Acta Scientific NUTRITIONAL HEALTH (ISSN:2582-1423)

Volume 7 Issue 2 February 2023

Research Article

Bioactive Compounds and Evaluation of Antioxidant and Antimicrobial Activity of Mountain Tea Extracts (*Sideritis*)

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Received: December 28, 2022

Published: January 06, 2023

Abstract

Objective: The aim of this study was to determine the effect of the extraction method on the yield of phenolics and flavonoids, as well as the profile of the main antioxidant components found in hybrid cultures of *Sideritis scardica* and *Sideritis syriaca*. In addition, the antioxidant and antimicrobial capacity of *Sideritis syriaca* extracts was studied.

Methodology: The methods used for the extraction of phenolic and flavonoid components were the following: wetting of the solid sample, ultrasonic assisted extraction, USAE (Ultrasonic Assisted Extraction) and microwave assisted extraction, MAE (Microwave Assisted Extraction). Also, bioactive components of the extracts were identified by liquid chromatography LC UVDAD/ESI-MS method. The presence of phenolic components was determined by the FolinCiocalteu method. Finally, DPPH and FRAP tests were performed to quantify the antioxidant capacity of the extracts, as well as tests against bacterial strains (*S.aureus, E.feacalis, E.coli*) to check their antimicrobial capacity.

Result: The most effective extraction method of the phenolic and flavonoid components of *Sideritis scardica* and *Sideritis syriaca* extracts, was the ultrasonic assisted method (USAE). Some of the bioactive compounds identified in the extracts were chlorogenic acid, phenyltannoic glycosides as well as flavonoid glycosides. Antioxidant and antimicrobial activities were found, which are related to the phenolic concentration of the extracts.

Conclusion: From the findings of the study, mountain tea can be considered a potential functional beverage and a valuable source of bioactive ingredients in a wide range of products.

Keywords: Mountain Tea (*Sideritis*); Phenolics; Antioxidant; Bioactive

Introduction

In recent years, herbal beverages, which are commonly characterised as tea, are quite popular among consumers who follow a healthy lifestyle, due to their content of bioactive ingredients. Generally, herbal drinks are prepared from natural ingredients of different parts of plants such as leaves, shoots, roots, fruits and flowers. They are sources rich in natural bioactive compounds such as carotenoids, phenolic acids, flavonoids, coumarins, alkaloids and terpenoids.

Scientific evidence shows that these bioactive compounds exert a multitude of biological effects, such as antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, antithrombotic and vasodilator effects.

Excessive production of reactive oxygen species (ROS) in the body causes oxidative stress, a harmful process that leads to the oxidation of biomolecules such as proteins, lipids, carbohydrates and DNA. Oxidative stress is known for the central role it plays in causing several non-communicable diseases (NCDs) such as cardiovascular diseases, arthritis, type 2 diabetes, different types of cancer, autoimmune diseases and neurodegenerative disorders.

The human body possesses endogenous antioxidant defence mechanisms, which simultaneously act against ROS. These mechanisms include enzymes, low molecular weight antioxidants (uric acid, glutathione, albumin) and certain vitamins (ascorbic acid, tocopherol) as well as carotenoids. However, endogenous antioxidant defence mechanisms are not sufficient in cases where the body is

burdened by excessive exposure to free radicals and other ROS, and external sources of antioxidants are often required to prevent oxidative damage to the human body.

As a result, there has been intense interest in natural non-nutritive antioxidant compounds with the aim of reducing the incidence and severity of non-communicable diseases (NCDs). Some of the best sources of plantderived antioxidants are fruits and vegetables, grains, legumes, oilseeds, teas and some spices.

The genus *Sideritis* (*Lamiaceae*) includes more than 150 plant species that are widespread mainly in the Mediterranean region and the Balkan peninsula. The name of the genus comes from the Greek word "iron" thus giving information about the use of these plants, in antiquity, for the treatment of wounds caused by weapons made of this material [8]. *Sideritis* species are traditionally used as teas, aromatic substances and in folk medicine as anti-inflammatory, anti-allergic, antimicrobial, antioxidant, antispasmodic and analgesic. The chemical components found in the genus *Sideritis* include terpenes, flavonoids, essential oils, coumarins, lignans and sterols [9]. Beverages made from *Sideritis* species are commonly called "mountain tea" as these species thrive in high altitude mountainous areas. The two species of *Sideritis* which are present in this study are *Sideritis scardica* and *Sideritis syriaca.*

Sideritis scardica is mainly found in the Balkan Peninsula, where it is widely used for the production of the drink "mountain tea". The name of the drink usually comes from the name of the mountain where it thrives: Olympus tea (Greece), Pyrenees tea (Bulgaria). It has a profile rich in compounds such as hydrocinnamic acids, phenylethanoid glycosides and flavonoid 7-O-glucosides, which are associated with a protective effect on the body [7].

