Asian Journal of Chemistry Vol. 21, No. 2 (2009), 1024-1028

Comparison of Antioxidant Phenolics of Ethanolic Extracts and Aqueous Infusions from *Sideritis* Species

GÜLCAN ÖZKAN
Department of Food Engineering, Faculty of Engineering and Architecture
Suleyman Demirel University, 32260 Isparta, Turkey
Fax: (90)(246)2111538; E-mail: gozkan@mmf.sdu.edu.tr

Water and ethanol as solvents for the manufacture of foodstuffs or food ingredients is permissible and it could be extraction flavourings from natural flavouring materials safely. In this study, comparison of phenolic composition of ethanolic extracts and aqueous infusions from *Sideritis caesarea* Duman, Aytaç et Baser, *Sideritis condensata* Boiss. & Heldr., *Sideritis ozturkii* Aytac & Aksoy, *Sideritis perfoliata* Linnaeus and *Sideritis pisidica* Boiss. & Heldr. were investigated. The yields of water extracts (10.56-22.12 %) were higher than yields of ethanol extract (3.52-7.98 %). Eleven phenolic compounds in ethanolic extract and ten phenolic compounds in aqueous infusions were identified by HPLC. Naringin was the major compound for both extracts and higher in ethanol extracts (23.87-222.90 mg/100 g dried herb); while gallic acid, apigenin-7-O-glucoside and eriodictiol were determined in only ethanol extracts, (+)-catechin and vitexin were in only water extracts. As a result, ethanol had higher performance for extracting phenolics but aqueous infusions more effective in extract yield.

Key Words: *Sideritis* spp., Phenolic composition, Ethanol extract, Aqueous infusion.

INTRODUCTION

The genus *Sideritis* (Lamiaceae) is represented by more than 150 species which are distributed especially in Mediterranean-Macronesian region[1] and this genus has 46 species in Turkey[2,3]. These are well known as herbal tea and locally named dag cayi[4]. Leaves, flowers and stem of *Sideritis* species have been also used as folk remedies to treat various ailments such as gastrointestinal ailments and common colds[4,5].

In previous studies, *Sideritis* species growing in Turkey had rich in essential oils[6-10], flavonoids and phenylethanoid glycosides[9-13] were reported. In addition to phenylethanoid glycosides and flavonoids which were isolated from various *Sideritis* species and investigated for their effects on gastric ulceration and antiinflammatory activity[13], iridoids and essential oils were investigated for their antimicrobial activity[14]. It was also confirmed diuretic, antiinflammatory[15], antispasmodic[16], antibacterial[17] and antioxidant activities[18] of different extracts from several *Sideritis* species growing in Turkey.

The present study was conducted to investigate the comparison of phenolic composition of ethanolic extracts and aqueous infusions from *Sideritis caesarea* Duman, Aytaç & Baser, *Sideritis condensata* Boiss. & Heldr., *Sideritis ozturkii* Aytac & Aksoy, *Sideritis perfoliata* Linnaeus and *Sideritis pisidica* Boiss. & Heldr.

EXPERIMENTAL

Sideritis caesarea Duman, Aytaç et Baser, *Sideritis condensata* Boiss. & Heldr., *Sideritis ozturkii* Aytac & Aksoy, *Sideritis perfoliata* Linnaeus and *Sideritis pisidica* Boiss. & Heldr., which were collected in the August. Herbarium specimens were deposited at the Department of Biology, Faculty of Science and Education, University of Suleyman Demirel, Isparta, Turkey.

Preparation of the ethanolic extracts and aqueous infusions: An amount of 1.5 g of dried leaves, flowers and stem of the herbs were ground to pass through 0.4 mm sieve and extracted with ethanol:acetic acid mixture (100 mL:100 µL) and boiling water:acetic acid mixture (100 mL:100 µL) with a magnetic stirrer (700 rpm) for 15 min. And then 10 mL sample in a test tube was centrifuged (Labofuge 400R, Heraeus Instruments, Germany) at 4500 rpm for 5 min. The supernatant was collected and before the analysis, the 2 mL extracts were filtered through filters (0.45 µm, membraPure, Bodenheim, Germany).

