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ORIGINAL PAPER

THE CONTENT OF MINERALS, BIOACTIVE COMPOUNDS AND ANTI-NUTRITIONAL FACTORS IN TEA INFUSIONS

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Abstract

Tea infusions are a valuable source of bioactive compounds. They are also a source of minerals, but anti-nutritional factors present in the leaves of *Camellia sinensis*, such as soluble oxalates, limit their availability. Tea leaves, in particular red tea which undergoes fermentation, can also contain ochratoxin A (OTA). The aim of this study was to compare the content of selected minerals, total polyphenols, tannins, soluble oxalates and OTA in three popular types of Chinese tea (green, black and red – 100 g packaged leaves) of five popular brands available on the Polish market. The antioxidant capacity (Trolox Equivalent Antioxidant Capacity) of tea infusions was also determined. Tea leaves differed significantly in their content of Cu, Mn, Fe, Mg, Na, K and P. Red tea leaves were characterized by particularly high levels of Na. The highest tannin content was found in red tea leaves. Red tea infusions were characterized by the highest antioxidant capacity, but the range of values for all tea infusions was relatively narrow: between 1.09 and 5.07 mM TE. The highest total phenolic content was noted in green tea infusions (114.3 mg in 200 ml). Black tea infusions had the highest content of compounds with adverse consequences for the consumers' health, such as soluble oxalates, whereas red tea infusions were characterized by the highest content of OTA, which reached 21.67 ng in 200 ml.

Keywords: tea, minerals, soluble oxalates, tannins, polyphenols, ochratoxin A, antioxidant capacity.

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INTRODUCTION

Tea is one of the most popular stimulant beverages consumed in the form of infusions. Different processing methods are applied to produce three main types of tea: green (non-fermented), red (semi-fermented) and black (fully fermented). Fermentation alters the sensory attributes of infusions and leads to changes in selected physicochemical parameters (Ferrara et al. 2001, Anesini et al. 2008, Khasnabis et al. 2015).

According to many authors, tea infusions and other herbal infusions are a rich source of minerals, and they are characterized by low levels of heavy metals. However, tea leaves have to be infused for at least 10 minutes for minerals to migrate from biological material into the solution (Ferrara et al. 2001, Özcan et al. 2008).

The health-promoting properties of tea have been demonstrated both *in vivo* and *in vitro*. These compounds are characterized by antiallergic, antioxidant, antimutagenic, anticarcinogenic, antiatherosclerotic and antibacterial activity (Imai et al. 1997, Cooper et al. 2005, Wolfram 2007, Sourabh et al. 2013, Jung et al. 2015). The preventive effects of tea infusions are observed when tea is consumed daily in significant amounts for many years. A 9-year prospective cohort study of a Japanese population revealed a negative correlation between green tea consumption and cancer incidence, especially among females drinking more than 10 cups (mostly 180 ml per cup) a day (Imai et al. 1997, Singh et al. 2011).

The beneficial effects of tea are linked with the presence of numerous phenolic compounds in tea leaves. Tea powders differ in their total polyphenol content, which can range from 22 to 32 g 100 g^{-1} in a batch of samples. The average total polyphenol content of Chinese green tea is estimated at 24% on a dry matter basis, and their catechin content ranges from several to more than 10% on a dry matter basis (Scott 2010, Sourabh et al. 2013). In the group of phenolic compounds, catechins are the major class of biologically active ingredients (Anesini et al. 2008).

Tannins migrate to infusions, in particular those brewed for a long time. Tannins represent a large and heterogeneous group of phenolic compounds with high molecular weight ($Mr > 500$) and high solubility in water. The tannin content of tea ranges from 13% to 30% on a dry matter basis. Tannins are responsible for the sensory attributes of infusions. Highly polymerized tannins containing a large number of hydroxyl groups are potent antioxidants, but they can also demonstrate anti-nutritional properties when their dietary intake is high (Khasnabis et al. 2015).

Tea leaves are also abundant in oxalic acid, an anti-nutritional compound that strongly inhibits dietary calcium intake. Tea infusions are regarded as a significant source of oxalate in the British diet, which is characterized by high consumption of tea (Charrier et al. 2002).

