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The Phenolic Composition and Antioxidant Activity of Tea with different Parts of *Sideritis condensate* at Different Steeping Conditions

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Abstract Tea with different parts (flower, leaf, seed) of *Sideritis condensate* infused at different temperatures (60 and 100°C) and times (5, 10 and 30 minutes) were assessed for their phenolic composition and antioxidant activities. Leaf tea had the highest total phenolic content where as seed tea had the lowest.Leaves soaked at 100°C for 10 minutes had the highest total phenolic content. Total phenolic content of flower tea increased with increase in extraction temperature and time. Radical scavenging activities of leaves infused at 60°C for 5, 10 and 30 minutes were statistically in the same group but lower than those of leaves soaked at 100°C for 5, 10 and 30 min. The major phenolic compound identified from almost all aqueous infusions was the *p*-coumaric acid. The conditions of tea prepared from leaves of the *Sideritis condensata* at 100°C for 5, 10 and 30 minutes are the most appropriate conditions in regard to extraction of the highest total phenolics and the strongest antioxidant activity.

Keywords: *Sideritis condensate, infusion, phenolic compounds, antioxidant activity*

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1. Introduction

Recently, awareness of health benefits of bioactive compounds has increased and people demand foods with a higher content of bioactive compounds and minerals [1]. Phenolic compounds known also as bioactive compounds having antioxidant, antiiflamatory, antiallergic and antineoplasic activities have received considerable attention because of their beneficial health effects [2]. Therefore, it is important to know the amount of these compounds in consumed foods and drinks. Tea and herbal infusions are considered as major source of phenolic compounds in the diet [3]. Being one of the widely consumed tea, water extract of *Sideritis condensate*is used as a popular drink in Turkey [23]. *Sideritis condensate* Boiss. Et Heldr. is a member of Lamiaceae family which comprises more than 150 species mainly distributed in the Mediterranean basin [4,5,6,7]. Locally known as "Dagcayı", *Sideritis condensate* Boiss. et Heldr., is an endemic plant of Turkey growing in Antalya province [8]. Sideritis species have been widely used in folk medicine as anti-inflammatory, anti-ulcer, cytostatic, antimicrobial, flu vaccine and stimulant circulatory agents and in treatment of cough, common cold, gastrointestinal disorders [9,10]. Flavonoid content of Sideritis species and their valuable chemical composition make contribution to these therapeutic properties of the plant and justify their popular uses in the traditional medicine [4,11,12]. Ezer et al. [13] characterized the *S.congesta*, *S. argyrea*, *S. perfoliata*, *S.condensata* species with high percentage of essential oil components. The dried aerial parts of *Sideritis condensate* is widely used as herbal tea by soaking these parts in hot water for half a minute or so in Turkey [23]. The ways of tea preparation (weight of tea soaked, the amount of water used for soaking, the time of tea in contact with water, the amount of stirring etc.), show variation among countries and individuals within countries [14]. It is well known that infusion conditions have asignificant effect on the antioxidant potential of tea [15,16]. Su et al. [17] found that antioxidant activity of tea solution increased with increase in extraction temperature and duration of soaking. Although presence of many researches on antioxidant activity of organic solvent (methanol, ethanol etc.) extracts of tea and herbs, there is little information about the phenolic profiles and antioxidant activity in infusion of herbs. (B). To our knowledge, there is no detailed research on the phenolic constituents and antioxidant activity of different parts (flower, leaf and seed) of *Sideritis condensate* tea infused under different conditions of steeping. Furthermore, it is not known which part of the

Sideritis condensate plant is rich in phenolic constituents and antioxidant activity. Herewith, this study was designed to investigate phenolic composition and antioxidant activities of each part (flower, leaf and seed) of *Sideritis condensate* infused with water under different time and temperature. So this study aimed to determine the effects of steeping conditions on phenolic composition and antioxidant activities of tea.

