JAP&T TPA-Timişoara

Available online at https://www.journal-of-agroalimentary.ro/

Journal of Agroalimentary Processes and Technologies 2023, *29* (3), 155-161

Journal of Agroalimentary Processes and Technologies

Comparision of bioactive properties and phenolic comounds of the aerial parts of mountain tea (*Sideritis phrygai* Bornm) treated by Ultrasonic and Water-Bath extraction systems at different temperature and times

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Abstract

Sideritis species are known as “mountain tea” in Turkey and are widely used as herbal tea. Total flavonoid amounts of the extracts of mountain tea treated by sonication and water-bath syatms at 90 ºC and different times were recorded between 65.24 (6min) and 68.01 mg QE/g (9 min) to 63.79 (9 min) and 66.99 mgQE/g (6 min), respectively. Total phenolic values of the extracts of mountain tea extracted by sonication at 25,80 and 90 ºC at different times were determined between 11.88 (3 min) and 12.60 mgGAE/g (9min), 9.74 and 16.34 mg GAE/g (6 mn) and 11.60 (3 min) and 16.62 mg GAE/g (9 min), respectively. Antioxidant activities of the mauntain tea extracts obtained by sonication system at 25,80, 90 ºC and different times (3,6,9 min) were measured between 3.67 (9 min) and 4.28 mmol/kg (3 min), 3.36 (6 min) and 3.75 mmol/kg (9 min) and 4.06 (9 min) and 4.21 mmol/kg (3 min), respectively. Rutin, catechin, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, 3,4-dihydroxybenzoic acid were the main phenoliccompunds of mountain tea extracts obtained by sonication and water-bath systems. Rutin amounts of the extracts of mountain tea extracted by sonication and water-bath systems depending on extraction temperatures and times were identified between 39.49 (25 ºC for 3 min) and 98.57 mg/100g (80 ºC for 6 min) to 81.99 (90 ºC for 9 min) and 544.23 mg/100g (90 ºC for 3 min), respectively. In general, the highest routine values were found in the samples extracted in water-bath at 25 and 80 ºC.

Keywords: *Sideritis* spp., mountain tea, total phenol, flavonoid, antioxidant activity, phenolic compounds

1.Introduction

Plants from the genus *Sideritis* (belongs to the *Lamiaceae* family) occur mainly in the Mediterranean area [8, 19, 23]. Sideritis phrygia Bornm. is distributed only on the northern slopes of Sultandağları, which is located on the common border of Afyonkarahisar, Isparta and Konya provinces in Turkey [10]. The Lamiaceae family consists of about 230 genera and 7100 species worldwide. Many species of this family are very important with their use in medicine, food industry and cosmetics. Sideritis species are used in folk medicine due to their antiulcerogenic, antimicrobial and anti-inflammatory properties [18].

Although it varies from region to region in Anatolia, Sideritis species, which are mostly called "mountain tea", "highland tea" or "tea grass", are mostly used as tea among the people due to their aromas (Chalchat and Özcan, 2005) [3, 9]. Sideritis (Lamiaceae), also called "shepherd's tea", is widely grown in the temperate climate of the northern hemisphere and is consumed as herbal tea (Chalchat and Özcan, 2005) [3, 8, 9]. Sideritis species have many biological effects such as antioxidant, analgesic, antiproliferative, anti-inflammatory, anti-ulcerogenic, anti-viral and anti-apoptotic [7, 9, 12, 13]. Ethanol extracts of *Sideritis* have been used as an antiseptic solution after tooth extraction, as well as for topical use on the skin [21].

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Ultrasound assisted extraction, which creates an acoustic cavitation effect that facilitates the penetration of the extraction solvent, has always become a technology that can be adapted to small or large scale (Ma et.) [14, 15, 16]. Plants are valuable sources of minerals, vitamins, phenolics and other important bioactive compounds. They are used not only in folk medicine, but also for the development of functional foods and pharmaceuticals [1,2]. Considering the extraction time, lower solvent consumption and increasing the quality of the extract, Ultrasonic assisted extraction has become more advantageous than other extraction systems [4, 22]. Turkey is among the countries rich in vegetation diversity. This wealth offers us very valuable endemic plants. One of them is Sideritis phrygia plant from the Lamiaceae family. The aim of current study was to investigate bioactive compounds, antioxidant activities and phenolic comounds of the aerial part extracts of mountain tea extracted by ultrasonic and water-bath extraction systems at different temperature and times.

