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Short Communication

Potentiometric Determination of Copper in Herbal Material and Hydrolats of *Veronica* Species (Family *Plantaginaceae*)

Ante Prkić[1,], Ivana Mitar[2], Josipa Giljanović[1], Marija Nazlić[3], Dario Kremer[4], Ivana Anđelić[2], Nenad Vuletić[2], Valerija Dunkić[3]*

[1] Department of Analytical Chemistry, Faculty of Chemistry and Technology, Ruđera Boškovića 35, 21000 Split, Croatia.
[2] Department of Chemistry, Faculty of Science, Ruđera Boškovića 33, 21000 Split, Croatia.
[3] Department of Biology, Faculty of Science, Ruđera Boškovića 33, 21000 Split, Croatia.
[4] Faculty of Pharmacy and Biochemistry, A. Kovačića 1, 10000 Zagreb, Croatia.
[*]E-Mail: prkic@ktf-split.hr .

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The aim of our work was an implementation of potentiometric determination of copper in samples of the genus *Veronica* (family *Plantaginaceae*). Genus *Veronica* herbs are widely used in e.g. cosmetic, traditional medicine and food industry. The copper content was potentiometrically analysed in 25 herbal samples of genus *Veronica* and 12 of their hydrolats. The analysed samples were herbal samples of *Veronicas* harvested mainly in three Croatian regions – Dalmatia, Lika and Slavonia as well as randomly selected samples of theirs hydrolats. *Veronicas*' samples were digested in a microwave oven by using nitric acid and hydrogen peroxide mixture. The potentiometric determination was performed by using commercially available CuISE for Cu^{2+}, by using potentiometric methods previously developed in our laboratory.

Keywords: copper, genus *Veronica*, potentiometric determination, ion-selective electrode

1. INTRODUCTION

The genus *Veronica* (Plantaginaceae) contains 450 species that grow mainly in the temperate zone of the Northern Hemisphere, while only a few species grow in the mountains of the tropical and temperate Southern Hemisphere and Australia [1]. Such a large number of species is indicative of the great ecological adaptability of the genus *Veronica*, within which it grows on humid and dry habitats, by the sea and in the mountains [2]. It is assumed that Latin name of *Veronica* derives from Sacred Veronica. *Veronica's* genus of Croatia's floral vegetation comprises of 40 plants of the genus

Veronica; 6 species belong to endangered species, 2 species are strictly protected, 1 species is endemic [3, 4].

Some of the species from the genus *Veronica* have been used in traditional medicine to treat flu, respiratory diseases, hemoptysis, laryngopharyngitis, cough, ulcers, cancer and as a diuretic wound healing process. Also, *Veronica'*s plant species have anti-inflammatory and cytotoxic activity. In the Balkan traditional medicine, the aerial parts of the *V. officinalis* were used to treat liver, spleen, kidney, and bladder, snake bites, wound healing, skin lesions, eczema and ulceration [5]. Aerial parts of numerous species from genus *Veronica* are edible, either raw or cooked [6].

Copper plays an essential role in physiological functions of plants, like photosynthesis, respiration, reproduction, regulation of auxins, lignification and phenol metabolism. Cu^{2+} and Cu^{+} ions are soluble in water and provide antifungal and antibacterial effects at low concentration levels, so their role in the production of fungicides is irreplaceable. Copper also plays an important function in the human diet, and in all higher organisms (plants, animals).

Copper deficiency can significantly reduce the activity of the phenol oxidases which are involved in the lignin synthesis, leading to formations of rather weak tissues and the distortion of leaves and stems. Cu presence affects the synthesis of phenol compounds which inhibit cell elongation [7].

Copper has a natural abundance of 60 mg/kg in the Earth's crust. In Europe, average ambient background Cu concentration in soils varies between 11.4 and 17 mg Cu/kg. Human Cu needs are low, but despite of that, Cu deficiency can occur because of its low concentration in edible plant tissues, especially when plants are grown on calcareous or alkaline soils in arid and semi-arid environments [8]. Cu is present in different species and varieties of plants especially in fruits and vegetables, nuts, seeds, chickpeas, liver, oysters and in some water [7]. Živković et al. investigated the composition of selected heavy metals (Cr, Zn, Cu, Fe, Mn) in three different *Veronica* species (*V.jacquinii*, *V.teucrium*, *V.urticifolia*). The content of Cu in plant samples varied between 6.04 and 12.8 mg/kg, depending on the location where the samples were collected [9]. Dunkić et al. investigated macroelements and essential trace elements of the species *Veronica spicata* and found that the content of Cu was below the limit of quantitation [10].

