Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Chemica Sinica, 2018, 9(1):599-604

ISSN : 0976-8505
CODEN (USA): CSHIA5

Analytical Control and Antioxidative Activity of Different Teas in Serbia

Vesna Veličković[1], Jelena M Mašković[2]*, Pavle Z Mašković[2]

[1]University of Kragujevac, Faculty of Science, Department of Chemistry, Radoja Domanovića 12, 34000 Kragujevac, Serbia
[2]University of Kragujevac, Faculty of Agronomy, Department of Chemistry and Chemical Engineering, Cara Dušana 34, 32000 Čačak, Serbia

ABSTRACT

Satureja montana, Ocimum basilicum, Camellia sinesis, Saliva officinalis, Althaea officinalis, Thymus camphoratus, Rosmarinus officinalis, Mentha piperita and Mountain tea were investigated for their quality (ash and moisture contents), as well as for chemical profile. HPLC analysis was applied to determine saccharide, vitamin C and caffeine contents in all teas samples. Obtained results demonstrated high quality of all tea samples (moisture of all samples was below 12%), while chemical analysis revealed presence of glucose, fructose, sucrose, vitamin C and caffeine in tested materials. Exception was Ocimum basilicum, where only glucose was detected and quantified. The results of total antioxidant acticity showed that tea of Althaea officinalis have the highest content (130.72 ± 0.54 mg AA/G) and the lowest content have Satureja montana (106.05 ± 0.40 mg AA/G). The highest content of polyphenol was detected in Satureja montana.

Keywords: Teas samples, Quality saccharide content, Caffeine content, Vitamin C content, Antioxidant activity, Polyphenol

INTRODUCTION

The plants with the antioxidant activity have occupied attention in recent years due to the development of a large number of different diseases [1, 2]. There are a number of natural antioxidants, e.g. α-tocopherol [3]; ascorbic acid [4 5]; retinol, thiamin and riboflavin, flavonoids [6] and phenolic acids [3,7], as well as a number of synthetic antioxidants [8]. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have a potentially harmful effect on human health, leading to various dysfunctions [8]. One of the most important trends in the food and pharmaceutical industry today is searching for natural antioxidants from the plant material. Phenolic compounds, important constituents in many plants, have received considerable attention as potentially protective factors against cancer and heart diseases because of their antioxidant potency and their ubiquity in a wide range of commonly consumed foods of plants origin [9].

Different herbs have been used in human diet as a source of compounds which are highly beneficial for health [10,11]. From such herbs, remedies are prepared and further used for the treatment of different types of infections, aliments and diseases [12]. Remedies may be prepared using almost every part of the plant: dry and fresh fruits, leaves, flowers, seeds, stems, etc., by pouring them with boiling water and left 5-10 minutes to steep [13]. Herbal infusions have been also considered as a valuable source of antioxidant species [14]. Antioxidants are capable of stabilizing, or deactivating free radicals before the latter attack cells and biological targets. They are therefore critical for maintaining optimal cellular and systemic health and well-being [15,16]. Today tea is used as a beverage and as a source of health benefit compounds. Due to the awareness of health benefit compounds, consumption of tea is becoming more and more popular in all world countries, including Serbia [11]. Many therapeutic properties such as neuroprotective, cardioprotective, chemoprotective, anticarcinogenic, hepatoprotective and anti-inflammatory have been attributed to herbal preparations [17,18], including teas. In Serbia, the production of teas and other herbal products have been increased at a rate of 10% annually [11].

Such importance of herbal beverages on human health put them in the spotlight of scientific community these days. For such reasons, aim of this study was to estimate chemical profile and quality of different teas from Serbia, as well

as to investigate and compare their biological activity. To accomplish this goal, modern and comprehensive analytical techniques were applied to determine saccharides, vitamin C and caffeine contents in teas. Quality parameters of plants material were ash and moisture contents.

MATERIALS AND METHODS

Plant samples

Satureja montana, *Ocimum basilicum*, *Camellia sinesis*, *Saliva officinalis*, *Althaea officinalis*, *Thymus camphoratus*, *Rosmarinus officinalis*, *Mentha piperita* and Mountain tea (mixture of several herbs) were collected in November 2015, in area of Čačak, Republic of Serbia. Collected material was dried naturally in the shade on draft for one month. Dried plants were grounded in the blender and kept in the paper bags prior the usage.

Determination of ash and moisture contents

Ash and moisture contents were determined using previously described standard methods (Ph.Jug.IV, 1987).