2.Material and methods

2.1.Material

The aerial parts of *Sideritis phrygai* Bornm [5] were collected from Konya (Kurşunlu) province in Turkey in 2022. After the samples were packed in paper bags, they were transported to the laboratory and dried in the open environment in the shade. Dried samples were ground in the laboratory mill and stored in a hermetic glass bottle in the refrigerator until analysis.

2.Methods

2.1.Moisture content

The % moisture content of dried plant samples was determined by Kern Dbs 60-3 moisture analyzer.

2.2.Sonication process

Extraction process was conductued as described previously [20] with minor changes. After *Sideritis phrygai* were powdered using a grinder. it was placed in Erlenmeyer flask (1 g).

After 15 ml of water (25. 80 and 90 °C) was added to the extract, the samples were sonicated for 3. 6 and 9 min 25. 80 and 90 °C in ultrasonic bath. Then, the extract solution was filtered by using a 0.45 µm membrane filter.

2.3.Total phenolic content

Total phenolic contents of Sideritis extracts were determined by using Folin-Ciocalteu (FC) reagent according to method described by Yoo et al. (2004) [24]. The extract (0.5 mL) was mixed with 2.5 ml of Folin Ciocalteu reagent and 2 ml of 7.5% Na_2CO_3 (sodium carbonate) solution, respectively. Then, the samples were stored at room temperature and in the dark after 2 hours. Absorbance was measured in a spectrophotometer (Shimadzu. Japan) at a wavelength of 725 nm. Results are stated as mg gallic acid equivalent (GAE)/g weight.

2.4.Total flavonoid content

The flavonoid contents of mountain tea extracts were measured using aluminum chloride method with little modifications. After pre-processing, the absorbance values of the extracts were measured at 510 nm. The results are described as mg quercetin (QE)/g [11].

2.5.Antioxidant activity

DPPH (1.1-diphenyl- 2- picrylhydrazyl) described by (Lee et al.. 1998) with some modifications was used for analysis of the free radical scavenging activity of extracts. The absorbance was detected at 517 nm.

2.6.Determination of phenolic compounds

HPLC (Shimadzu) equipped with a PDA detector and an Inertsil ODS-3 (5 µm; 4.6 mm × 250 mm) column was applied for analysis of phenolic compounds and their quantities of mountain tea extracts. The injection volume for test was injected as 20 µL [17].

2.3.Statistical analyzes

Minitab 16 statistical program was used to interpret the data obtained as a result of the research. The data were subjected to analysis of variance. The averages of the main variables whose differences were found to be statistically significant were compared with the Tukey multiple comparison test and were considered to be of statistical significance. The mean values and standard deviations of the obtained results are given (Düzgüneş et al., 1987).

3.Results and Discussion

3.1.Bioactive properties of the aerial parts of the extracts of mountain tea (Sideritis phrygai)

Bioactive compounds and antioxidant activities of aerial parts of mountain tea (*Sideritis phrygai*)