2. Material and Methods

2.1. Chemical Agents

HPLC grade phenolic standards,gallic acid, protocatecuic acid, *p*-hydroxy benzoic acid, catechin, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, epicatechin, *p*-coumaric acid, ferulic acid, rutin, myricetin, fisetin, apigenin, kaempferol, isorhamnetin and the internal standard (IS) propyl paraben were purchased from Sigma-Aldrich, quercetin was from Fluka. HPLC grade acetonitrile, methanol, ethanol, ethyl acetate, hexane were from Merck, ether was from ACS, Reag. PhEur for analysis, acetic acid, HCl (hydrochloric acid) and Folin-ciocalteau reagent were purchased from Merck. Sodium carbonate, DPPH (1,1-Diphenyl-2-picrylhydrazyl radical, 2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl) were supplied from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Trolox (6- hydroxy-2, 5, 7, 8-tetramethylchroman-2carboxylic acid) was from FlukaChemie GmbH (Switzerland). HPLC syringe filters (RC-membrane, 0.2 µm) were from Sartorius Minisart RC 15, Sartorius (Germany).

2.2. Location of Plant Material

Location and voucher number of *Sideritiscondensate* was as follows: C3 Antalya: Akseki, between Kuyucak and İbradı, 1226 m, 21 vii 2008, O. Tugay 5498 &Gümüşçü (KONYA).

2.3. Extraction Conditions of *Sideritis condensate*

Collected plant materials were dried at room temperature. Then flower, leaf and seed parts of the plant were separated and ground to fine powder with a grinder. 2.5 g powdered material was weighed into socket flask with reflux condenser in a water bath and 60 ml distilled water was added. Extraction was accomplished at60 ° C and 100°C for 5, 10 and 30 minutes. 5 ml of each extract were kept for antioxidant analyses. Following the water extraction, liquid-liquid extraction was performed with 5 ml diethyl ether and 5 ml ethyl acetate, consecutively in shaker (HeidolphPromax 2020 Reciprocating Shaker, Schwabach / Germany). The liquid liquid extractions were repeated three times (Endaleet al.2005, Kim et al. 2006).In each step, organic phases were collected and then organic phases were combined together. After evaporation (IKA RV 05 Staufen, Germany)of organic phases at 60° C, the dried extracts were dissolved in 2.5 mL of methanol and stored at 4°C.

2.4. Determination of Total Phenolic Content

The total phenolic content of the each part was determined with Folin-Ciocalteu's phenol reagent method, using gallic acid as the standard (Singleton and Rossi 1965).Briefly, 20 µL samples (1 mg/mL), 400 µL of 0.5 Folin-Ciocalteu reagents and 680 µL of distilled water were mixed and the mixture was vortexed. Following 3 min. incubation, 400 µL of Na_2CO_3 (10%) solution was added and vortexed. Absorbances of the mixtures were measured at 760 nm after 2 h. Results were expressed as g of gallic acid equivalents per kg of dry weight, by using a standard curve for gallic acid in the concentration range between 0.015 and 0.5 mg/mL ($r^2 = 0.998$).

2.5. Determination of Phenolic Compounds

HPLC analyses of phenolic compounds were carried out on Thermo Finnigan Surveyor HPLC equipped with UV-Vis detector supplying double wavelength simultaneously. C18 column (150 mm × 4.6 mm id, 5 µm particle; Fortis) was used.Benzoic acid derivatives (such as gallic acid, protocatechuic acid, *p*-hydroxy benzoic acid, vanillic acid, and syringic acid) and flavonols (such as catechin and epicatechin) were analyzed at 280 nm while cinnamic acid derivatives such as chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid and flavonols such as rutin, myricetin, fisetin, quercetin, apigenin, kaempferol, and isorhamnetin were analyzed at 315 nm. Propyl paraben (IS) was performed at 280 nm and normalization calibration method was used. Gradient elution was used for HPLC analyses modifying the method developed by De Villiers et al. [18]. 2% acetic acid in water (A) and 70:30 acetonitrile/water mixture (B) mixture of mobile phase with a flow rate of 1.2 ml/min at 30°C with injection volume of 25 µL was used. The gradient was as follows: 0-3 min 5 % B; 3-8 min 5-15 % B; 8-10 min 15-20 % B; 10-12 min 20-25 % B; 12-20 min 25-40 % B; 20-30 min 40-80 % B, before returning to the initial conditions.