Determination of saccharides content

Determination of saccharides content was performed using Varian liquid chromatograph coupled with RI detector. Column was Zorbax Carbohydrate (150 mm x 4.6 mm), flow was 1.4 mL/min, mobile phase was mixture of acetonitrile and water (80:20, V/V), and column temperature was 40°C, while injected volume was 5.0 μL.

Determination of caffeine content

Determination of saccharides content was performed using Varian liquid chromatograph coupled with DAD detector. Column was Zorbax Carbohydrate (150 mm x 4.6 mm), flow was 1.0 mL/min, mobile phase was mixture of acetonitrile and KH_2PO_4 (20:80, V/V; pH 3.5), column temperature was 30°C, while injected volume was 5.0 μL.

Determination of vitamin C content

Determination of saccharides content was performed using Varian liquid chromatograph coupled with DAD detector. Column was RP Chrompack C18 (150 mm × 4.6 mm), flow was 1.0 mL/min, mobile phase was mixture of acetonitrile and KH_2PO_4 (20:80, V/V; pH 3.5), column temperature was 30°C, while injected volume was 5.0 μL.

Determination of total phenolics and flavonoids contents

Total phenolics (TPC) and total flavonoids (TFC) contents were determined using the previously described methods [19, 20]. Final contents for TPC and TFC were expressed as milligrams of gallic acid equivalents per g of dry extract (mg GAE/g), while result for TFC were expressed as milligrams of rutin equivalents per g of dry extract (mg RU/g).

Determination of condensed tannins and gallotannins

Condensed tannins (CT) were determined according to previously described method which relies on the precipitation of proanthocyanidins with formaldehyde, while gallotannins (GA) were determined using the described potassium iodate assay [21]. Both contents were expressed as gallic acid equivalents.

Determination of anthocyanins

Anthocyanins were determined according the previously described procedure [22,23] using pH single and differential methods. Total anthocyanins content (TAC) was expressed as cyanidin-3-glucoside per g of dry extract (mg C_3G/g).

Determination of antioxidant activity

Antioxidant activity of obtained extracts was determined using following, previously described assays: total antioxidant capacity [24], lipid peroxidation assay, hydroxyl radical scavenging activity [25] and DPPH radical scavenging activity [26] with slight modification [27]. Total antioxidant capacity (TA) was expressed as milligrams of ascorbic acid per gram of dry extract (mg AA/g). Results for lipid peroxidation assay are expressed as ILP50 in mg/mL, for hydroxyl radical scavenging activity as OH50 in mg/mL and for DPPH as IC50 in mg/mL.

RESULTS AND DISCUSSION

Quality parameters of investigated plants: *Satureja montana*, *Ocimum basilicum*, *Camellia sinesis*, *Saliva officinalis*,

Althaea officinalis, *Thymus camphoratus*, *Rosmarinus officinalis*, *Mentha piperita* and Mountain tea (mixture of several herbs) are presented in **Table 1**. General requirement for moisture content is below 12%. Regarding this requirement, presented results showed that all samples fulfil it and is of good quality. Highest moisture content was noticed in *Saliva officinalis* and Mountain tea (10.20%), while the lowest was in *Ocimum basilicum* sample (7.60%).

Table 1: Ash and moisture contents in investigated teas.

Plant material	Ash content (%)	Moisture content (%)
Satureja montana	13.65	8.7
Ocimum basilicum	27.57	7.6
Camellia sinesis	9.35	8.9
Saliva officinalis	16.23	10.2
Althaea officinalis	9.27	8.9
Thymus camphoratus	17.38	8.3
Rosmarinus officinalis	17.16	9.3
Mentha piperita	15.84	9.2
Mountain tea	10.48	10.2

Results for ash content revealed the highest percentage in *Ocimum basilicum* sample (27.57%). On the other hand, the lowest ash content was noticed in the *Althaea officinalis* sample (9.27%), followed by *Camellia sinesis* sample (9.35%). *Thymus camphoratus* and *Rosmarinus officinalis* showed similar results (17.38% and 17.16%, respectively).

Saccharides composition in the investigated teas is presented in **Table 2**. Presented results did not show that fructose nor was sucrose not detected in *Ocimum basilicum* sample. The highest content of glucose was notice in *Camellia sinesis* sample (58.00 g/L), while lowest was in the *Mentha piperita* sample (21.00 g/L). In the case of fructose, the highest content was notice in *Rosmarinus officinalis* sample (91.00 g/L), while lowest was in the *Althaea officinalis* sample (11.00 g/L).

Table 2: Saccharides contents in investigated teas.