Analysis of phenolic constituents: The procedure for quantitation of the phenolic compounds has been described by Caponio *et al.*[19]. The reversed phase-high performance liquid chromatography (RP-HPLC) was used. Detection and quantification was carried out with a SCL-10 Avp System controller, a SIL-10AD vp Autosampler, a LC-10AD vp pump, a DGU-14a degasser, a CTO-10 A vp column heater and a diode array detector set at 278 nm. The 250 mm × 4.6 mm i.d. C18 column used was filled with Agilent Eclipse XDB C-18 (250 mm × 4.6 mm), 5 µ. The flow rate was 0.8 mL/min, injection volume was 10 mL and the column temperature was set at 30 ºC. Gradient elution of two solvents was used: solvent A consisted of acetic acid-water (2:98, v/v), solvent B: methanol and the gradient programme used is given Table-1. The data were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software System (Chiyoda-ku, Tokyo, Japan). The extract samples, standard solutions and mobile phases were filtered by a 0.45 µm pore size membrane filter (Vivascience AG, Hannover, Germany). The amount of phenolic compounds in the extract was calculated as mg 100 g^{-1} herb using external calibration curves, constructed for each phenolic standard.

TABLE-1
SOLVENT GRADIENT CONDITIONS WITH LINEAR GRADIENT

Final time	3	20	28	35	45	60	62	70	75	80
A %	95	75	72	70	65	63	55	50	20	0
B %	5	25	28	30	35	37	45	50	80	100

A (solvent) = Acetic acid:water (2:98 v/v), B (solvent) = Methanol.

Statistical analysis: Results of the research were tested for statistical significance by one-way ANOVA. Differences were considered statistically significant at the $p \leq 0.01$ level.

RESULTS AND DISCUSSION

The comparison of phenolic composition from *S. caesarea, S. condensata, S. ozturkii, S. perfoliata* and *S. pisidica* ethanolic extracts and aqueous infusions were investigated in the study and their yields, phenolic acid and flavonoid compositions presented as detailed (Table-2). There were statistically significant differences among yields, solvents using extraction and species at the $p \leq 0.01$ level.

TABLE-2
YIELDS, PHENOLIC ACID AND FLAVONOID COMPOSITION OF ETHANOLIC EXTRACTS AND AQUEOUS INFUSIONS FROM *S. pisidica, S. condensata, S. ozturkii, S. caesarea* AND *S. perfoliata* (mg/100 g dried herb)

Phenolic compounds	Ethanol extracts				
	S. pisidica	*S. condensata*	*S. ozturkii*	*S. caesarea*	*S. perfoliata*
Gallic acid	3.30±0.10c	4.37±0.03b	4.37±0.03b	5.87±0.00a	1.97±0.03d
(+)-Catechin	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c
Caffeic acid	3.83±0.03b	0.67±0.00f	1.40±0.00d	2.57±0.23c	7.30±0.03a
(-)-Epicatecin	3.80±0.27e	7.00±0.13d	82.70±0.23a	2.57±0.30f	18.27±0.07b
Vitexin	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c
Rutin	10.87±0.27b	7.03±0.30c	6.63±0.03d	3.50±0.10ef	12.10±0.43a
Naringin	49.97±0.77d	42.40±1.07e	23.87±0.60g	222.90±5.77a	76.33±3.07b
Apigenin-7-O-glucosid	0.00±0.00b	2.37±0.03a	0.00±0.00b	0.00±0.00b	0.00±0.00b
Rosmarinic acid	0.00±0.00e	25.87±1.53b	0.00±0.00e	48.73±0.60a	0.00±0.00e
Eriodictiol	2.30±0.03a	0.00±0.00b	0.00±0.00b	3.07±1.33a	2.30±2.30a
Quercetin	1.80±0.07b	0.00±0.00c	1.43±0.03b	0.00±0.00c	0.00±0.00c
Naringenin	1.70±0.10b	1.40±0.00b	0.00±0.00c	3.33±0.07a	2.67±0.07ab
Luteolin	4.57±0.03b	3.27±0.07c	2.70±0.10c	4.77±0.17b	4.47±0.13b
Yield (%)	7.79±0.00e	6.53±0.00ef	3.52±0.00g	7.98±0.00e	5.93±0.00f
	Aqueous infusions				
Gallic acid	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00±0.00e
(+)-Catechin	3.17±0.37b	5.03±1.03a	0.00±0.00c	4.77±0.90a	2.60±0.27b
Caffeic acid	0.93±0.07e	0.40±0.07g	0.00±0.00h	0.73±0.00f	1.43±0.17d
(-)-Epicatecin	0.00±0.00g	2.00±0.13f	10.57±0.03c	0.00±0.00g	3.03±0.17e
Vitexin	12.33±0.00b	49.70±0.50a	0.00±0.00c	0.00±0.00c	0.00±0.00c
Rutin	3.47±0.13ef	1.40±0.07g	2.43±0.03f	3.50±0.03ef	4.03±0.10e
Naringin	18.63±0.57h	15.33±0.60h	6.40±0.27j	35.20±0.40f	71.00±0.80c
Apigenin-7-O-glucosid	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b
Rosmarinic acid	0.00±0.00e	6.60±0.07d	0.00±0.00e	17.70±0.63c	0.00±0.00e
Eriodictiol	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b
Quercetin	3.23±0.10a	0.00±0.00c	3.83±0.10a	0.00±0.00c	0.00±0.00c
Naringenin	1.50±0.03b	0.90±0.03bc	1.07±0.13b	1.03±0.03b	1.20±0.07b
Luteolin	7.93±0.13a	8.03±0.10a	0.00±0.00d	0.00±0.00d	0.00±0.00d
Yield (%)	22.12±0.00a	14.36±0.00c	18.94±0.00b	10.56±0.00d	18.10±0.00b