treated by Sonication and Water-Bath systems at different temperature and times (3,6 9 min) are shown in Table 1. Results exhibited some fluctuations depending on extraction type, temperatures and times. Moisture content of *Sideritis phrygai* was determined as 8.46%. Total flavonoid contents of the etxracts of mountain tea treated by sonication and water-bath systems at 25 ^{o}C and different times were determined between 45.21 (3 min) and 47.76 mg QE/g) to 45.56 (6 min) and 48.99 mg QE/g (3 min), respectively. While total flavonoid contents of mountain tea extracts treated by sonication at 80 ^{o}C and different times change between 59.27 (9 min) and 64.24 mg QE/g (6 min), total flavonoid amounts of mountain tea treated by water-bath system at 80 ^{o}C and different times were reported between 58.01 (3 min) and 64.90 mg QE/g (6 min). In addition, total flavonoid values of mountain tea treated by sonication and water-bath syatms at 90 ^{o}C and different times were recorded between 65.24 (6min) and 68.01 mg QE/g (9 min) to 63.79 (9 min) and 66.99 mg QE/g (6 min), respectively (Fig.1). Total phenolic amounts of the extracts of mountain tea treated by sonication at 25,80 and 90 ^{o}C at different times were determined between 11.88 (3 min) and 12.60 mgGAE/g (9min), 9.74 and 16.34 mg GAE/g (6 mn) and 11.60 (3 min) and 16.62 mg GAE/g (9 min), respectively. In addition, total phenolic amounts of the mountain tea extracts obtained by water*bath system at 25,80 and 90 ^{o}C at different times (3,6 and 9 min) were found between 10.31 (9min) and 16.69 mgGAE/g (3 min), 12.43 (9 min) and 12.59 (6 min) and 9.48 (3 min) and 14.10 mg GAE/g (9 min), respectively. In addition, antioxidant activities of the mauntain tea extracts obtained by sonication system at 25,80, 90 ^{o}C and different times (3,6,9 min) were measured between 3.67 (9 min) and 4.28 mmol/kg (3 min), 3.36 (6 min) and 3.75 mmol/kg (9 min) and 4.06 (9 min) and 4.21 mmol/kg (3 min), respectively. Also, antioxidant activity of the extracts of mountain tea extracted by water-bath at different temperatures (25,80, 90 ^{o}C) and times (3,6,9 min) were recorded between 3.96 (6 min) and 4.49 mmol/kg (3 min), 3.32 (3 min) and 7.55 nnol/kg (9 min) and 3.39 (3 min9 and 4.24 mmol/kg (9 min), respectively. As seen Table 1, in mountain tea extracts extracted by sonication, the highest total flavonoid contents were obtained at 90 °C for different times, and this was observed in samples extracted at 80 °C and 25 °C in decreasing order. In addition, the highest total phenol and antioxidant activity values were determined at 25 ^{o}C in the extracts obtained by sonication system, followed by processes at 90 ^{o}C and 80 ^{o}C in decreasing order. In mountain tea extracts obtained by water-bath, the highest total flavonoid was obtained at different times of the samples extracted at 90 °C, followed by the extraction process at 80 °C and 25 °C, in decreasing order. In general, the bioactive component and antioxidant activity values of mountain tea extracts extracted by sonication system were slightly higher than those extracted in water-bath. Total phenolic contents of aerial parts of S phrygia and *S. bigerana* plants determined using Folin-Ciocalteu reagent were 1.73±0.02 µgGAE/mL and 1.62±0.04 µgGAE/mL, respectively (Tekeli, 2012). Total phenolic, flavonoid and antioxidant activity values of S. lycia and S. libanotica subsp linearis were determined between 16.05–18.04 and 9.16–10.49 g gallic acid equivalent/kg dw, 9.70–14.30 and 5.41–9.68 g catechin equivalent/kg dw and 6.57 –8.71 and 13.88–19.04 g dw/g DPPH, respectively [6]. Total phenolic and flavonoid profiles of methanol extracts of *S. congesta* plants were 139 mg GAE/g and 70.9 mg QE/g, respectively (Bardakcı et al. 2020). FRAP values of *S. phrygia* and *S. bilgerana* extracts were measured as 7.61 µg/mL and 7.15 µg/mL (trolox equivalent) respectively (Tekeli, 2012). The antioxidant activity values for DPPH, ABTS and CUPRAC of S. Ozturkii extracts were 32.48–137.30 mg TE/g, 46.78–176.45 mg TE/g and 132.47–428.61 mg TE/g, respectibvely [25]. When the results were compared with the results of previous studies on some Sideritis species, differences were observed. These differences are probably due to harvest time, part used, climatic factors and location.