Plant material	Glucose (g/L)	Fructose (g/L)	Sucrose (g/L)
Satureja montana	36	34	40
Ocimum basilicum	35	-	-
Camellia sinesis	58	44	114
Saliva officinalis	49	28	29
Althaea officinalis	22	11	206
Thymus camphoratus	45	36	48
Rosmarinus officinalis	60	91	20
Mentha piperita	21	57	47
Mountain tea	22	34	57

Sucrose was the dominant saccharide in the most cases with the exception of *Saliva officinalis*, *Rosmarinus officinalis* and *Mentha piperita*. The highest content of sucrose was reported in *Althaea officinalis* (206.00 g/L), followed by *Camellia sinesis* (114.00 g/L), while all other contents were far lower. The lowest content of this saccharide was noticed in *Rosmarinus officinalis* sample (20.00 g/L) followed by *Saliva officinalis* sample (29.00 g/L).

Vitamin C and caffeine contents are presented in **Table 3**. Results showed that *Camellia sinesis* was the richest plant with the vitamin C (2.20 g/L), while *Thymus camphoratus*, with 0.26 g/L, was the poorest plant with this essential vitamin. Vitamin C is well known by its antioxidant activity, while lack of this molecule in organism leas to various disorders and diseases.

Table 3: Vitamin C and caffeine contents in investigated teas.

Plant material	Vitamin C (g/L)	Caffeine (g/L)
Satureja montana	0.9	17.5
Ocimum basilicum	1.1	0.49
Camellia sinesis	2.2	41
Saliva officinalis	0.56	0.33
Althaea officinalis	0.35	0.03

Thymus camphoratus	0.26	17.6
Rosmarinus officinalis	0.38	1.43
Mentha piperita	0.55	14.3
Mountain tea	0.67	16.7

Caffeine is alkaloid compound, and is well known by its application in Coca Cola and other drinks, as well as the main compound of coffee. It affects the hearth rate and pulse, as well as blood pressure, and is widely used against somnolence. Result of HLC analysis showed that *Camellia sinesis* possessed the highest amount of this compound (41.00 mg/L), followed by *Thymus camphoratus* (17.60 g/L), *Satureja montana* (17.50 g/L), Mountain tea (16.70 g/L) and *Mentha piperita* (14.30 g/L). On the other hand, the lowest content of caffeine was notice in *Althaea officinalis* (0.03 g/L).

The results for TPC, TFC, CT, GA and TAC obtained using spectrophotometric assays are presented in **Table 4**. According to the results, the highest contents of total phenolic content were detected in tea Satureja montana (93.07 ± 0.48 mg GAE/g), and the lowest one was in tea of *Ocimum basilicum* (90.60 ± 0.40 mg GAE/g). Results of total flavonoids content showed that tea of *Camellia sinesis* is the richest in these compounds (21.72 ± 0.48 mg RU/g). Results of total flavonoids content showed that tea of *Camellia sinesis* is the richest in these compounds (21.72 ± 0.48 mg RU/g). The tea of *Thymus camphoratus* showed the highest content of condensed tannins, while *Ocimum basilicum* had the lowest content. The highes total anthocyanins content were detected in tea of *Althaea officinalis* (46.94 ± 0.83 mg C3G/g) and the lowest in *Saliva officinalis* (41.11 ± 0.42 mg C3G/g). The highest content of gallotannins is present in tea of *Rosmarinus officinalis* (37.60 ± 0.54 mgGAE/mL).

Table 4: Chemical profile of teas extracts obtained by spectrophotometric assays.

Extract	TPC (mg GAE/g)vece	TFC (mg RU/g)	CT (mg GAE/g)	GA (mg GAE/g)	TAC (mg C3G/g)
Satureja montana	98.07 ± 0.48	20.80 ± 0.40	23.59 ± 0.34	33.36 ± 0.77	44.33 ± 0.43
Ocimum basilicum	90.60 ± 0.40	16.11 ± 0.12	20.35 ± 0.20	32.19 ± 0.55	46.29 ± 0.39
Camellia sinesis	94.18 ± 0.77	21.72 ± 0.48	19.39 ± 0.39	35.16 ± 0.74	45.86 ± 0.46
Saliva officinalis	93.50 ± 0.42	15.96 ± 0.29	27.86 ± 0.59	35.71 ± 0.75	41.11 ± 0.42
Althaea officinalis	97.38 ± 0.78	15.52 ± 0.43	28.77 ± 0.42	36.96 ± 0.38	46.94 ± 0.83
Thymus camphoratus	95.58 ± 0.71	16.23 ± 0.14	29.88 ± 0.49	36.39 ± 0.22	41.10 ± 0.27
Rosmarinus officinalis	98.34 ± 0.26	17.11 ± 0.89	28.14 ± 0.13	37.60 ± 0.54	42.25 ± 0.73
Menttha piperita	93.48 ± 0.46	18.09 ± 0.55	26.58 ± 0.29	32.33 ± 0.08	44.39 ± 0.43
Mountain tea	94.14 ± 1.10	18.73 ± 0.32	26.16 ± 0.88	33.17 ± 0.73	42.59 ± 0.62