[1]Differences between columns indicated by the same letters are not statistically significant (Duncan's multiple range test, $p \leq 0.01$).

Aqueous infusions yields were found higher ethanol extract yields. Yields (%) of *S. pisidica*, *S. condensata*, *S. ozturkii*, *S. caesarea* and *S. perfoliata* were 7.79, 6.53, 3.52, 7.98 and 5.93 for ethanol extracts and 22.12, 14.36, 18.94, 10.56 and 18.10, respectively. Findings in this study supported the observations of some other researchers. Marinova and Yanishlieva[20], Dapkevicius *et al.*[21] and Durling *et al.*[22] reported that different solvents using extraction of phenolics effected on extract yields.

Naringin was the most abundant phenolic and it was higher in ethanol extracts. Ethanol extracts of *S. pisidica, S. condensata, S. ozturkii, S. caesarea* and *S. perfoliata* had naringin as 49.97, 42.40, 23.87, 222.90 and 76.33 mg/100 g dried herbs, respectively. However, naringin in aqueous infusions was between 6.40 and 71.00 mg/100g dried herbs. (-)-Epicatecin, vitexin and rosmarinic acid were followed naringin. (-)-Epicatecin was determined as 2.57-82.70 mg/100 g dried herbs in all ethanol extracts, but it was found only in aqueous infusions of *S. condensata, S. ozturkii* and *S. perfoliata* as between 2.00-10.57 mg/100 g dried herbs. Vitexin was 49.70 and 12.33 mg/100 g dried herbs in aqueous infusions of *S. pisidica* and *S. condensata*. Rosmarinic acid was also the highest in ethanol extract of *S. caesarea* (48.73 mg/100 g dried herb) and followed it *S. condensata* ethanol extract and aqueous infusions of *S. caesarea* and *S. condensata*. Similarly, some researchers reported that there are significantly important differences phenolic composition of herbs according to using solvents and solvent mixures[23-27].

Gallic acid, (+)-catechin, caffeic acid, rutin, apigenin-7-O-glucoside, eriodictiol, quercetin, naringenin and luteolin were minor components of *Sideritis* species. (+)-Catechin, vitexin were not found in all ethanol extracts of *Sideritis* species and, gallic acid, apigenin-7-O-glucoside and eriodictiol were not in all aqueous infusions. Apigenin-7-O-glucoside were detected in only ethanol extract of *S. condensata*. Ozturkoside A, B and C were isolated from the active phenolic fraction of *S. ozturkii*[9,28]. However, there are no data of phenolic composition of *S. pisidica, S. condensata, S. caesarea* and *S. perfoliata* and only a few sets of data on the flavonoids of other *Sideritis* species. Similarly, luteolin and apigenine were found in extracts of other *Sideritis* species[29]. Fiamegos *et al.*[30] were reported that GC-MS analysis of herbal aqueous infusions of *Sideritis cretica* (μg/mL) had ferulic acid (4.878), *p*-hydroxy benzoic acid (2.791), syringic acid (1.186), 4-hydroxy cinnamic acid (1.004), homovanillic acid (0.847), *trans*-cinnamic acid (0.615) and vanillic acid (0.040).

Based on these results, it is possible to conclude that ethanol extracts of *Sideritis* species had higher amount of phenolics as compared to aqueous infusions, but the aqueous infusions had higher extract yields. Ethanol could be prefering extraction solvent of flavourings from *Sideritis* species using food ingredients.

REFERENCES

1. C. Obon de Castro and D. Rivera-Nunez, in ed.: J. Cramer, A toxonomic Revision of the Section *Sideritis* (Genus *Sideritis*), Berlin-Stuttgart (1994).
2. H. Duman, in: Flora of Turkey and the East Aegean Islands, University Press, Edinburgh, pp. 201-204 (2000).
3. Z. Aytaç and A. Aksoy, *Flora Mediterranean*, **10**, 181 (2000).