Sideritis syriaca is also found in mountainous, rocky areas and mainly in the mountains of Crete, under the name "malotira". Its extracts include flavonoids and a high phenolic content. Some of its main bioactive components are: apigenin 7-O-glucoside, 1coumarol, dihydrocaffeoyl chlorogenic acid and isoscutellarein [7].

Materials and Methods

To study the antioxidant constituents of *Sideritis scardica* and *Sideritis syriaca* species, plants were initially grown and collected in Bulgaria, in an area near Sofia. The plant parts were air-dried and ground to prepare for the extraction [2].

The chemical compounds used for the extraction and identification of the bioactive components were Methanol (99.9%), sodium carbonate, gallic acid and Folin-Ciocalteu reagent [2]. Methanol extraction was performed by wetting the solid sample, as well as by ultrasonic assisted extraction, USAE (Ultrasonic Assisted Extraction) and microwave assisted extraction, MAE (Microwave Assisted Extraction). The efficacy of the extraction was evaluated by its yield of biologically active components, i.e., total phenolics and flavonoids.

Extraction of the dry and powdered plant material was carried out at a liquid-solid ratio of 15:1 (ml/g). Increasing the liquid-solid ratio leads to a higher total amount of extracted compounds, but without affecting the amount of polyphenols and flavonoids valuable for the study. Different ethanol-water solvent mixtures were also used in ratios (20/80, 30/70, 70/30, 80/20). Equilibrium values were estimated after 24hours.

The total amount of the extracted material (g extract/g dry solid) was determined by the method of gravimetric analysis, after evaporation of the solvent. The extracts were then filtered to remove unwanted solid particles, diluted to the required degree of spectrophotometric analysis and finally the concentration of total phenols and flavonoids they contained was determined [2].

The amount of total phenolics was determined according to the Folin - Ciocalteu method. The reaction mixture consisted of 0.5 mL of the extract (2 mg/mL), 5 mL of distilled water, and 0.5 mL of the Folin-Ciocalteu reagent. After 3 min, 1 mL of saturated sodium carbonate solution (7.5%) was added. The mixture was mixed and left for 1 hour at room temperature. Absorbance was measured at 765 nm (Jen way 6505 UV - vis spectrophotometer, 160A, Keison Products, Essex, England). Each measurement was repeated three times. The results of total phenolic content were expressed as mg of gallic acid equivalents (GAE) per 100 g of dry matter (DM), using a standard calibration curve, which was constructed from a standard solution of gallic acid (0.03-0.25 mg/mL) [10].

The content of individual phenolics and flavonoids in *Sideritis* species was analysed by liquid chromatography LC UV-DAD/ESIMS followed by isolation of the compounds. The identification of the compounds was carried out based on the fragmentation patterns obtained during mass spectrometry, MS and comparing their data from NMR analysis with those reported in the literature [2].

The determination of the antioxidant activity was carried out by the following methods: (i) DPPH method, which is based on electron transfer and specifically the ability of phenolic compounds to react with DPPH and decolorise its solution and (ii) FRAP method

which is based on the reduction of slightly yellow of Fe3+-TPTZ complex in the blue coloured form of the ferrous compound, in the presence of the antioxidant phenolic components of the sample.

In the first method, 2 mL of extract was mixed with 1 mL of 0.3 mmol/L DPPH solution in methanol. Then, it was incubated in a dark place for 30 minutes and the absorbance of the mixture was measured at 517 nm. Different concentrations (50-5000 μg mL-1) were tested from each sample and the % free radical scavenging capacity was determined by the following equation

% Binding capacity = 100 - [(Sample Ab - Blank Ab) * 100/Blank Ab]

EC_{50} values refer to the concentration of the extract at 50% of its antioxidant capacity [10].