Values of limit of detection (LOD) were calculated according to the EPA method as an S/N level of 3 and limit of quantification (LOQ) was calculated as an S/N level of 10. Calculated amounts per compound wereas follows; 0.5 mg/L for gallic acid, protocatechuic acid, *p*-OH benzoic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, *p*- coumaric acid, rutin and propylparaben, 1 mg/L for ferulic acid, myricetin, fisetin, apigeninkaempferol, and isorhamnetin, 2 mg/L for catechin, epicatechin and quercetinwere prepared and mixture was injected 7 times to verify the LOD and LOQ of each compound then calculated as the percentage relative standard deviation %RSD of peak area and retention time.

2.6. Antioxidant Activity: DPPH Radical Scavenging Activity

The ability of different parts of *Sideritis condensate* extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined spectrophotometricallyat 517 nm [19]. 0.75 ml extract of each part of*Sideritis condensate* was mixed with 0.75 mL of a 0.1 mM of DPPH in methanol. Trolox was used as a positive control. The values are expressed as SC_{50} (mg sample/ml) required to scavenge 50 % of the initial DPPH. Lower SC_{50} values

indicate higher antioxidant activity. The experiments were performed in triplicate.

2.7. Statistical Analyses

Triplicate measurements of total phenolics and DPPH values were statistically evaluated by analysis of variance (ANOVA) procedure using SPSS statistical software ver. 9.0 (SPSS Inc., Chicago, IL). The differences among the means were compared using Duncan's multiple comparison.

3. Results and Discussion

Antioxidant activities of water extract of the each aerial parts of *Sideritis condensate* had different levels of antioxidant activity. DPPH is a widely used method to evaluate radical scavenging capacity [20]. Phenolic constituents and antioxidant activities of *Sideritis condensate* varied according to plant parts, infusion conditions and extraction medium. Lower DPPH values show higher antioxidant activity [19]. According to Table 1, the highest total phenolics was obtained with water in the leaves of *Sideritis condensate* soaked at 100°C for 10 minutes and thereby the strong DPPH scavenging activity was determined. The amount of total phenolics of leaves infused at 100°C for 5 and 30 min were in the same group but lower than those of soaked at 100°C for 10 minutes. Although total phenolics of leaves infused at 100°C for 10 minutes is higher than the others (5 and 30 min.),the DPHH activities were statistically in the same group. Infusion of leaves at 100°C for 5, 10 and 30 minutes seems to be the most efficient condition to provide good antioxidant activities. The second highest total phenolic was obtained in leaves soaked at 60°C for 30 minutes. Radical scavenging activities of leaves infused at 60°C for 5, 10 and 30 minutes were statistically in the same group but lower than those of leaves soaked at 100°C for 5, 10 and 30 min. Seed part of the plant infused both at 60°C and at 100°C had the lowest antioxidant activities. Increasing infusion temperatures resulted in a decrease of DPPH radical scavenging activity and a increase of total phenolic contents.

Table 1. Antioxidant activities of *Sideritis condensate* of aqueous extract*

Temperature		Steeping time (min.)	Total phenolics[1] [mgGAE/ g]	DPPH[2] SC_{50}[mg/mL]
		5	2.727 ± 0.05 f	0.562 ± 0.002 g
	Flower	10	3.035 ± 0.028 g	0.448 ± 0.007 fg
		30	3.592 ± 0.089 h	0.403 ± 0.016 defg
		5	8.293 ± 0.24 l	0.165 ± 0.006 abc
60°C	Leaf	10	7.57 ± 0.011 k	0.175 ± 0.018 abc
		30	9.441 ± 0.072 m	0.115 ± 0.003 abc
		5	0.608 ± 0.045 b	4.889 ± 0.300 j
	Seed	10	0.507 ± 0.016 b	4.528 ± 0.188 i
		30	0.371 ± 0.011 a	4.397 ± 0.248 i
		5	4.586 ± 0.078 i	0.413 ± 0.007 efg
	Flower	10	4.642 ± 0.067 i	0.286 ± 0.005 cdef
		30	5.518 ± 0.056 j	0.237 ± 0.009 bcd
		5	12.171 ± 0.091 n	0.057 ± 0.009 a
100°C	Leaf	10	12.99 ± 0.051 o	0.044 ± 0.001 a
		30	12.132 ± 0.095 n	0.049 ± 0.001 a
		5	0.75 ± 0.022 c	0.806 ± 0.022 h
	Seed	10	1.062 ± 0.017 d	0.822 ± 0.010 h
		30	2.041 ± 0.031 e	0.790 ± 0.009 h
Trolox				0.255 ± 0.000[3]