4. T. Baytop, Therapy with Medicinal Plants, Past and Present, Nobel Tip Publications, Istanbul, p. 193 (1999).
5. E. Yesilada, G. Honda, E. Sezik, M. Tabata, T. Fujita, T. Tanaka, Y. Takeda and Y. Takaishi, *J. Ethnopharmacol.*, **46**, 133 (1995).
6. N. Ezer, R. Vila, S. Cañigueral and T. Adzet, *Phytochemistry*, **41**, 203 (1996).
7. K.H.C. Baser, *Pure Appl. Chem.*, **74**, 527 (2002).
8. G. Topcu, A.C. Goren, T. Kilic, Y.K. Yildiz and G. Tumen, *Nat. Prod. Lett.*, **16**, 33 (2002).
9. F.P. Sahin, N. Ezer and I. Çalis, *Phytochemistry*, **65**, 2095 (2004).
10. F.P. Sahin, D. Tasdemir, P. Rüedi, N. Ezer and I. Calis, *Phytochemistry*, **66**, 125 (2005).
11. N. Ezer, M.K. Sakar, B. Rodriguez and M.C. De la Torre, *Int. J. Pharmacogn.*, **30**, 61 (1992).
12. N. Ezer and Y. Akcos, *H.U. J. Fac. Pharm.*, **15**, 81 (1995).
13. Y. Akcos, N. Ezer, I. Çalis, R. Demirdamar and B.C. Tel, *Pharm. Biol.*, **36**, 1 (1999).
14. Y. Akcos, N. Ezer, B. Özcelik and U. Abbasoglu, *FABAD J. Pharm. Sci.*, **23**, 99 (1998).
15. E. Yesilada and N. Ezer, *Int. J. Crude Drug Res.*, **27**, 38 (1989).
16. N. Ezer, E. Sezik, K. Erol, K. Erol and M. Özdemir, in: K.H.C. Baser, Proceedings of the 9th Symposium on the Plant Drugs Eskisehir, 16-19 May 1991, Proceeding Book, Anadolu University Publications, pp. 83-93 (1992).
17. N. Ezer, G. Usluer, I. Günes and K. Erol, *Fitoterapia*, **65**, 549 (1994).
18. A. Güvenc, P.J. Houghton, H. Duman, M. Coskun and P. Sahin, *Pharm. Biol.*, **43**, 173 (2005).
19. F. Caponio, V. Alloggio and T. Gomes, *Food Chem.*, **64**, 203 (1999).
20. E.M. Marinova and N.V. Yanishlieva, *Food Chem.*, **58**, 245 (1997).
21. A. Dapkevicius, R. Venskutonis, T.A. van Beek and J.P.H. Linssen, *J. Sci. Food Agric.*, **77**, 140 (1998).
22. N.E. Durling, O.J. Catchpole, J.B. Grey, R.F. Webby, K.A. Mitchell, L.Y. Foo and N.B. Perry, *Food Chem.*, **101**, 1417 (2007).
23. U. Justesen and P. Knuthsen, *Food Chem.*, **73**, 245 (2001).
24. V. Exarchou, N. Nenadis, M. Tsimidou, I.P. Gerothanassis, A. Troganis and D.Boskou, *J. Agric. Food Chem.*, **50**, 5294 (2002).
25. C.T. Matsingou, N. Petrakis, M. Kapsokefalou and A. Salifoglou, *J. Agric. Food Chem.*, **51**, 6696 (2003).
26. M. Kosar, H.J.D. Dorman and R. Hiltunen, *Food Chem.*, **91**, 525 (2005).
27. M. Skerget, P. Kotnik, M. Hadolin, A.R. Hras, M. Simonic and Z. Knez, *Food Chem.*, **89**, 191 (2005).
28. E. Kupeli, F.P. Sahin, I. Çalis, E. Yesilada and N. Ezer, *J. Ethnopharmacol.*, **112**, 356 (2007).
29. O.M. Palomino, P. Gomez-Serranillos, E. Carretero and A. Villar, *J. Chromatogr. A*, **731**, 103 (1996).
30. Y.C. Fiamegos, C.G. Nanos, J. Vervoort and C.D. Stalikas, *J. Chromatogr. A*, **1041**, 11 (2004).

(*Received*: 1 December 2007; *Accepted*: 19 September 2008) AJC-6870