Copper (Cu) is an essential trace element in both humans and animals. The human body contains approximately 100 mg Cu (1.4-2.1 mg per kilogram of body weight). According to the WHO (World Health Organization) recommended daily intake for adults ranges from 1-1.5 mg/day [11]. Bost et al. found that the minimal amount of dietary Cu required to achieve a null balance lies somewhere between 0.8 and 2.4 mg/day [12]. Copper is a cofactor of many redox enzymes and beyond its role in iron metabolism, the need for Cu also comes from its involvement in many biological processes, including antioxidant defence, neuropeptide synthesis, and immune function. In the skin, copper: a) stimulates dermal fibroblasts proliferation; b) upregulates collagen (types I, II, and V) and elastin fibre components (elastin, fibrillins) production by fibroblasts; c) serves as a cofactor of lysyl oxidase needed for efficient extracellular matrix (ECM) protein cross-linking; d) stabilizes the skin ECM once formed, as increased crosslinking of collagen and elastin matrices occur in a copper dose-dependent manner; e) serves as a cofactor of superoxide dismutase, an antioxidant enzyme present in the skin, important for protection against free radicals, inhibits cellular oxidative effects such as

membrane damage and lipid peroxidation and f) serves as a cofactor of tyrosinase, a melanin biosynthesis essential enzyme responsible for skin and hair pigmentation [13]. Cu absorption occurs mainly in the proximal part of the small intestine. It is transported from the small intestine into the liver via the portal vein. Parameters such as age, food type, gender and other affect the absorption rate of Cu taken through food intake and can cause the absorption rate to vary between 12 and 71%. Research shows that the gastrointestinal Cu secretion plays a major role in the control of Cu homeostasis which confirms the fact that most of the endogenous Cu secreted into the gastrointestinal lumen is reabsorbed through the intestinal epithelium. Urinary Cu excretion is low (10-25 μg/day) when compared to faecal excretion and so far, there has been insufficient researches to prove whether urinary excretion plays a role in the Cu homeostasis control. Copper is an essential micronutrient for man, but at high levels it is toxic. An overload of Cu can lead to Fenton-type redox reactions, resulting in oxidative cell damage and cell death [12]. Although Cu is toxic at high levels, poisoning is very rare because the excess Cu is excreted through gastrointestinal tract, and it is mostly reported in patients with genetic inheritable Wilson disease [7]. Body of a person with Wilson disease retains excess copper because the liver does not release copper into bile as it should. Coper damages the organ as it builds up in the liver. After enough damage, the liver releases the copper directly into the bloodstream, which carries the copper throughout the body. In the end, the copper damages build up caused to the kidneys, brain, and eyes, and if not treated, it can lead to death [14].

Hydrolats, essential waters or hydrosols are a by-product of essential oils. In most cases, these by-products were discarded after water distillation because the less concentrated mixtures were seen as completely useless. These aqueous residues, hydrolats, also contain dissolved volatile components but in a smaller amount than in essential oils. In other words, of the same amounts of plant essence inside, just in a larger amount of water. This practice of discarding hydrolats changed a few years ago when people started seeing that those less-diluted amounts could be used in all sorts of different applications [15]. Hydrolats are widely used in healing, cosmetic, and culinary applications of aromatherapy. Hydrosols are also used as flavorings and curables. Herbal distillates have been used historically as a topical agent for cosmetics and medicinal use. Since herbal distillates are less concentrated than essential oils, they are more suitable for some applications, such as for the use of those who are sensitive, such as the elderly or young children [16]. Boyraz and Ozcan investigated the fungicidal effect of some spice hydrolats and the results showed that 15% dose of *Satureja hortensis* possesses a complete fungicidal effect by 100% inhibition of mycelial growth of *Rhizoctonia solani*, *Botrytis cinerea* and *Alternaria citri* [17]. Tornuk et al. investigated how different plant hydrosols protect fresh-cut apples treated with *Staphylococcus aureus* and they concluded that plant hydrosols are successful in reducing the count of *S. aureus* inoculated on fresh-cut apples. The remarkable decreasing effect of each hydrosol was observed even at the treatment time (20 min) [17]. According to these results, we can say that hydrosols may not only be used in cosmetics and medicine but in agriculture, too, although there is still a lot of research to be done in this field.