Table 1. Bioactive properties of sonicated *Sideritis phrygai*

Process	Temperature	Time	Total Flavonoid Concent (mg QE/g)	Total Phenolic Concent (mg GAE/g)	Antioxidant activity (mmol/kg)
Ultrasonic	25°C	3min	45.21±1.30^{IJ*}	11.88±0.90^{DEFG}	4.28±0.05^{BC}
		6 min	46.50±0.23^{D}	12.37±0.43^{DEF}	4.21±0.10^{BC}
		9 min	47.76±0.81^{BD}	12.60±0.86^{DEF}	3.67±0.22^{CDEF}
	80°C	3min	60.81±0.87^{EF}	9.74±0.75^{GH}	3.64±0.27^{CDEF}
		6 min	64.24±0.37^{CD}	16.34±0.72^{AB}	3.36±0.20^{EF}
		9 min	59.27±1.04^{FG}	10.60±0.59^{FGH}	3.75±0.18^{CDEF}
	90°C	3min	65.73±0.43^{ABC}	11.60±0.24^{EFGH}	4.21±0.05^{BC}
		6 min	65.24±1.29^{BC}	14.19±1.01^{BCD}	4.14±0.09^{BCD}
		9 min	68.01±0.83^{A}	16.62±0.38^{A}	4.06±0.13^{BCD}
Water-Bath	25°C	3min	48.99±0.26^{H}	16.69±0.39^{A}	4.49±0.05^{B}
		6 min	45.56±0.48^{IJ}	15.46±1.40^{ABC}	3.96±0.05^{BCDEF}
		9 min	46.84±0.51^{IHJ}	10.32±1.17^{FGH}	3.99±0.05^{BCDE}
	80°C	3min	58.01±0.18^{G}	12.46±0.62^{DEF}	3.32±0.25^{F}
		6 min	64.90±0.32^{BCD}	12.59±0.35^{DEF}	3.53±0.05^{DEF}
		9 min	62.53±0.54^{DE}	12.43±0.14^{DEF}	7.55±0.07^{A}
	90°C	3min	65.87±0.31^{ABC}	9.48±0.80^{H}	3.39±0.43^{EF}
		6 min	66.99±1.65^{AB}	13.19±0.8^{CDE}	3.50±0.17^{DEF}
		9 min	63.79±0.65^{CD}	14.10±0.75^{BCD}	4.24±0.00^{BC}

*Values are mean ± standard deviation and those with different letters in each column are significantly different (p≤0,01)

Table 2. Phenolic components of *Sideritis phrygai* samples

Process	Temperature	Time	Gallic acid	3.4-Dihydroxy benzoic acid	Catechin	Caffeic acid	Syringic acid	Rutin
Ultrasonic	25°C	3min	4.72±1.62*	8.71±4.21^{B}**	115.08±6.00^{abc}***	17.76±3.16^{ab}	23.08±7.65****	39.49±10.85^{F}
		6 min	8.62±0.81	17.28±1.32^{B}	186.32±1.66^{a}	24.66±0.89^{a}	22.44±8.01	71.92±9.01^{EF}
		9 min	7.85±0.51	13.76±0.43^{B}	131.52±66.45^{ab}	19.66±7.74^{ab}	31.71±0.49	76.89±11.33^{EF}
	80°C	3min	3.90±0.96	12.38±3.19	58.23±43.00^{bc}	12.20±2.76^{ab}	14.91±2.89	97.98±5.13^{E}
		6 min	5.99±0.73	35.78±2.17^{A}	36.79±1.67^{c}	19.41±0.71^{ab}	13.50±0.71	98.57±0.66^{E}
		9 min	3.82±1.48	19.22±1.01^{AB}	34.43±1.58	16.06±0.99^{ab}	13.81±0.72	86.84±1.78^{EF}
	90°C	3min	5.83±0.66	16.96±1.06^{B}	65.94±43.89^{bc}	12.38±5.44^{ab}	16.45±3.37	82.71±24.86^{EF}
		6 min	4.46±0.41	20.02±0.64^{AB}	34.44±2.09^{c}	16.51±0.49^{ab}	16.37±0.43	87.37±1.34^{EF}
		9 min	5.18±0.54	17.72±7.37^{B}	34.23±3.64^{c}	17.83±3.21^{ab}	17.75±0.29	87.25±5.85^{EF}
Water-Bath	25°C	3min	7.68±3.48	16.58±2.17^{B}	29.23±16.50^{c}	9.63±1.90^{b}	33.16±16.68	504.5±5.43^{AB}
		6 min	8.54±2.70	10.80±4.96^{B}	90.31±23.43^{bc}	10.20±0.93^{b}	24.67±2.75	430.59±5.96^{CD}
		9 min	6.39±1.88	9.80±3.42^{B}	96.17±29.58^{abc}	14.97±4.30^{ab}	30.86±4.56	421.23±12.54^{D}
	80°C	3min	6.69±2.11	12.98±7.07^{B}	35.42±7.92^{c}	11.08±4.01^{b}	14.52±1.76	476.49±17.32^{BC}
		6 min	4.18±1.82	14.64±5.61^{B}	33.53±14.15^{c}	15.84±1.60^{ab}	13.52±1.47	533.89±39.55^{A}
		9 min	4.18±1.47	15.63±7.30^{B}	36.95±8.16^{c}	16.75±1.39^{ab}	15.83±1.04	515.28±7.07^{AB}
	90°C	3min	3.94±1.06	12.86±2.67^{B}	28.20±11.88^{c}	13.11±2.71^{ab}	14.31±2.99	544.23±22.55^{A}
		6 min	6.14±1.00	21.12±1.00^{AB}	51.25±1.00^{bc}	17.42±8.00^{ab}	16.80±2.00	89.24±5.00^{EF}
		9 min	3.15±1.98	11.52±5.36^{B}	28.67±1.99^{c}	12.02±4.17^{b}	15.56±0.23	81.99±5.05^{EF}

