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Full Length Research Paper

The analysis of quality and antioxidant activity of green tea extracts

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The aim of the research was to analyze the composition of biologically active compounds, to determine the correlation between the concentration of biologically active compounds and antioxidant activity, the dependence of extractive compounds on the extraction time, the quality of different tea preparation forms (daily tea, infusions, decoctions) of those teas found in Lithuanian market. Four specimens of green tea from different regions of the world have been chosen as the object of analysis. Quality criteria's have been analyzed by using gravimetric and spectrophotometric methods. Detailed analysis has been performed using high performance liquid chromatography (HPLC) technique. With reference to analysis results it can be stated that in the aspect of relation to antioxidant activity/phenolic compounds, the teas with highest numbers in these dimensions were those from China region. The least numbers in antioxidant activity, dry residue and phenolic compounds were of teas from Sri Lanka. We tested the hypothesis that all green teas have phenolic compounds as their biological active compounds and because of this group of active compounds they distinguish antioxidant activity. The study gave strong evidence that green tea as a daily preparation and as well as pharmaceutical form (infusions, decoctions, capsules of green tea extract) may be used as preventive measures for cancerogenous processes, prostate cancer, renal or liver diseases because of it's antioxidant and free radical binding properties.

Key words: Green tea, aqueous extracts, infusions, decoctions, phenolic compounds, antioxidant activity.

INTRODUCTION

Tea refers to the products of the leaves, leaf buds, and internodes of the *Camellia sinensis* or *Camellia assamica* plant prepared by different methods, such as daily preparation, infusions, decoctions etc. Tea also refers to the aromatic beverage prepared from the cured leaves by combination with hot or boiling water (Shapiro et al., 2006). At the moment tea is the second most popular beverage in the world (after water) (Cheng, 2006). The detailed analysis of green tea is relevant in the terms of preventive effect on metastasis of lung, breast cancer (Ruhl et al., 2005) prevention of inflammation, thrombosis (as the reasons of primary heart attacks and cardiovascular diseases) (Tsubono et al., 2001), preventive effect on atherosclerosis and positive effect on decreasing cholesterol concentration in the blood (Katiyar et al., 1997), positive effect of it's antimutagenic and anticancerogenic properties, antioxidant activities established by ability to bind to free radicals and neutralize them (Khan et al., 2007) and the effect of decreasing the risk of renal calculi by 30% (Rijke et al., 2003). Continuously and increasingly new articles are published in scientific literature concerning preventive or healing properties of green tea, therefore it is obvious that either scientific community, either society is interested in it's properties. Whilst analyzing directly one of the main scientific databases of science, it has been calculated that in year 2007 there have been 1866 articles published about green tea, respectively 2072 articles in 2008 and 2297 articles in 2009. The amount of publications increases approximately 10% per year. Whilst evaluating the quality by the composition of green

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tea catechins (one of the fractions of flavonoids), such as (-)-catechin, (-)-epicatechin, (-)-epigallocatechin, (-)-catechin-3-gallate, (-)-epicatechin-3-gallate, (-)-gallocatechin-3-gallate, (-)-epigallocatechin-3-gallate, theaflavin, theaflavin-3-gallate, theflavin-3,3-bigallate are indicated as major part of biologically active substances (there are up to 30% of catechins in the leaves of dry tea stock and up to 200 mg in one cup of tea) (Bonoli et al., 2003). Flavonoids (and their fraction – catechins) are the basic phenolic compounds in green tea responsible for antioxidant activities such as neutralization of free radicals that are formed in the process of metabolism (Horzic et al., 2009; Cai et al., 2002). Free radicals are the main factors responsible for the initiation of formation of cancer cells. Active oxygen forms participate in the pathogenesis of different diseases in molecular level by deformation of low-density lipoproteins (LDL), formation of secondary products (aldehydes), rapid decomposition of proteins, reduced activity of enzymes, contravention of cell membrane, DNA mutations, changes of carbohydrate receptors. This leads to the development of cancerogenesis process, sugar diabetes, establishment of cataract, renal insufficiencies, myocardial infarct, arthritic diseases, sensitivity of central nervous system, Alzheimer's disease (Horzig et al., 2009).

The study was performed to analyze different kinds of teas of different regions existing in Lithuanian market. Evaluation of the quantity of their qualitative parameters (the amount of phenolic compounds antioxidant activity and dry residue) has been the most important subject of this study. The object of most established studies evaluating these parameters has been different kinds of teas (not exceptionally green tea) (Joubert et al., 2008, Yao et al., 2006) or other kinds of tea (such as roiboos or black tea) (Raynolds et al., 2010; Owour et al., 2006). Therefore, we decided that a study concerning only different kinds of green tea would be substantial.