2. EXPERIMENTAL

2.1. Reagents and chemicals

All needed solutions were prepared by solving a certain amount of chemicals in ultrapure water. Ultrapure water (declared conductivity of 0.04 μS cm^{-1}) was prepared by Millipore Simplicity (USA). The following chemicals were used: Sodium nitrate, $NaNO_3$, p.a., Sodium acetate, CH_3COONa, p.a., Sodium hydroxide, NaOH, p.a., Acetic acid, CH_3COOH, p.a. and Copper (II) nitrate×3 H_2O, p.a. which were obtained from Kemika (Croatia). Nitric acid, HNO_3, s.p. (suprapure), Hydrogen peroxide, H_2O_2, s.p. and Pentane (C_5H_{12}, M = 72.15 g mol^{-1}), HPLC grade, were obtained from Merck (Germany).

The ionic strength of buffer solutions was adjusted by dissolving a needed mass of $NaNO_3$ to reach the value of 0.1 M.

Herbal samples digestion was described in our previous research [18-20]. Potentiometric measurements of copper in plants was thoroughly described in our previous research [21].

2.2. Apparatus

The indicator electrode that was used was copper ion-selective electrode (CuISE) Orion 94-29A from Orion (USA). Reference electrode used for measurement was Orion 90-02 double junction reference electrode (Orion, USA). Potentiometric measurements were conducted in the double-wall glass vessel and the data were recorded with a millivoltmeter (Seven Excellence Mettler Toledo, USA) connected to a personal computer.

Figure 1 provides a response of the CuISE to Cu^{2+} at pH = 4.75 used in our experiment.

Standard copper solution (0.01 M Cu^{2+}) was prepared by dissolving a required mass of copper salt in ultrapure water. The above-mentioned solution was used for the construction of a calibration curve by successive dilution method by certain pH = 4.75 solution with constant ionic strength, Figure 1.

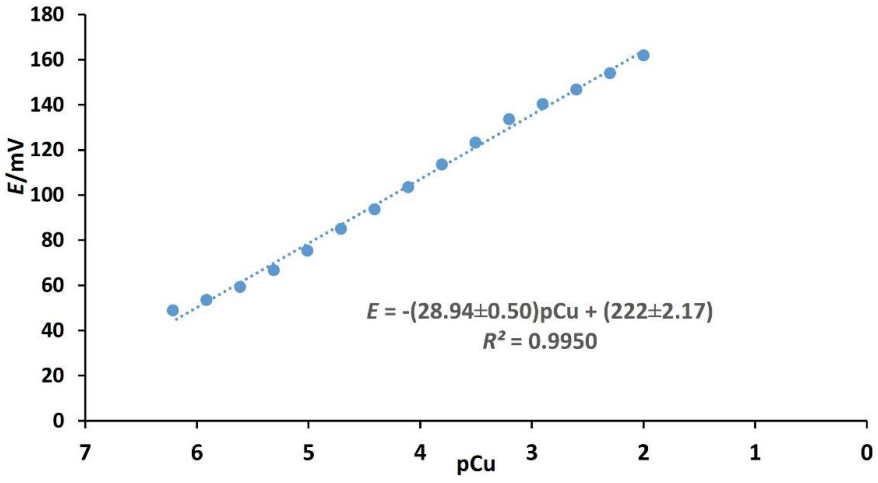

Figure 1. The response of CuISE for copper ions at pH = 4.75

The potential change of 28.94 mV per decade for copper concentration, with the correlation coefficient of 0.9950 was recorded, which is very much in line with the theoretical Nernstian slope for divalent ions (29.6 mV per decade).

Ultrapure water was used for all measurements in order to be certain that measured copper comes from a plant material.

Due to the high acidity of microwaved samples (pH ≈ 0.5), 10.0 mL of each sample was partially neutralized by adding 1.52 mL of 10% NaOH and subsequently diluted by acetic buffer, pH = 4.75 for copper measurement mixed with 0.1 M $NaNO_3$ in 50 mL flask to keep both ionic strength and pH constant. During the measurement, solutions were constantly mixed, and the temperature was kept constant at 25 °C. Measurements were finished as soon as potential had obtained a constant value. The final value is given in Table 1, an article represents a mean of five consecutive measurements.

2.3. Isolation of the hydrolats

Aerial parts of the chosen species were air-dried and subjected to hydrodistillation in a Clevenger-type apparatus for 3 hours at the water boiling temperature (100 °C), according to Kremer et al. method [22]. 2 mL of pentane and 40 mL of water was added to the inner part of the graduated tube at each distillation. At the end of the process, the organic and the water layer –hydrolat was separated and refrigerated until the analysis was done.

3. RESULTS AND DISCUSSION

Table 1 shows data for analyzed *Veronicas* samples.

