Kabata – Pendias, 2011)

| **Chemical element** | **MPK – maximal permitted concentrations mg/kg** |
|---|---|
| Manganese (Mn) | 2 000 |
| Iron (Fe) | 50 000 |
| Lead (Pb) | 100 |
| Nickel (Ni) | 50 |
| Chromium (Cr) | 100 |
| Copper (Cu) | 100 |
| Cadmium (Cd) | 3 |
| Zinc (Zn) | 300 |
| Calcium (Ca) | - |
| Magnesium (Mg) | - |

Table 4. Bioconcentration factor (BCF) and Translocation factor (TF) of specie *Sanguisorba minor*

| **Metal** | **Bioconcentration** | **Translocation root/herba** | **Translocation root/flower** |
|---|---|---|---|
| Mn | 0.170830882 | 0.354374646 | 1.035529012 |
| Ni | 0.107373737 | 0.058889934 | 1.232361242 |
| Ca | 5.829753189 | 0.81090557 | 1.301598895 |
| Mg | 1.944623327 | 0.816545182 | 1.255897183 |
| Fe | 0.100918423 | 0.092645914 | 0.931951389 |
| Zn | 0.526214253 | 0.397239595 | 0.838473151 |
| Cr | 0.159520028 | 0.080807522 | 0.972068584 |
| Pb | 0.139344825 | 0.183341548 | 0.721701988 |
| Cu | 0.169652266 | 0.386956522 | 0.951345756 |

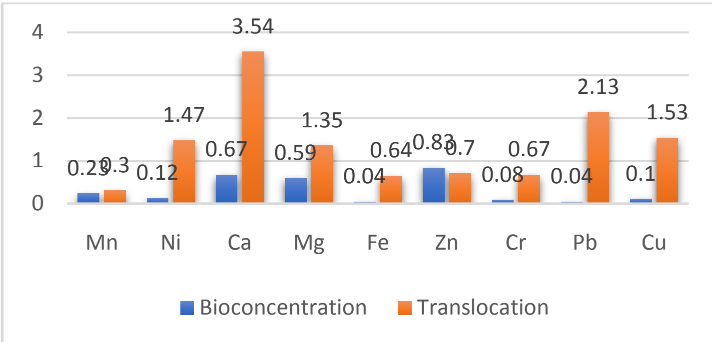


Figure 1.Bioconcentration factor (BCF) and Translocation factor (TF) of specie*Sanguisorba minor*

| | | root | | | | | | | | | herba | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mn | Ni | Ca | Mg | Fe | Zn | Cr | Pb | Cu | Mn | Ni | Ca | Mg | Fe | Zn | Cr | Pb | Cu |
| root | Mn | 1 | | | | | | | | | | | | | | | | | |
| | Ni | -0.5 | 1 | | | | | | | | | | | | | | | | |
| | Ca | **0.92*** | -0.46 | 1 | | | | | | | | | | | | | | | |
| | Mg | -0.02 | -0.41 | -0.32 | 1 | | | | | | | | | | | | | | |
| | Fe | 0.07 | 0.68 | -0.07 | -0.13 | 1 | | | | | | | | | | | | | |
| | Zn | 0.36 | 0.49 | 0.22 | -0.01 | 0.83 | 1 | | | | | | | | | | | | |
| | Cr | **-0.89*** | 0.77 | -0.82 | -.008 | 0.5 | 0.06 | 1 | | | | | | | | | | | |
| | Pb | 0.17 | -0.39 | 0.03 | 0.72 | 0.29 | 0.24 | -0.08 | 1 | | | | | | | | | | |
| | Cu | -0.35 | 0.04 | -0.07 | -0.22 | 0.33 | -0.16 | 0.41 | 0.32 | 1 | | | | | | | | | |
| herba | Mn | 0.8 | 0.1 | 0.7 | -0.38 | 0.34 | 0.7 | -0.5 | -0.1 | -0.36 | 1 | | | | | | | | |
| | Ni | -0.21 | -0.52 | -0.24 | 0.23 | **-0.95*** | **-0.88*** | -0.23 | -0.28 | -0.28 | -0.56 | 1 | | | | | | | |
| | Ca | -0.43 | 0.19 | -0.31 | 0.13 | 0.59 | 0.12 | 0.59 | 0.59 | 0.87 | -0.39 | -0.46 | 1 | | | | | | |
| | Mg | -0.14 | 0.86 | 0.1 | -0.75 | 0.65 | 0.53 | 0.46 | -0.51 | 0.1 | 0.45 | -0.64 | 0.07 | 1 | | | | | |
| | Fe | 0.56 | -0.69 | 0.76 | -0.36 | -0.56 | -0.45 | -0.73 | -0.15 | 0.13 | 0.22 | 0.31 | -0.29 | -0.27 | 1 | | | | |
| | Zn | 0.58 | -0.37 | 0.81 | -0.66 | -0.36 | -0.23 | -0.64 | -0.41 | 0.04 | 0.47 | 0.1 | -0.4 | 0.1 | **0.9*** | 1 | | | |
| | Cr | **0.88*** | -0.11 | 0.86 | -0.23 | 0.36 | 0.67 | -0.6 | 0.17 | -0.14 | **0.93*** | -0.62 | -0.17 | 0.25 | 0.34 | 0.48 | 1 | | |
| | Pb | 0.5 | 0.01 | 0.75 | -0.68 | 0.36 | 0.31 | -0.26 | -0.04 | 0.44 | 0.62 | -0.59 | 0.17 | 0.47 | 0.55 | 0.71 | 0.72 | 1 | |
| | Cu | 0.49 | -0.48 | 0.44 | -0.11 | -0.78 | -0.36 | -0.74 | -0.5 | -0.7 | 0.29 | 0.61 | **-0.93*** | -0.29 | 0.57 | 0.58 | 0.15 | -0.09 | 1 |

Figure 2. Pearson's Correlation of heavy metals among root and herbaof specie*Sanguisorba minor*

According to our results, Mn(458.78mg $kg^{-1}$) and Zn (92.764 mg $kg^{-1}$) in root is in much lower concentration(Soriano *et al*., 2010; Kabata-Pendias, 2011). Results obtained for Pb (121.74 mg $kg^{-1}$ in root, and 22.32 mg $kg^{-1}$stem and leaf and 87.86 in flower) is lower that the maximum allowed concentration in plant except the content in the root where is higher contrecationtham allowed (Soriano *et al*., 2010; Kabata - Pendias, 2011). The obtained results for heavy metals show that BAC<1, which means that *S. minor*heavy metal excluders. The BF and TF of Zn were both less than 1 (0.62, 0.96), which coincides with the research (Kothe and Varma, 2012) for *S. minor* BF and TF of Zn were both less than 1 (0.7, 0.7). This indicate that *S. minor*roots are able to take up the metals (in dissolved form from low levels in the soil).

## Conclusion

The impact of the chemical composition and bioactivities of different parts of the Sanguisorba minor plant was assessed in order to highlight its important role in human diet and health. Based on the obtained results, the investigated soil was found to be moderately polluted with metals such as Mn, Pb, Ni, Cr, Fe, Cd, Cu, Ca, and Mg. However, the Sanguisorba minor plant was found to grow successfully on this type of soil. The solvents used in this study revealed that acetone was a very efficient medium for extraction, as it resulted in the highest amounts of total phenols, flavonoids, and antioxidant activity. All these results indicate that Sanguisorba minor could be an important source of bioactive compounds, with good antioxidant properties, and thus can be used in various treatments. However, it is necessary to carry out detailed investigations beforehand, especially if the plant species originates from an area contaminated with metals.

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