Tea may contain undesirable compounds, including mycotoxins such as ochratoxin A (OTA) (Haas et al. 2013, Abd El-Aty et al., 2014). OTA is a deleterious mycotoxin with several toxic effects, and one of the most widespread mycotoxins (Centre for Food Safety 2006). It is carcinogenic (group 2B), nephrotoxic, hepatotoxic, reprotoxic, teratogenic, immunotoxic and neurotoxic (Centre for Food Safety 2006, Pfohl-Leszkowicz, Manderville 2007). This mycotoxin is produced worldwide in food, including cereals, nuts, spices such as red pepper, liquorice, grapes, beer, wine, green coffee and roasted coffee, by *Aspergillus* Section Circumdati and *Aspergillus* Section Nigri, mostly in subtropical and tropical areas, or by *Penicillium*, especially *Penicillium verrucosum* and *P. nordicum*. If not properly stored, tea could be a substrate for molds which constitute a considerable source of OTA in a diet (Malir et al. 2013, Abd El-Aty et al. 2014, Malir et al. 2014).

The aim of this study was to compare the content of selected minerals, total polyphenols, tannins, soluble oxalates and OTA in three popular types of Chinese tea (green, black and red) available on the Polish market. The antioxidant capacity of the analyzed types of tea was also determined. The intake of minerals from tea infusions was estimated.

MATERIALS AND METHODS

Samples

Three popular types of loose leaf tea – green, black and red – were purchased in retail. Five brands of each type of tea were purchased in packages 100 g in weight. Five brands of each tea type were acquired (produced in China). They differed considerably in the degree of leaf crushing, leaf age and color. None of the products cost more than EUR 3 per 100 g in retail. All analyses were performed in triplicate.

Determination of total phenolic content

Tea infusions were prepared by adding 1.5 g of plant material to 200 ml of boiled deionized water and left for 5 min at room temperature. The contents of the beaker were shaken several times during the stabilization period. Infusions were prepared in accordance with the producers' recommendations. The beaker was capped to prevent water from evaporating. The infusions were filtered through filter paper and adjusted to 200 ml. The total content of phenolic compounds was determined by the spectrophotometric method of Folin-Ciocalteu (ISO 14502-1:2005) and expressed as gallic acid equivalents.

Determination of tannin content

Extraction was carried out by heating a 1 g tea sample with distilled water in a boiling water bath for 30 min (in four replicates). The extract was combined with copper acetate, acetic acid and potassium iodide, and the produced iodine was titrated with sodium thiosulfate using a starch solution as the reference (Plust et al. 2011).

Determination of antioxidant capacity

The antioxidant capacity of tea infusions (pouring 200 ml of distilled water temp. 85°C over 2 g of tea for 5 min) was determined in the Trolox Equivalent Antioxidant Capacity (TEAC) assay (Re et al. 1999). Solutions of ABTS (7 mM) and potassium peroxodisulfate (140 mM) in water were mixed to a final concentration of 2.45 mM $K_2S_2O_8$. ABTS radical solution was diluted to an absorbance of 0.70 ± 0.02 at 734 nm. All measurements were performed as follows: 30 µl of diluted extracts was added to 3 ml of ABTS radical solutions and absorbance was read after 30 min against the appropriate reagent blank. The results were expressed as Trolox equivalents.

Determination of soluble oxalate content

Tea infusions were prepared by adding 2 g of sample to 100 ml of boiled deionized water and left for 5 min at room temperature. Oxalates were extracted with hot water, the sediment was precipitated with calcium chloride buffer and centrifuged. The precipitate was dissolved in a solution of sulfuric acid and titrated with a potassium permanganate solution (Sperkowska, Bazylak 2010*a*).