Table 2. *(continuation)*

Process	Temperature	Time	p-Coumaric acid	Ferulic acid	Resveratrol	Quercetin	Cinnamic acid	Kaempferol
Ultrasonic	25°C	3min	16.90±0.12*	25.10±1.57^{efg}***	5.90±6.13****	13.25±17.67	2.76±2.28	19.52±0.23^{BC}**
		6 min	19.42±5.24	21.77±2.60^{fg}	14.16±2.62	36.33±1.82	3.11±1.12	18.22±0.82^{BC}
		9 min	25.12±4.22	34.48±10.55^{bcdefg}	12.56±6.73	33.63±0.81	6.84±2.58	17.82±0.68^{BC}
	80°C	3min	19.54±2.16	67.63±2.57^{bc}	10.38±4.34	19.01±12.32	4.71±1.64	32.90±0.95^{A}
		6 min	26.36±2.52	62.32±0.56^{bcd}	15.42±1.97	7.55±4.36	4.52±0.79	6.61±0.20^{D}
		9 min	19.68±0.62	120.11±21.08^{a}	12.01±7.43	23.75±15.46	5.98±1.12	7.42±0.60^{D}
	90°C	3min	26.68±5.19	63.81±0.33^{bcd}	11.46±3.96	19.40±13.35	3.72±1.06	13.51±5.17^{C}
		6 min	7.58±1.00	69.92±1.18^{b}	5.19±1.24	15.65±17.07	3.45±0.63	5.59±1.04^{D}
		9 min	20.66±9.57	25.10±1.57^{efg}	10.52±3.44	12.00±13.22	4.90±0.36	17.61±1.73
Bath	25°C	3min	20.76±4.16	22.70±3.45^{fg}	17.70±9.17	61.16±32.40	6.25±2.04	18.33±0.45^{BC}
		6 min	27.96±0.31	18.81±5.30^{fg}	9.35±3.75	49.21±34.27	6.30±2.80	18.49±1.72^{BC}
		9 min	15.59±9.35	12.92±1.73^{g}	10.11±6.89	22.96±15.00	5.84±4.18	20.04±0.85^{B}
	80°C	3min	14.68±11.04	29.68±9.41^{defg}	7.02±1.99	7.14±7.87	3.83±0.57	3.50±1.22^{D}
		6 min	24.41±3.46	44.81±9.15^{bcdefg}	9.94±3.40	15.44±8.71	6.26±1.48	6.57±1.57^{D}
		9 min	23.18±3.78	31.97±3.95^{cdefg}	10.99±4.02	3.88±0.61	3.79±1.23	4.35±0.90^{D}
	90°C	3min	50.16±20.88	60.53±4.30^{bcde}	8.04±4.91	24.95±6.93	6.10±0.59	3.96±1.14^{D}
		6 min	26.26±4.08	44.58±1.00^{bcdefg}	11.25±7.00	16.52±8.00	3.73±7.00	6.22±1.00^{D}
		9 min	30.41±2.60	49.58±18.92^{bcedf}	11.25±0.48	34.90±3.97	3.80±0.67	5.07±0.37^{D}

*Mean±standard deviation; ** $p \leq 0,01$; *** $p \leq 0,05$; **** $p < 0,05$.

