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Optimization infusions conditions for improving phenolic content and antioxidant activity in *Sideritis scardica* tea using response surface methodology

Maria Irakli*, Kortessa Tsifodimou, Eirini Sarrou, Paschalina Chatzopoulou

Hellenic Agricultural Organization – Demeter – Plant Breeding and Genetic Resources Institute, PO Box 60458, 57001 Thermi, Thessaloniki, Greece

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ABSTRACT

Sideritis scardica (Mountain Tea), an endemic plant of the Balkan Peninsula, is generally acknowledged as a very popular consumed beverage with multiple pharmacological properties including antioxidant activity. This study was designed to optimize the infusion conditions, aiming to maximize the phenolic compounds and antioxidant activity of the tea-infusion, made from different parts of *Sideritis* plants. The influence of different infusion temperatures (60, 80 and 93 °C) and steeping times (10, 20 and 30 min) either as aerial parts or as stems, flowers, leaves of *Sideritis* plants was determined using response surface methodology. The results showed good fit of data and the optimal infusion conditions were steeping time no more than 10 min and temperature water ranged between 87.5–99.8 °C for all infusions under investigation. The highest bioactive content and antioxidant activity was exhibited in leaves infusions followed by flower, aerial parts, and stems. Under this condition, chlorogenic acid was detected as the major phenolic acids in all *Sideritis* infusions. Moreover, the most abundant minerals were recorded in the order: K > P > Ca > Mg > Na > Fe. Stems tea-infusions contained the highest amount of minerals followed by those obtained from flowers, aerial parts and leaves, respectively.

1. Introduction

Herbal infusions have been traditionally used in the folk medicine for the ailment of various disorders. Nowadays, their consumption has been very popular due to the vast variety of tea flavors marketed worldwide and their health benefits, attributed to their bioactive compounds. Besides polyphenols, which are mainly associated to the antioxidant activity and scavenging harmful free radicals, teas may contribute to the intake of a number of essential minerals (Szymczycha-Madeja et al., 2012).

The genus *Sideritis* comprises more than 150 species and several taxa are occurring in Greece and other Mediterranean countries. *Sideritis* species are used traditionally for the preparation of herbal tea, commonly known as “Mountain tea”, which is widely consumed due to its properties, known from folk medicine; against common cold, including fever, flu and bronchitis, to relieve gastric disorders and mucous membrane inflammation, as analgesic, sedative, diuretic, antimicrobial, antibacterial, antioxidant and anti-inflammatory (Bojović et al., 2011; Gonzales-Burgos et al., 2011; Goulas et al., 2014). Recently, *Sideritis scardica* (*S. scardica*) extracts, were proved effective as triple monoamine reuptake inhibitors, and are recommended therefore for the treatment of neurodegenerative diseases, such as anxiety disorders, Mild Cognitive Impairement, (Knorle, 2012), while memory enhancing action, has been reported as well (Dimpfel et al., 2016).

S. scardica is an endemic to Balkan species, widespread in mountainous areas of North – Central Greece. The traditional uses of four *Sideritis* species, among them *S. scardica*, in the form of herbal tea, has been adopted by HMPC, in EMA/HMPC/39453/2015 herbal monograph (EMA/HMPC, 2015). Previous studies have been reported on the content of phenolic compounds, terpenoids, hydrocarbons and related compounds, and the essential oil composition as well (Todorova and Trendafilova, 2014).

Herbal teas are made from any part of plants, including leaves, flowers, seeds, or whole aerial parts. “Mountain tea” is prepared traditionally as infusion, by steeping flowering stems or aerial parts in boiling water, for 10–30 min. Moreover, due to the fact that different plant parts may deliver different groups of compounds in hot water

Abbreviations: AP, aerial parts; CA, caffeic acid; CCD, central-composite design; CLA, chlorogenic acid; CNA, cinnamic acid; FA, ferulic acid; F, flowers; GA, gallic acid; L, leaves; pCA, *p*-coumaric acid; PRCA, protocatechuic acid; RSA-DPPH, DPPH radical scavenging activity; RSM, response surface methodology; S, stems; SRA, syringic acid; *Sideritis scardica*, *S. scardica*; TFC, total flavonoid content; TPC, total phenolic content; VA, vanillic acid; 4HBA, 4-hydroxybenzoic acid

* Corresponding author.
E-mail address: irakli@cerealinstitute.gr (M. Irakli).

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used for infusion, the contribution of various *Sideritis* plant parts, in water soluble phenolic compounds is variable. Additionally, hot water temperature and the time of steeping, may affect the proportion of phenolics obtained during the infusion preparation (Ferreira et al., 2014; Hajiaghaalipour et al., 2016). While the phytochemical composition of *S. scardica* extracts, obtained by different solvents, has been extensively investigated (Janeska et al., 2007; Alipieva et al., 2010; Danesi et al., 2013; Petreska et al., 2015), limited studies have been reported on the phenolic compounds of water infusions (Alipieva et al., 2010). The phenolic compounds detected, belong to different classes; phenylethanoid glycosides, flavonoid-7-*O*-diglycosides, flavonoid acetylglycosides and hydroxycinnamic acids (Petreska et al., 2011). Moreover, little is known about the influence of tea preparation conditions on bioactive constituents, and mainly the phenolic compounds, released in a cup of Mountain Tea. Polyphenol rich foods may prevent degenerative diseases such as cardiovascular diseases, cancer and other degenerative disorders and their daily intake by different types of foods and beverages, is mainly in the form of hydroxycinnamates and flavonoids (estimated about 1/3 and 2/3 of the total intake respectively) (Mateos et al., 2006).