In the second method for determining antioxidant capacity, a sample containing 3 mL of FRAP solution (0.3 mol L-1 acetate buffer containing 10 mmol L-1 TPTZ and 40 mmol L-1 $FeCl_3 10H_2O$) and 100 μL of extract (5 mg mL-1). It was then incubated at 37°C for 4 min and absorbance was measured at 593 nm. In addition, a standard solution of 500 μmol L-1 L-ascorbic acid in distilled water was prepared. The difference in absorbance was converted to a FRAP value by relating the difference in absorbance at 593 nm of the test sample to that of the L-ascorbic acid standard solution. Finally, the results were expressed as mmol of ascorbic acid (AsA) equivalents per 100 g of dry matter (DM).

The antimicrobial capacity of the decoction from *Sideritis syriaca* was tested on a set of bacterial strains from the American Type Culture Collection (USA). These strains were one Gram-negative strain of *Escherichia coli* and two Gram-positive strains of *Enterococcus faecalis* and *Staphylococcus aureus* [10].

Results and Discussion

Increasing the amount of solvent led to a significant increase in extraction efficiency, however, it had very little effect on the extraction of bioactive components (Table 1). Based on these results, a plant material to solvent ratio of 1:15 was applied in the study.

USAE extraction with methanol was performed at 25oC and 50oC in order to determine the effect of extraction time (Table 2).

During MAE extraction with methanol, irradiation was applied for 10s and then the sample solution was cooled for 10s. This procedure was implemented with a different number of irradiation/cooling cycles for each sample, using two power levels. In the case of longer MAE irradiation times (relative to USAE), phenolic and flavonoid yields decreased. This is apparently due to the high input energy, which leads to chemical changes (probably oxidation) of phenolics and flavonoids and to lower amounts of these substances in the extracts. Also, increasing the power used in the MAE method resulted in a higher extraction rate of bioactive components, but the difference was not statistically significant (Table 3) [2].

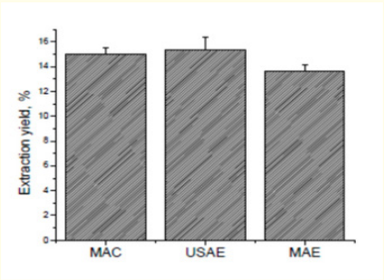

Figure 1: Performance of different methanol extraction methods.

Ratio plant material/solvent, w/ v (mg/mL)	Extraction yield, %	Total phenolics, mg_{CAE}/g	Total flavonoids mg/g
1:15	11.7 ± 0.5	24.4 ± 0.5	8.5 ± 0.5
1:30	12.0 ± 0.9	26.9 ± 3.6	8.6 ± 0.6
1:50	15 ± 0.5	35.1 ± 0.3	10.5 ± 0.4

Table 1: extraction yield, total phenolics and flavonoids in extracts.

Time, (min)	Extraction yield, %	Total phenolics, mg_{CAE}/g	Total flavonoids mg/g
	25oC		
5	7.1 ± 1.2	8.8 ± 3.1	5.5 ± 0.5
15	10.3 ± 0.6	12.6 ± 0.5	6.1 ± 0.3
30	10.8 ± 0.6	18.1 ± 3.4	6.6 ± 0.2
60	11.9 ± 0.7	19.3 ± 1.6	7.9 ± 0.4
	50oC		
5	19.3 ± 0.1	15.6 ± 0.5	7.1 ± 0.1
15	11.2 ± 0.1	18.3 ± 0.2	8.1 ± 0.4
Time, (min)	Extraction yield, %	Total phenolics, mg_{CAE}/g	Total flavonoids mg/g
30	14.6 ± 1.1	25.6 ± 3.1	14.3 ± 0.9
60	15.3 ± 1.1	28.9 ± 1.0	14.5 ± 1.1

Table 2: USAE with methanol. Extraction yield, total phenolics and flavonoids in the extracts.

Time, (s)	Extraction yield, %	Total phenolics, mg_{CAE}/g	Total flavonoids mg/g
	160 W		
10	10.6 ± 1.2	19.2 ± 1.7	11.0 ± 1.9
3 x 10	12.2 ± 0,3	22.2 ± 0.4	12.1 ± 1.3
6 x 10	11.0 ± 0.5	20.9 ± 1.0	11.7 ± 0.7
9 x 10	12.4 ± 0.8	21.7 ± 0.2	11.5 ± 0.2
	400 W		
10	11.0 ± 0.6	19.3 ± 0.4	10.0 ± 0.2
3 x 10	13.6 ± 0.5	23.6 ± 0.9	12.9 ± 0.4

Table 3: MAE with methanol. Extraction yield, total phenolics and flavonoids in the extracts.