Determination of ochratoxin A content

The OTA content was determined according to the method of Iha and Trucksess (2010). Infusions were prepared by pouring 200 ml of hot water over 2 g of tea and for 10 minutes. 90 ml of the infusion was combined with 100 ml of 3% $NaHCO_3$. The mixture was filtered through glass microfiber filter paper, and 50 ml of the filtrate was passed through a VICAM Ochra Test WB immunoaffinity column (IAC). The column was washed with 5 ml of 10 mM PBS (pH=7.6) and 5 ml of water and dried by passing air through the column with a glass syringe. OTA was eluted twice with 0.75 ml of methanol, collected into a 2 ml volumetric flask and diluted with water. After mixing, 50 µl of the mixture was injected into the HPLC (conditions: column-LC Phenomenex C18, 5 µm, 250x4.6 mm I.D., mobile phase–acetonitrile/water/acetic acid (40/50/10, v/v/v), flow rate: 1 ml min^{-1}, detector: 333 nm excitation and 467 nm emission wavelength). A linear calibration curve was plotted by measuring the area under OTA peaks for six standard methanolic solutions (concentrations of 0.10, 0.25, 0.50, 1.00, 1.50 and 2.00 ng OTA ml^{-1}). OTA concentrations were quantified using the calibration curve. Recovery was

from 76% to 83%. The detection limit (LOD) was 0.30 ng g^{-1} and the quantification limit (LOQ) was 0.45 ng g^{-1}.

Determination of mineral content

The content of Cu, Mn, Fe, Zn, Mg, Ca, Na, K and P in dry tea material was determined. Mineralization was carried out in a VELP DK 20 digester with an aluminum heating block and temperature control (VELP Scientifica, Italy). The content of Fe, Mg, Ca, Zn, Mn and Cu was determined by flame atomic absorption spectroscopy (air-acetylene flame) in a Thermo Scientific iCE 3000 Series Atomic Absorption Spectrometer (United Kingdom) with a GLITE data station, background correction (deuterium lamp) and cathode lamps. The Ca content was determined with the addition of 10% aqueous solution of lanthanum chloride to a final concentration of 0.5% La^{+3}. The content of Na and K was determined by atomic emission spectroscopy in the emission mode. The content of minerals was expressed in mg (Mn, Mg, Ca, K, P) and μg (Cu, Fe, Zn, Na) per 1 g of dried tea leaves. In tea infusions, the mineral content was determined by adding 200 ml of boiled deionized water do 2 g of tea sample and left for 8 min in room temperature. The infusion was decanted, cooled and filtered. Filtrates were analyzed using method described above.

Dry matter content

The dry matter content of the analyzed products was determined in a gravimetric analysis (PN-ISO 1573:1996).

Adulteration

The degree of adulteration was evaluated with the use of copper(II) acetate. Tea without the addition of extracted leaves contains tannins which change the color of the reagent from blue to green or green-blue (Plust et al. 2011).

pH

The pH of tea infusions was determined by measuring the potential difference between electrodes immersed in standard and test solutions (Seven Compact pH – meter, Mettler Toledo).

The results were processed statistically by calculating means with standard deviation and comparing mean values in a one-way analysis of variance (ANOVA) at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

The results of water content and acidity (pH) in teas samples are shown in Table 1. The observed differences were statistically significant (Table 1).

Table 1

Water and tannin content of tea leaves and the pH of tea infusions

Specification	Green tea		Black tea		Red tea		ANOVA	
	±SD	range	±SD	range	±SD	range	*F*	*p*
Water content (%)	6.3 ± 1.1	5.11-7.66	8.36 ± 0.61	7.60-8.96	9.55 ± 0.96	8.25-10.78	50.5	**<0.001**
Tannin content (%)	18.9 ± 3.9	15.4-25.1	12.2 ± 2.8	8.0-15.2	19.2 ± 5.1	14.6-26.6	10.9	**<0.001**
pH of tea infusion	5.55 ± 0.01	5.54-5.55	6.64 ± 0.01	6.62-6.64	3.75 ± 0.01	3.74-3.75	8.9	0.003

The tannin content differed significantly between the analyzed teas, and the lowest average tannin content of 12.26% was found in black teas, whereas one equal 19.15% was noted in red teas (Table 1). In a study by KHASNABIS et al. (2015), the average tannin content was determined at 13.5% in black teas and 2.65% in green teas.

The content of phenolic compounds in tea is influenced by cultivation treatments, horticultural practices, leaf age, climate, environmental stress and processing technology (WANG et al. 2000, LIN et al. 2003). Processing technology can significantly lower the content of phenolic compounds in green tea leaves by up to 15%. Tea infusions are a source of bioactive compounds for consumers.

The differences in the polyphenol content of green, black and red tea infusions were not statistically significant (Table 2). However the highest polyphenol content was determined in green tea infusions. In black tea infusions, the average content of phenolic compounds was lower, and it fluctuated within a relatively wide range of values.