The results of antioxidant activities in obtained teas are present in **Table 5**. The results of total antioxidant capacity showed that tea of *Althaea officinalis* have the highest content (130.72 ± 0.54 mg AA/G) and the lowest content have *Satureja montana* (106.05 ± 0.40 mg AA/G). The highest content of polyphenol has *Satureja montana* (20.80 ± 0.40 mg RU/mL). Tea of *Ocimum basilicum* has the nighest content of lipid peroxidation (29.95 ± 0.50 mg/mL). Hydroxyl radical scavenging activity is the most powerfull in tea *Ocimum basilicum* (48.41 ± 0.76 mg/mL), while the tea of *Saliva officinalis* has it in lowest content (40.15 ± 0.93 mg/mL). Antioxidant activity determinated as DPPH radicals show that tea of Salvia officinalis has the most powerful scavenging activity (58.33 ± 0.18 mg/mL).

Table 5: Antioxidant activity of obtained teas extracts.

Extract	TA (mg AA/G)	ILP50 (mg/mL)	OH50 (mg/mL)	IC50 (mg/mL)
Satureja montana	106.05 ± 0.40	33.55 ± 0.44	43.51 ± 0.19	53.18 ± 0.19
Ocimum basilicum	118.63 ± 0.73	29.95 ± 0.50	49.41 ± 0.76	52.54 ± 0.43
Camellia sinesis	124.56 ± 0.19	34.44 ± 0.39	44.38 ± 0.29	50.54 ± 0.73
Saliva officinalis	130.72 ± 0.54	33.55 ± 0.23	40.15 ± 0.93	58.33 ± 0.18
Althaea officinalis	131.32 ± 0.28	30.56 ± 0.81	49.14 ± 0.18	51.06 ± 0.52
Thymus camphoratus	106.81 ± 0.40	34.23 ± 0.63	41.69 ± 0.78	50.17 ± 0.38
Rosmarinus officinalis	118.63 ± 0.73	43.82 ± 0.75	46.48 ± 0.18	54.66 ± 0.29
Mentha piperita	119.24 ± 0.06	32.55 ± 0.30	47.54 ± 0.18	53.20 ± 0.30
Mountain tea	120.43 ± 0.33	31.15 ± 0.83	45.76 ± 0.31	50.76 ± 0.43

CONCLUSION

Satureja montana, *Ocimum basilicum*, *Camellia sinesis*, *Saliva officinalis*, *Althaea officinalis*, *Thymus camphoratus*, *Rosmarinus officinalis*, *Mentha piperita* and Mountain tea were investigated regarding their quality and chemical profile. Obtained results demonstrated high quality of tested plants, which is important data for the market in Serbia and abroad. Investigation of chemical composition showed the presence of vitamin C and caffeine in all teas. Saccharide analysis proved existence of glucose, fructose and sucrose in all teas, with the exception of *Ocimum basilicum*, which lacked in fructose and sucrose. Presence of vitamin C is especially valuable due to its significant effect on human health, while caffeine justifies application of some teas instead of coffee. The scavenging activity is very good for all assay teas. The highest content of gallotannins is present in tea of *Rosmarinus officinali.*

REFERENCES

[1] Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, et al. (**2003**) Antioxidant and antimicrobial activity of the essential oil and methanol extracts of Achillea millefolium subsp. millefolium Afan.(Asteraceae). *Journal of ethnopharmacology* 87: 215-220.

[2] Zhu KX, Lian CX, Guo XN, Peng W, Zhou HM (**2011**) Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ. *Food Chemistry* 126: 1122-1126.

[3] Fukumoto LR, Mazza G (**2000**) Assessing antioxidant and prooxidant activities of phenolic compounds. *Journal of agricultural and food chemistry* 48: 3597-3604.

[4] Kitts DD (**1997**) An evaluation of the multiple effects of the antioxidant vitamins. *Trends in Food Science & Technology* 8: 198-203.