Table 1. Copper content in herb samples from *Veronicas* and their hydrolats, mg kg^{-1}

Genus name	Herbs	Hydrolats	Harvesting location
Veronica arvensis L.	147.05	0.022	Dalmatia
Veronica arvensis L.	150.76	0.103	Lika
Veronica austriaca L. ssp. *jacquinii* (Baumg.) Eb. Fisch.	170.73	0.130	Dalmatia
Veronica austriaca L. ssp. *jacquinii* (Baumg.) Eb. Fisch.	226.37	nm*	Lika
Veronica chamaedrys L.	155.84	0.210	Slavonia
Veronica chamaedrys L.	366.26	nm	Lika
Veronica chamaedrys L.	31.17	0.191	Slavonia
Veronica cymbalaria Bodard	234.01	nm	Dalmatia
Veronica cymbalaria Bodard	53.44	nm	Dalmatia

Veronica cymbalaria Bodard	69.69	nm	Dalmatia
Veronica hederifolia L.	51.70	nm	Dalmatia
Veronica hederifolia L.	93.95	0.001	Dalmatia
Veronica longifolia L. (=*Pseudolysimachion longifolium* (L.) Opiz	158.45	nm	Slavonia
Veronica officinalis L.	260.66	nm	Slavonia
Veronica officinalis L.	258.50	0.196	Lika
Veronica persica Poir.	78.93	nm	Slavonia
Veronica persica Poir.	46.03	nm	Dalmatia
Veronica polita Fr.	109.99	nm	Dalmatia
Veronica saturejoides Vis. ssp. *saturejoides*	235.96	nm	Bosnia and Herzegovina
Veronica saturejoides Vis. ssp. *saturejoides*	40.31	0.099	Dalmatia
Veronica serpyllifolia L.	39.32	nm	Lika
Veronica serpyllifolia L.	41.67	0.236	Slavonia
Veronica spicata L.	158.45	0.272	Dalmatia
Veronica spicata L.	302.64	0.216	Lika
Veronica spicata L.	307.70	0.262	Dalmatia

*nm – not measured

It can be seen that there is significantly higher copper content in *Veronicas* than in other plants according to available data [18-20, 23-25], at least twice and for a dozen cases, almost 100 times. On the other hand, knowing the importance of *Veronicas* in various applications e.g. cosmetic, traditional medicine and food industry, it is reasonable to propose a suitable quality control of raw material for metal content. By searching literature, it has not been found any work with this topic. A method used for quality control of raw material should be inexpensive, simple, fast and not to require a specifically trained operator. Potentiometry satisfies everything of above mentioned. Alternatively, potentiometry is very robust and is suitable for outdoor application. It has been proven useful and statistically similar to sophisticated techniques, e.g. atomic absorption spectrometry [21]. Since the hydrolats can, too, contain copper, we have done an analysis of randomly selected samples. It is important emphasize a few directions that we took: samples with the highest copper content and low content.

Since human skin has been recognized as a possible way for copper intake [26] and application of *Veronicas* in various cosmetics, it is reasonable to determine and propose a method for quality control of raw material in cosmetics. Some investigations [26] have shown that copper absorbed through skin accumulates in and around skin cells nuclei. This phenomenon can be potentially harmful to human health as a cancer precursor. Although there are no records, by our knowledge, of the high absorption rate through human skin, there is a result for cat skin (0.1 mg cm^{-2} day^{-1}) in the form of a

complex with organic ligands. If the total surface of human skin is taken to be 1.5-2 m^2 what is equal to 15 000-20 000 cm^2 and assuming that human and cat skins have the same absorption rate of copper, it gives a possibility of daily copper intake of 1500-2000 mg what is about thousand times higher than Recommended Dietary Allowance (RDA) cooper intake proposed by WHO [11].

Our investigation has shown that is practically the same extraction rate of copper, 7 out 12 samples, in hydrolats of all analyzed *Veronicas,* Table 1. The measured values of copper in hydrolats is almost twice higher (0.2 mg) than copper mass rate absorption through skin and this finding is horrifying, especially for those who use cosmetic products on a daily base. Even with reduction of applying cosmetics on every square centimetre of skin and a low content of copper in cosmetics, over a longer time of use, it is expecting to have bad outcome for health.

Because *Veronicas,* obviously, accumulate copper from soil, the *Veronicas* can be used for treating soils polluted by copper [27], but this will be our further investigations.

4. CONCLUSION

Since the potentiometry is a simple-to-use, inexpensive and fast analytical method with reasonably low limit of detection (around 1×10^{-6} mol L^{-1}), this work proves the possibility of using potentiometry in quality control of raw material as main analytical method for evaluation of heavy metals content for cosmetic, food, pharmaceutical industries etc. The priority of modern analytical chemistry is to be the tool for assuring and preventing consumers from being intoxicated with various heavy metals, e.g. lead, mercury, copper, iron etc. In case of finding heavy metal concentration within the range of limit of detection, such raw material can be easily discarded from industrial process and make a significant cost reduction in various industries.