* Means followed by the same letter within each column are not significantly different and values are means ± standard deviations of measurements of three measurements

[1] Total phenolics are expressed in mg of gallic acid per g of sample of *Sideritis condensate*

[2] SC_{50} values are expressed in mg per mL.

[3] Trolox was used and expressed as µg/mL for only DPPH assays.

In this research, 20 different constituents such as phenolicsgallic acid, protocatechuic acid, *p*-OH benzoic acid, catechin, chlorogenic acid, vanillic acid, caffeic acid,syringic acid, epicatechin, *p*-coumaric acid, ferulic acid, *o*-coumaric acid, rutin, myricetin, fisetin, quercetin, apigenin, kaempferol, isorhamnetin, rhamnetin were investigated. The phenolic constituents are presented in Table 2. There was considerable variability in phenolic profile of flower, leaf and seed parts of the *Sideritis condensate* infusions. Protocatechuic acid, *p*-OH benzoic acid, catechin, chlorogenic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulicacid were found in water infusions. Different steeping temperatures and times of water infusions resulted in extraction of different phenolic compounds. Reis et al. [21] showed water as a good solvent to extract the considerable amount of phenolic compounds in apple pomace. This is in accordance with our results. Flowers infused at 60°C for 10 and 30 minutes were the richest part in phenolicsi.e. protocatechuic, chlorogenic, caffeic, *p*-coumaric, ferulic, rutin, kaempferol followed by flowers infused at 60 and 100°C for 5 min., leaves and seeds infused at 60 and 100°C for 5, 10, and 30 minutes. Isorhamnetin, exerts anticancer effects (Kim et al., 2011) was detected only in infusion of flowers at 100°C for 10 min. The major phenolic compound identified from almost all aqueous infusions was the *p*-coumaricacid. *p*-coumaric acid and caffeic acids were previously reported as main phenolic acids of *Sideritislycia L.* and *Sideritislibanotica subsp. Linearis L.* (Dinçer et al. 2010).Coumaric Acid is capable of scavenging hydroxyl radical, inhibiting lipid peroxidation in vivo, and thereby reducing serum LDL cholesterol levels (Zang et al. 2000).*p*-OH benzoic acid and quarcetin were found only in extracts of flowers prepared under the

infusion conditions of 100°C for 5, 10 and 30 minutes. Apigenin and chlorogenic acid were identified only from steeping conditions of 60°C for 5, 10 and 30 minutes. The quantity of apigenin was at the same level with that of *S.raeseri* analyzed by Janeska et al [12]. Although high content of total phenolics and strong DPPH activities of leaves infused at 100°C for 5, 10 and 30 min., polyphenolic profile of flowers infused under the steeping conditions of 60°C for 5, 10 and 30 min were richer than those of leaves prepared at 100°C for 5, 10 and 30 min.Despite the low number of detected phenolics in leaves soaked at 100°C, the high antioxidant activity of leaves infusion may be related to synergism among the identified phenolicscatechin, caffeic acid, *p*-coumaric acid, rutin and kaempferol. Either, the other phenolics with high antioxidant activity which were not tested in this study may contribute to high antioxidant capacity. Flavonoids present in mixture can interact, and their interactions can affect the total antioxidant capacity of a solution [22].The amount of extracted total phenolics increased with increasing infusion time and temperatures, however, the amounts of individual phenolics decreased. Some phenolic compounds i.e. chlorogenic acid, rutin, apigenin and kaempferol identified at 60°C were not detected at 100°C, which might be due to loss of phenolic compounds.These findings indicated the variation of phenolic profile of different part of *Sideritis condensate*at different steeping times, temperatures and extraction mediums.