[5] Wall J (**2000**) Antioxidants in prevention of reperfusion damage of vascular endothelium. *TSMJ* 1: 67.

[6] Aquino R, Morelli S, Tomaino A, Pellegrino M, Saija A, et al. (**2002**) Antioxidant and photoprotective activity of a crude extract of Culcitium reflexum HBK leaves and their major flavonoids. *Journal of ethnopharmacology*, 79: 183-191.

[7] Silva BA, Malva JO, Dias AC (**2008**) St. John's Wort (Hypericum perforatum) extracts and isolated phenolic compounds are effective antioxidants in several in vitro models of oxidative stress. *Food Chemistry* 110: 611-619.

[8] Ito N, Fukushima S, Tsuda H (**1985**) Carcinogenicity and modification of the carcinogenic response by BHA, BHT, and other antioxidants. *CRC Critical Reviews in Toxicology* 15: 109-150.

[9] Rice-Evans C (**2001**) Flavonoid antioxidants. *Current medicinal chemistry* 8: 797-807.

[10] Čestić S, Radojković M, Cvetanović A, Mašković P, Đurović S (**2016**) Influence of steeping time on biological activity of black mulberry tea. *Acta Periodica Technologica* 47: 177-191.

[11] Vidović S, Cvetkovic D, Ramić M, Dunjić M, Malbaša R, et al. (**2013**) Screening of changes in content of health benefit compounds, antioxidant activity and microbiological status of medicinal plants during the production of herbal filter tea. *Industrial crops and products* 50: 338-345.

[12] Čestić S, Radojković M, Cvetanović A, Mašković P, Đurović S (2016) Phytochemical profile and biological potential of mulberry teas (Morus nigra L.). *Acta Agriculturae Serbica* 21: 25-35.

[13] Piljac-Žegarac J, Šamec D, Piljac A (**2013**) Herbal teas: A focus on antioxidant properties. In *Tea in Health and Disease Prevention* 129-140.

[14] Alarcón E, Campos AM, Edwards AM, Lissi E, López-Alarcón C (**2008**) Antioxidant capacity of herbal infusions and tea extracts: A comparison of ORAC-fluorescein and ORAC-pyrogallol red methodologies. *Food Chemistry* 107: 1114-1119.

[15] Percival M (**1998**) Antioxidants. Clinical Nutrition Insight 31: 1-4.

[16] Atoui AK, Mansouri A, Boskou G, Kefalas P (**2005**) Tea and herbal infusions: their antioxidant activity and phenolic profile. *Food chemistry* 89: 27-36.

[17] Campanella L, Bonanni A, Tomassetti M (**2003**) Determination of the antioxidant capacity of samples of different types of tea, or of beverages based on tea or other herbal products, using a superoxide dismutase biosensor. *Journal of pharmaceutical and biomedical analysis* 32: 725-736.

[18] Visioli F, Borsani L, Galli C (**2000**) Diet and prevention of coronary heart disease: the potential role of phytochemicals. *Cardiovascular Research* 47: 419-425.

[19] Brighente IMC, Dias M, Verdi LG, Pizzolatti MG (**2007**) Antioxidant activity and total phenolic content of some Brazilian species. *Pharmaceutical Biology* 45: 156-161.

[20] Singleton VL, Rossi JA (**1965**) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture* 16: 144-158.

[21] Wilfred V, Nicholson R (**2006**) Phenolic compound biochemistry. *Springer.*

[22] Cheng GW, Breen PJ (**1991**) Activity of phenylalanine ammonia-lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. *Journal of the American Society for Horticultural Science*, 116: 865-869.

[23] Vulić JJ, Tumbas VT, Savatović SM, Đilas SM, Ćetković GS, et al. (**2011**) Polyphenolic content and antioxidant activity of the four berry fruits pomace extracts. *Acta periodica technologica* 42: 271-279.

[24] Prieto P, Pineda M, Aguilar M (**1999**) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry* 269: 337-341.

[25] Hinneburg I, Dorman HD, Hiltunen R (**2006**) Antioxidant activities of extracts from selected culinary herbs and spices. *Food chemistry* 97: 122-129.

[26] Takao T, Kitatani F, Watanabe N, Yagi A, Sakata K (**1994**) A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. *Bioscience, Biotechnology, and Biochemistry*, 58: 1780-1783.

[27] Kumarasamy Y, Byres M, Cox PJ, Jaspars M, Nahar L, et al. (**2007**) Screening seeds of some Scottish plants for free radical scavenging activity. *Phytotherapy Research* 21: 615-621.