The figures below (1 and 2) show the comparison between the three extraction methods: maceration (MAC), USAE and MAE. The data presented in these figures correspond to the highest yields of bioactive compounds obtained by each method.

Wetting (1:50 solid/solvent) gave the highest yield for total phenolics. On the other hand, the USAE method gave the highest yield of total flavonoids. But considering that the maceration process (MAC) takes 24 hours and requires 50 mL/g of solvent in contrast to USAE which takes only 1 hour and requires only 15 mL/g of solvent, it is evident that the optimal procedure for extracting phenolics and of flavonoids with methanol is USAE [2].

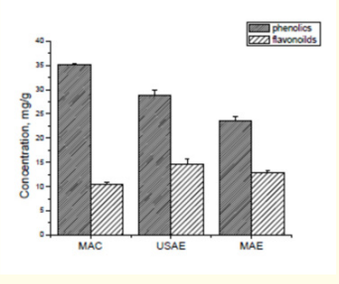

Figure 2: Phenolic and flavonoid yield from different extraction methods.

The Folin-Ciocalteu method on mountain tea decoction revealed its high phenolic content, which was equivalent to 1863 mg GAE per100 g of dry matter.

The results (Table 4) showed that the mountain tea decoction had high antioxidant activity with an EC50 value equal to 468 µg dry matter/mL by the DPPH method and 13.4 mmol AsA/100 g DM by the FRAP method

[10].

The minimum inhibitory concentration (MIC) values obtained of *Sideritis syriaca* decoction against the three bacterial strains are shown in table 4.

Thus it was observed that mountain tea decoction from *Sideritis syriaca* provides strong inhibition (MIC ≤ 0.5 mg/mL) against *S. Aureus* (MIC = 0.47 mg/mL) and moderate (0.6 ≤ MIC ≤ 1 .5 mg/mL) against *E. faecalis* (MIC

= 0.62 mg/mL) and *E. coli* (MIC = 1.25 mg/mL) [1].

Thus, among the three microorganisms included in the present study, both Grampositive bacteria presented the lowest MIC values, suggesting a stronger antimicrobial capacity of the decoction against these groups. In addition, the outer membrane of Gram-negative bacteria is composed of lipopolysaccharides and proteins, which allows selective permeability to xenobiotics and thus controls their access to bacterial structures. This feature is of particular importance as it makes Gram-negative bacteria less susceptible to plant extracts compared to Gram-positive bacteria [5].

The identification of the compounds was carried out based on MS fragmentation patterns and compared with standards and data from already isolated compounds from the same *Sideritis* variety.

Antioxidant activity	Total phenolics (mg GAE/100g DM)
Antimicrobial activity	1863
	DPPH (EC50 µg/mL) 468
	FRAP (mmolAsA/100g DM) 13.4
	Phosphmolybdenum (mol AsA/100 g DM) 24.0
	E. coli (mg/mL) 1.25
	E. faecalis (mg/mL) 0.62
	S. aureus (mg/mL) 0.47

Table 4: Phenolic content, antioxidant and antimicrobial capacity of mountain tea.

Thus, the presence of chlorogenic acid [3]., three phenyltannoid glycosides and eight flavonoid glycosides was confirmed in the samples.

Conclusion

While there are many different extraction methods and conditions of the phenolic and flavonoid components of *Sideritis scardica* and *Sideritis syriaca* extracts, the most effective one seems to be the ultrasonic assisted method, (USAE). This method has the advantage, as it is the one that has the shortest duration (1h), requires a smaller solvent volume (15 mg/mL) and most importantly, does not destroy valuable components that we want to get from the extraction.

Regarding the bioactive compounds of the extracts, a multitude of them were identified, such as chlorogenic acid, phenyltannoic glycosides as well as flavonoid glycosides, using the LC UV-DAD/ESI-MS liquid chromatography [13].

Another important finding is that the concentration of these compounds and the antioxidant capacity of the extracts are shown to be directly related to each other. The antioxidant capacity of mountain tea extracts was confirmed and their contribution was quantified, using the applied test methods of DPPH and FRAP.

In addition, through tests against bacterial strains, the antimicrobial capacity of the extracts was demonstrated, mainly against Gram positive bacteria (*S. aureus*) and to a lesser extent against Gram negative (*E. feacalis, E. coli*).

Therefore, mountain tea can be considered to have the potential to be marketed as a functional beverage and used as a valuable source of bioactive ingredients in a wide range of products.

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