Due to the growing interest on *S. scardica* and the functionality of green herbal beverages, demanded by the consumers, it would be interesting to investigate and standardize the parameters of making “mountain tea”, with increased health benefits. response surface methodology (RSM) is a collection of statistical and mathematical techniques for optimization study and is widely used to optimize conditions for extracting active compounds from herbs. With RSM, the relationships of several factors can be reflected with limited data, and intuitive models can be build, that may assist us to get optimal results quickly. To date, there is little information about optimizing the tea infusions conditions using RSM (Jeszka-Skowron and Zgoła-Grzeskowiak, 2014; Kim et al., 2016). Hence, in the present study an attempt was made to optimize the parameters involved in mountain tea infusion and more specifically the time and temperature steeping as well as the influence of different *S. scarcica* parts using RSM based on the response of total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity. Additionally, mineral content and phenolic acid profile of the *S. scarcica* infusions was assessed under the optimal conditions.

2. Materials and methods

2.1. Chemicals

1,1-Diphenyl-2-picrlthydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin–Ciocalteu reagent and standards of 4-hydroxybenzoic acid (4HBA), caffeic acid (CA), syringic acid (SRA), *p*-coumaric acid (pCA) and ferulic acid (FA) were purchased from Sigma–Aldrich (Steinheim, Germany); whereas chlorogenic acid (CLA), was supplied by Extrasynthese (Genay Cedex, France). Protocatechuic acid (PRCA) and gallic acid (GA) were from Alfa Aesar, (Heysham, UK), whereas vanillic acid (VA) and cinnamic acid (CNA) were from Fluka (Steinheim, Germany). All other solvents/chemicals obtained from Chem-Lab (Zedelgem, Belgium) were of analytical grade or high-performance liquid chromatography (HPLC) grade.

2.2. Plant material

Aerial parts of *S. scardica* plants were collected during flowering, from the experimental field of Plant Breeding and Genetic Resources Institute (Hellenic Agricultural Organization-Demeter), during June 2015. The plant material was air dried under shadow, and then divided into two portions: one portion was treated as it is – aerial part (AP) (including stems, leaves and flowers) and the second part was manually separated into three parts; leaves (L), flowers (F) and stems (S). All samples were cut into small pieces, milled to a 0.75 mm by a laboratory mill (Retzch, Haan, Germany) and stored at room temperature until used for further analysis.

2.3. Tea infusions

Tea infusions of different parts of mountain tea L, F, S and AP were prepared as follows: 1 g of dried and ground sample was covered with 100 mL of distilled water. Different temperatures (60–93 °C) and steeping times (10–30 min) were applied. Each water extract was then filtrated through filter paper and the filtrates were collected. 2 mL extracts were centrifuged (Heraeus Instruments, Germany) at 12000 rpm for 10 min and stored at 4 °C in sealed plastic tubes till analysis. All the experiments were repeated triple.

2.4. TPC

The analyses of TPC from four tea infusions were performed according to Singleton et al. (1999). Briefly, 0.2 mL of tea infusion was transferred into a test tube and mixed with 0.8 mL of Folin–Ciocalteu (dilution 1:10 v/v with water). After incubation for 2 min, 2 mL sodium carbonate (7.5% w/v) solution was added to the reaction mixture and the volume was adjusted to 10 mL with distilled water. The mixture was allowed to stand for 1 h in a dark place and then the absorbance at 725 nm was recorded. The analyses were performed in triplicate and results were expressed as mg of GA equivalents (GAE) per g of dry weight of herbal mass (mg GAE/g dw).

2.5. TFC

The concentration of TFC was determined using the aluminum chloride colorimetric method of Bao et al. (2005) with minor modification. Aliquots (0.2 mL) of tea infusions were mixed with 0.15 mL 5% $NaNO_2$. After 5 min, 0.15 mL 10% $AlCl_3{\cdot}6H_2O$ solution was added, and the mixture was allowed to stand for another 5 min, and then 0.5 mL 1 M NaOH was added. The mixture was incubated for 30 min at room temperature and the absorbance was measured at 510 nm. The TFC was estimated using a standard calibration curve with catechin as a standard and expressed as milligrams catechin equivalent (CATE) per g of dry weight of herbal mass (mg CATE/g dw).

2.6. DPPH radical scavenging activity (RSA-DPPH)

RSA-DPPH of tea infusions was determined according to Yen and Chen (1995) with some modifications. An aliquot of 2.85 mL of 0.1 mM DPPH in methanol was mixed with 100 μL of tea infusions and the decrease in absorbance was measured at 516 nm after 5 min of reaction. The calibration curve consisted of a solution of Trolox at different concentrations (100–1000 μM). The analyses were performed in triplicate and results were expressed as mg Trolox equivalents (TE) per g dry weight of herbal mass (mg TE/g dw).

2.7. Phenolic acids profile by HPLC analysis

HPLC analyses of phenolic compounds were performed using an Agilent 1200 system (Agilent Technology, Urdorf, Switzerland) applying to chromatographic conditions as described by Skendi et al. (2017). Chromatographic separations were carried out on 250 mm × 4.6 mm Nucleosil $100C_{18}$ 5 μm column thermostatted at 30 °C. The mobile phase consisted of three solvents: water–acetic acid (1%) (A), acetonitrile (B) and methanol (C). A linear gradient starting with 5% B and 5% C was installed to reach 4% B and 16% C at 10 min, 5% B and 20% C at 25 min, 5% B and 20% C at 30 min, 5% B and 30% C at 31 min, and finally 0% B and 60% C at 37 min. The flow rate was 1.3 mL/min and the injection volume 20 μL. Spectral data from all peaks were accumulated in range 240–400 nm and chromatograms