Table 2

Antioxidant capacity of tea infusions and the intake of phenolic compounds, soluble oxalates and OTA from 200 ml of tea infusions prepared from 1.5 g of tea leaves

Specification	Green tea		Black tea		Red tea		ANOVA	
	±SD	range	±SD	range	±SD	range	*F*	*p*
Antioxidant capacity (mM TE)	2.05 ± 0.97	1.09-3.64	1.15 ± 0.18	1.12-1.54	2.61 ± 1.35	1.45-5.07	8.6	**0.001**
Phenolic compounds (mg)	77.4 ± 21.7	61.6-114,3	70.6 ± 21.9	36.3-96.3	56.7 ± 13.1	45.1-72.7	0.19	0.19
Soluble oxalates (mg)	20.8 ± 5.8	13.2-27.9	48.3 ± 9.9	34.0-61.5	28.3 ± 12.3	10,2-32.2	32.1	**<0.001**
Ochratoxin A (ng)	2.55 ± 1.32	1.20-4.01	3.30 ± 2.85	1.20-8.03	5.8 ± 8.8	1.39-21.67	24.9	0.001

In a study by Fu et al. (2011), the polyphenol content of commercial preparations of Chinese tea was determined in the range of 253 to 867 mg L^{-1}. In our study, the maximum total phenolic content of one cup (200 ml) of tea infusions made from 1.5 g of green tea leaves was determined at 114.3 mg, with an average content of 77.4 mg. Our results fall within the mid-range of values reported by other authors.

The concentrations of phenolic compounds in finely ground (<0.5 mm) dried tea leaves after extraction with 70% methanol are several times higher than in tea infusions. Most tea infusions are prepared in accordance with the producer's instructions. The total polyphenol content of the examined teas (Table 2) corresponds to the actual concentrations of phenolic compounds in infusions (brewed for 5 min). Dried leaves of Argentinian green tea characterized by significant fluctuations in polyphenol concentrations, contained 14 to 21 g of phenolic compounds per 100 g (Anesini et al. 2008), whereas the polyphenol content of Chinese teas was 24 g 100 g^{-1} (Scott 2010). In our study, polyphenol concentrations expressed per 1 g of dried tea leaves used for preparing infusions were lower.

The antioxidant capacity of teas against ABTS radical cations are showed in Table 2. Samples differed significantly in their antioxidant capacity which was lowest in black tea and highest in red tea infusions. Higher levels of radical scavenging activity were noted in the teas analyzed by Gorjanović et al. (2012) at 7.88 mM TE g^{-1} for green teas and 2.90 mM TE g^{-1} for black teas. In a study by Kopjar et al. (2015), the average antioxidant capacity was determined at 3.28 mM TE g^{-1} for green teas and 2.61 mM TE g^{-1} for black teas.

Tea is a good source of natural antioxidants when consumed in the amount of several cups per day (Re et al. 1999, Gorjanović et al. 2012). Despite the above, the antioxidant capacity of the analyzed teas, which involves the ability to scavenge free radicals, was not as high as expected. This could suggest that older leaves were used in the production process.

All tea samples were analyzed for the OTA content, which exceeded 0.45 ng g^{-1} (LOQ) – Table 2, Figure 1. Commission Regulation (EC) No 1881/2006 does not specify OTA limits for tea, however, the limit for coffee beans (5 µg kg^{-1}) would not be exceeded in the sample with the highest OTA contamination. Most tea producers or distributers recommend the first brew be discarded to remove some of the water-soluble or suspended contaminants. However, OTA has very low solubility in water (<1 mg ml^{-1}). Further research is needed to determine the extent to which mycotoxins can be detected in infusions with different brewing duration and water temperature. The presence of contaminants in tea has to be strictly controlled to minimize the health risks associated with the consumption of tea infusions contaminated by toxigenic fungi and their metabolic products, including OTA.