References

1. D.C. Albach, M.M. Martínez-Ortega, M.A. Fischer, M.W. Chase, *Taxon*, 53 (2004) 429.
2. J. Sharifi-Rad, D. Mnayer, G. Tabanelli, Z. Stojanović-Radić, M. Sharifi-Rad, Z. Yousaf, L. Vallone, W. Setzer, M. Iriti, *Cell. Mol. Biol.*, 62 (2016) 57.
3. T. Nikolić, J. Topić, State Institute for Nature Protection, Zagreb, (2005).
4. S. Pignatti, Flora d\'Italia, 1982.
5. J.H. Kwak, H.J. Kim, K.H. Lee, S.C. Kang, O.P. Zee, *Arch. Pharmacal Res.*, 32 (2009) 207.
6. D. Stojković, J. Petrović, M. Soković, J. Glamočlija, J. Kukić-Marković, S. Petrović, J. *Sci. Food Agric.*, 93 (2013) 3205.
7. D. Rusjan, R. Veberič, M. Mikulič-Petkovšek, *Eur. J. Plant Pathol.*, 133 (2012) 965.
8. B. Printz, S. Lutts, J.-F. Hausman, K. Sergeant, *Frontiers in plant science*, 7 (2016) 601.
9. J. Živković, S. Ražić, J. Arsenijević, Z. Maksimović, *J. Serb. Chem. Soc.*, 77 (2012) 959.
10. V. Dunkic, I. Kosalec, I. Joze Kosir, T. Potocnik, A. Cerenak, M. Zovko Koncic, D. Vitali, I. Dragojevic Muller, M. Kopricanec, N. Bezic, *Curr. Drug Targets*, 16 (2015) 1660.
11. http://www.who.int/whr/1996/en/ (31.7.2018.)
12. M. Bost, S. Houdart, M. Oberli, E. Kalonji, J.-F. Huneau, I. Margaritis, *J. Trace Elem. Med. Biol.*, 35 (2016) 107.
13. G. Borkow, Use of biocidal surfaces for reduction of healthcare acquired infections, Springer, (2014)

14. https://liverfoundation.org/for-patients/about-the-liver/diseases-of-the-liver/wilson-disease (31.7.2018.)
15. https://www.hydrosolworld.com/what-are-hydrosols/ (31.7.2018.)
16. R. Mulvaney, N.J. Abram, R.C. Hindmarsh, C. Arrowsmith, L. Fleet, J. Triest, L.C. Sime, O. Alemany, S. Foord, *Nature*, 489 (2012) 141.
17. N. Boyraz, M. Özcan, *Fitoterapia*, 76 (2005) 661.
18. A. Prkić, J. Giljanović, S. Petričević, M. Brkljača, M. Bralić, *Anal. Lett.*, 46 (2013) 367.
19. A. Prkić, A. Jurić, J. Giljanović, N. Politeo, V. Sokol, P. Bošković, M. Brkljača, A. Stipišić, C. Fernandez, T. Vukušić, *Open Chem.*, 15 (2017) 200.
20. A. Prkić, N. Politeo, J. Giljanović, V. Sokol, P. Bošković, M. Brkljača, A. Stipišić, *Open Chem.*, 16 (2018) 228.
21. A. Prkić, I. Mitar, J. Giljanović, V. Sokol, P. Bošković, I. Dolanc, T. Vukušić, *Int. J. Electrochem. Sci.*, 13 (2018). (in press)
22. D. Kremer, V. Matevski, V. Dunkic, N. Bezic, E. Stabentheiner, *Rec. Nat. Prod.*, 10 (2016) 228.
23. S. Ražić, S. Đogo, L. Slavković, *Microchem. J.*, 84 (2006) 93.
24. S. Ražić, A. Onjia, S. Đogo, L. Slavković, A. Popović, *Talanta*, 67 (2005) 233.
25. S.S. Randjelovic, D.A. Kostic, G.S. Stojanovic, S.S. Mitic, M.N. Mitic, B.B. Arsic, A.N. Pavlovic, *Cent. Eur. J. Chem.*, 12 (2014) 1144.
26. J.J. Hostýnek, R.S. Hinz, C.R. Lorence, M. Price, R.H. Guy, *Crit. Rev. Toxicol.*, 23 (1993) 171.
27. J. Sardans, F. Montes, J. Peñuelas, *Spectrochim. Acta B,* 65 (2010) 97.