3.2.The phenolic compounds of the aerial parts of the extracts of mountain tea (Sideritis phrygai)

The phenolic compounds and their quantitative values of the extracts of mountain tea aerial parts treated by sonication and water-bath systems at different temperatures (25, 80 and 90 ºC) and times (3,6 and 9 min) are shown in Table 2. Rutin, catechin, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, 3,4-dihydroxybenzoic acid were the main phenoliccompunds of mountain tea extracts obtained by sonication and water-bath systems. Rutin amounts of the extracts of mountain tea extracted by sonication and water-bath systems depending on extraction temperatures and times were identified between 39.49 (25 ºC for 3 min) and 98.57 mg/100g (80 ºC for 6 min) to 81.99 (90 ºC for 9 min) and 544.23 mg/100g (90 ºC for 3 min), respectively. Genel olarak, en yüksek rutin değerleri water-bath'de 25 ve 80 ºC'da ekstrakte edilen örneklerde tespit edilmiştir. Ferulic acid contents of mountain tea extracts treated by sonication system at different temperatures (25, 80, 90 ºC) and times (3,6, 9 min) were detected between 21.77 (9min) and 25.10 mg/100g (9 min), 62.32 (6 min) and 120.11 mg/100g (9 min) and 25.10 (9 min) 69.92 mg/100g (6 min), respectively. In addition, ferulic acid amounts of extracts of mountain tea extracted by water-bath system at different temperatures and times were detected between 12.92 (9min) and 22.70 mg/100g (3 min), 29.68 (3 min) and 44.81 mg/100g (6 min) and 44.58 (6 min) and 60.53 mg/100g (3 min), respectively. While catechin amounts of the extracts of mountain tea treated by sonication at different temperatures and times vary between 34.23 mg/100g (90 ºC for 9 min) and 186 mg/100g (25 ºC for 6 min), catechin amounts of the mountain tea extracts treated by water-bath at different temperatures and times were detected between 28.20 mg/100g (90 ºC for 3 min) and 96.17 mg/100g (25 ºC for 9 min). Also, caffeic acid and syringic acid contents of the extracts of mountain tea treated by sonication system at different temperatures and times were determined between 12.20 mg/100g (80 ºC for 3 min) and 24.66 mg/100g (25 ºC for 6 min) to 13.50 (80 ºC for 6 min) and 31.71 mg/100g (25 ºC for 9 min), respectively. Caffeic acid and syringic acid contents of the extracts of mountain tea treated by water-bath system at different temperatures and times were reported between 9.63 (25 ºC for 3 min) and 14.97 mg/100g (25 ºC for 3 min), respectively. In previous study, The extract of *S. phrygia* contained 112.3 µg/g catechin, 48.8 caffeic acid, 69.2 ferulic acid, 687.1 apigenin glucoside, 41.1 luteolin, 1247.0 apigenin (Tekeli, 2012). In general, the phenolic compounds of mountain tea extracts extracted by sonication at different temperatures and times were higher than those extracted by water-bath in most

cases. In addition, the highest phenolic compounds of the mountain tea extracts extracted with both extraction systems were observed in the samples extracted at different times at 25 °C, followed by the extraction processes applied at 80 °C and 90 °C in descending order. In addition, there were phenolic compounds that varied in quantity depending on the type of extraction, duration and temperature applied. These differences were probably due to factors such as applied temperature, time, plant particle size, homogeneity of the samples. Looking at the results, it can be said that mountain tea extracted with both extraction systems is a good source of rutin.

Conclusion

Results showed some differences depending on extraction type, temperatures and times. In mountain tea extracts obtained by water-bath, the highest total flavonoid was obtained at different times of the samples extracted at 90 °C, followed by the extraction process at 80 °C and 25 °C, in decreasing order. In general, the bioactive component and antioxidant activities of mountain tea extracts extracted by sonication system were slightly higher than those extracted in water-bath. Rutin, catechin, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, 3,4-dihydroxybenzoic acid were the main phenoliccompunds of mountain tea extracts obtained by sonication and water-bath systems. In general, the phenolic compounds of mountain tea extracts extracted by sonication at different temperatures and times were higher than those extracted by water-bath in most cases. In addition, the highest phenolic compounds of the mountain tea extracts extracted with both extraction systems were observed in the samples extracted at different times at 25 °C, followed by the extraction processes applied at 80 °C and 90 °C in descending order.

Conflict of Interest. Author has declared that no competing interests exist.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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