Soluble oxalates are natural antinutrients found in plant origin foods. Dietary oxalates form insoluble salts with other food ingredients (in calcium),

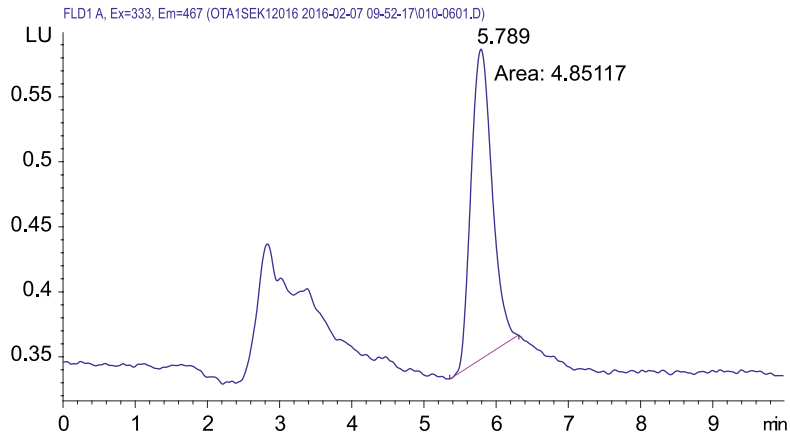

Fig. 1. Chromatographic separation of ochratoxin A from a sample of oolong tea

and contribute to the formation of kidney stones (Reynolds 2005). The oxalate content in teas is presented in Table 2. The statistical analysis revealed significant differences in the oxalate content between the teas. Fluctuations in the oxalate content of different tea types were noted by other authors. Jabłońska-Ryś (2012) determined the oxalate content of tea (brewed 5 min) at 17.00-37.40 mg g^{-1} DM in black, 23.82-33.83 mg g^{-1} DM in green, and at 43.82 mg g^{-1} DM in red teas. In a study by Sperkowska and Bazylak (2010*a*), black teas contained higher oxalate content of 9.6 to 21 mg g^{-1} whereas those in green teas ranged from 6.4 to 13 mg g^{-1} (Sperkowska, Bazylak 2010*b*). According to Charrier et al. (2002), non-fermented teas contain less soluble oxalates than fermented teas, and the longer the fermentation period, the higher the oxalate content. Hönow et al. (2010) observed that the oxalate content was influenced by the harvest time because leaves harvested in the fall were larger and contained more oxalates than fine leaves from the spring.

The mineral content in dry material and infusions (expressed in infusion prepared form 1g of tea) is presented in Table 3. Teas differed significantly in their content of Cu, Mn, Fe, Mg, Na, K and P. Significant differences were not observed in the content of Ca and Zn. Red tea contained considerably higher Na concentrations, which could point to high salinity levels in biological material. Results reported by Olivier et al. (2012) in infusions prepared from 2.5 g of dried tea leaves and 250 ml of water. The authors showed the consumption of 3 to 4 cups of black or green tea meets the daily mineral requirements for Mn only, whereas the K amount supplied with one cup of tea corresponds to less than 1% recommended daily intake. According to Euromonitor International, the average tea consumption in Poland is 1 kg per capita for year. When preparing tea, consumers usually take 1.8 - 2.2 g of leaves for one glass. Table 4 presents an average daily intake of mineral compounds from tea infusions. Tea is not a good source of minerals because

Table 3

Mineral content in 1 g of tea leaves (l) and infusions* (i)