Table 2. Phenolic constituents of the *Sideritis condensate* by HPLC-UV

	60°C								
	Flower [a]			Leaf [a]			Seed [a]		
Compounds	5 min	10 min	30 min	5 min	10 min	30 min	5 min	10 min	30 min
Protocatechuic acid	128.36	207.58	172.60	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
p-OH benzoic acid	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
Catechin	ND[b]	ND[b]	ND[b]	171.01	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
Chlorogenic acid	200.45	316.78	280.63	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
Vanillic acid	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	190.5	201.86	205.92
Caffeic acid	477.62	558.67	1044.72	92.95	144.34	393.43	ND[b]	ND[b]	ND[b]
p-coumaric acid	126.35	234.31	398.11	97.39	150.36	176.29	10.25	11.26	115.78
Ferulic acid	12.38	67.49	106.14	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
Rutin	302.28	608.34	988.66	133.53	197.11	850.29	ND[b]	ND[b]	225.62
Quercetin	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
Apigenin	ND[b]	808.91	908.03	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
Kaempferol	1113.36	1207.20	1965.04	ND[b]	1672.29	939.43	ND[b]	ND[b]	ND[b]
İsorhamnetin	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
	100°C								
	Flower [a]			Leaf [a]			Seed [a]		
Compounds	5 min	10 min	30 min	5 min	10 min	30 min	5 min	10 min	30 min
Protocatechuic acid	124.84	144.50	200.00	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
p-OH benzoic acid	858.06	1097.38	1178.43	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
Catechin	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	208.92	ND[b]	ND[b]	ND[b]
Chlorogenic acid	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
Vanillic acid	1362.90	1674.55	1569.22	ND[b]	ND[b]	ND[b]	492.89	494.37	708.43
Caffeic acid	ND[b]	48.59	54.31	42.81	167.28	601.23	ND[b]	ND[b]	ND[b]
p-coumaric acid	144.52	ND[b]	ND[b]	103.69	200.96	226.87	36.24	60.56	248.67
Ferulic acid	ND[b]	ND[b]	59.61	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
Rutin	ND[b]	ND[b]	ND[b]	206.21	411.56	879.12	ND[b]	ND[b]	504.58
Quercetin	2961.94	2527.12	1902.35	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
Apigenin	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]
Kaempferol	ND[b]	ND[b]	ND[b]	ND[b]	1056.85	ND[b]	ND[b]	ND[b]	ND[b]
İsorhamnetin	ND[b]	15284.40	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]	ND[b]

[a]Results are expressed in µg phenolic compound per g dry samples.
[b]Non-detected. Gallic acid, syringic acid, epicatechin, o-coumaric acid, myricetin, fisetin and rhamnetin were not detected at all in *Sideritis condensate*

4. Conclusions

The results of our study indicated that antioxidant activity of tested plant is high and phenolic composition of *Sideritis condensate* changed according to infusion temperatures, times and different parts of plant. Significant differences were observed between different parts of plant especially in regard to phenolic composition. Our study reveals that tea preparation from leaves of the *Sideritis condensate* at 100°C for 5, 10 and 30 minutes are the most appropriate conditions in regard to extraction of the highest total phenolics and the strongest antioxidant activity. However, infusion of all three parts flower, leaf and seed of the *Sideritis condensate* had different polyphenolic profile and it is a good choice for consumers to use mix of these parts as aherbal tea. Our results justifies the widely consumption of Sideritis infusions as a

herbal tea and tested plant is regarded as medicinal plant from the ancient times.

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Abbreviations

ANOVA	Analysis of variance
DPPH	2,2-diphenyl-1-picrylhydrazyl
GAE	Gallic acid equivalent
HPLC	High performance liquid chromatography
IS	Internal Standard
LOD	Limit of detection
LOQ	Limit of quantification
RSD	Relative Standard deviation

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