Mineral		Green tea		Black tea		Red tea		ANOVA	
		±SD	range	±SD	range	±SD	range	*F*	*p*
Cu (μg)	l	12.9 ± 1.7	10.8-14.8	18.9 ± 2.9	15.3-22.3	14.6 ± 6.5	2.6-20.7	8.10	0.001
	i	3.72 ± 1.26	3.18-4.27	8.55 ± 2.25	6.55-10.38	2.85 ± 1.23	0.55-4.23	7.45	0.002
Mn (mg)	l	1.34 ± 0.45	0.66-1.95	1.44 ± 0.89	0.60-2.95	0.53 ± 0.27	0.05-0.79	10.49	<0.001
	i	0.33 ± 0.29	0.13-0.52	0.59 ± 0.74	0.19-1.65	0.14 ± 0.09	0.01-0.22	9.63	<0.001
Fe (μg)	l	102 ± 31	72-159	76 ± 34	34-117	129 ± 55	41-168	6.03	0.005
	i	2.47 ± 1.02	1.45-3.48	0.93 ± 0.48	0.51-1.47	8.7 ± 3.7	2.51-11.23	6.54	0.006
Zn (μg)	l	22.8 ± 2.2	19.2-25.3	27.3 ± 6.0	22.2-38.4	28.4 ± 9.9	10.5-38.9	2.85	0.069
	i	8.58 ± 1.34	7.23-9.91	12.5 ± 4.2	9.9-18.2	7.7 ± 2.9	3.1-10.5	3.01	0.071
Mg (mg)	l	1.45 ± 0.31	1.12-1.97	1.81 ± 0.15	1.62-2.05	1.93 ± 0.23	1.58-2.14	17.23	<0.001
	i	0.59 ± 0.21	0.38-0.79	0.91 ± 0.20	0.79-1.18	0.70 ± 0.09	0.61-0.78	18.15	0.001
Ca (mg)	l	5.52 ± 0.62	4.46-6.27	6.35 ± 0.88	5.09-7.23	5.5 ± 1.9	1.76-6.72	2.22	0.119
	i	0.51 ± 0.23	0.39-0.55	0.38 ± 0.04	0.35-0.43	1.09 ± 0.47	0.38-1.32	2.09	0.121
Na (μg)	l	17.6 ± 8.4	6.5-26.0	24.1 ± 8.7	11.3-34.4	507 ± 901	66-2247	4.38	0.019
	i	13.9 ± 8.3	5.1-21.6	13.7 ± 4.9	6.8-19.1	481 ± 889	60-2105	4.01	0.022
K (mg)	l	13.3 ± 1.6	12.3-16.2	17.4 ± 1.8	14.9-19.3	17.0 ± 7.6	2.6-22.4	3.45	0.041
	i	12.9 ± 2.1	11.5-15.7	16.4 ± 1.5	14.2-18.6	15.4 ± 6,3	2.3-19.7	3.66	0.038
P (mg)	l	2.27 ± 0.07	2.21-2.39	2.80 ± 0.31	2.52-3.23	3.1 ± 1.5	0.35-4.41	3.39	0.043
	i	0.82 ± 0.03	0.81-0.85	1.51 ± 0.18	1.28-1.73	1.63 ± 0.88	0.16-2.37	3.15	0.048

* infusion prepared from 1 g of tea

Table 4

Average daily intake of minerals from tea leaves infusions (mg) prepared from 2 g

Mineral	Recommended daily intake*	Green tea	Black tea	Red tea
Cu	0.7–1.0	0.007	0.017	0.006
Mn	0.7-1.0	0.66	1.18	0.28
Fe	8-18	0.005	0.002	0.017
Zn	8-11	0.017	0.025	0.015
Mg	240-420	1.18	1.82	1.4
Ca	1000-1300	1.02	0.76	2.18
Na	1200-1500	0.028	0.027	0.96
K	4500-4700	25.8	28.4	30.8
P	700-1250	1.64	2.56	3.26

* Estimated daily intake based on the Recommended Dietary Intake (RDI, estimated by the National Academy of Sciences. Food and Nutrition Board, USA).

only a small percentage is transferred to infusions, and several cups of tea per day meet the requirements for only one micronutrient.

CONCLUSIONS

The leaves of the three examined types of tea differed in their average content of Cu, Mn, Fe, Mg, Na, K and P. The evaluated infusions were a poor source of the analyzed elements, excluding Mn. Soluble oxalates were determined in the highest concentration in black tea infusions. The presence of carcinogenic OTA was noted in all infusions. The highest OTA content was noted in red tea infusions, where a 200 ml cup of tea made from 1.5 g of leaves contained more than 21.5 ng of OTA. Relatively high concentrations of OTA could pose a health risk for high consumers of tea.

Green tea infusions were characterized by the highest total phenolic content, but average polyphenol concentrations were similar in all three types of tea. The above could point to considerable variations in the quality of dried tea leaves (leaf age, processing technology, period and method of storing unprocessed leaves and the end product). The antioxidant capacity of tea infusions was relatively low, and it was determined within a narrow range of values. Black tea was characterized by the lowest antioxidant capacity.

Green, red and black tea infusions differed considerably in their content of the analyzed compounds (except phenolic compounds), minerals (except Zn and Ca) or antioxidant capacity. Based on the results of this study, none of the examined teas could be singled out as a product that offers greater health benefits for consumers than the remaining ones.

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