MATERIALS AND METHODS

The objects for analysis were four specimens of most popular green teas (accordingly to the statistics submitted by the largest corporation of Lithuanian markets) among Lithuanian consumers from three different regions of the world: N1 – "Bancha" from Japan; N2 - "Maxima" from Sri Lanka; N3 – "Majski" form China; N4 – "Dilmah" from Sri Lanka. Fundamental properties required for the stock: the leaves must be pure, without any impurities or additional components (such as substances for obtaining scent, colour or taste), on the matter of species it must be a stock only of one (*C. sinensis*) – not a mixture of different herbs or species of tea.

Preparation of green tea aqueous extracts

Daily method of tea making (household preparation) from the stock of green teas has been used to prepare aqueous extracts. The aqueous extract is prepared in ratio 1:10 with the consideration of the absorption coefficient of green tea leaves (which is 2). Technology of preparation – 5 g of tea leaves of diameter lower than 5 mm (under articles FS-B22:2002 and Eur.Ph.01/2002,2.1.4) are poured with 60 ml of boiling water. Time is given for the extraction to cool down and the quality of these extracts has been evaluated after 10, 20 and 30 min.

Infusions and decoctions of green tea have been produced

Infusion is prepared in the adequate proportions as daily prepared extracts with the consideration of absorption coefficient of green tea leaves. Tea is put into a special device for making infusions/decoctions and poured with calculated amount of water. Infusion is being heated for 15 min. and cooled down for 45 min. Decoction technology is analogical to that of infusion. Only time periods of heating (30 min.) and cooling (15 min) differ (European Pharmacopoeia 2007).

Spectrophotometric analysis has been applied for evaluation of the total quantity of phenolic compounds and antioxidant activity, using, Unicam Helios alfa" spectrophotometer (Unicam Cambridge, UK).

Total amount of phenolic compounds has been estimated by using Folin-Ciocalteu and sodium carbonate solutions. Data has been calculated by using the calibration diagram linear regression equation of the gallic acid standard. Gallic acid is selected under the reason of ability to evaluate it in quantity measures and it's significant part in the composition of the biologically active compounds of green tea (Cai et al., 2002). Total amount of phenolic compounds is being evaluated by using Folin-Ciolcateu reagent (Sigma, Switzerland). Folin-Ciolcateu is diluted with water (proportion 1:10). Afterwards 5 ml of this diluted solution is poured into 1 ml of aqueous solution that is being analyzed. Bluish color is obtained by addition of 4 ml 7.5% of Na_2SO_4 (Reachim, Russia) solution in distilled water. Spectrophotometry is performed after period of 30 min. in the fixed wavelength of 765 nm (Povilaitytė et al., 2001).

Antioxidant activity is evaluated using the reaction of DPPH* binding. $DPPH^*$ solution is prepared in this manner: 0.01183 g of $DPPH^*$ (Sigma-Aldrich, USA) is dissolved in 96% ethanol (UAB „Stumbras", Lithuania) up to 100 ml. 2.5 ml of this solution is mixed with 0.5 ml of aqueous extract of green tea. After time period of 16 min absorption is being measured in the wavelength of 515 nm with 96% ethanol as a comparative solution (Kasparavičienė et al., 2003; Briedis et al., 2003; Kumazawa et al., 2004).

$ABTS^*$ radical is obtained in the reaction of $ABTS^*$ (Sigma-Adrich, USA) and potassium persulfate $K_2S_2O_8$ (Merck, Germany). Free radical is obtained by storing mixture of $K_2S_2O_8$ and $ABTS^*$ in the dark for 16 h. At first two solutions must be prepared: (1) 0.0549 g of $ABTS^*$ is dissolved in 50 ml of PBS (phosphate buffered saline) solution; (2) 0.0038 g $K_2S_2O_8$ is dissolved in 0.2 ml of water. The mixture of these two solutions, stored in the room temperature for 15 to16 h may be used as an agent for evaluation of antioxidant activity. PBS solution (pH 7,4 phosphate buffer) is prepared in this manner - 8.18 g NaCl (Carl Roth GmbH Co. Lauersruhe, Germany), 0.27 g KH_2PO_4 (Carl Roth GmbH Co. Lauersruhe, Germany), 1.42 g Na_2HPO_4 (Carl Roth GmbH Co. Lauersruhe, Germany) and 0.15 g KCl (BDH Laboratory Supplies Poole, Great Britain) are dissolved in 1 L of ultra clean water.

Gravimetric analysis (dry residue) is performed in the temperature of 105°C by pouring 2 ml of aqueous extract of every kind of tea and its preparation form to a porcelain plate. The porcelain plate is being heated in the temperature of 105°C for 2 h. Dry residue is calculated by subtracting the mass of porcelain plate

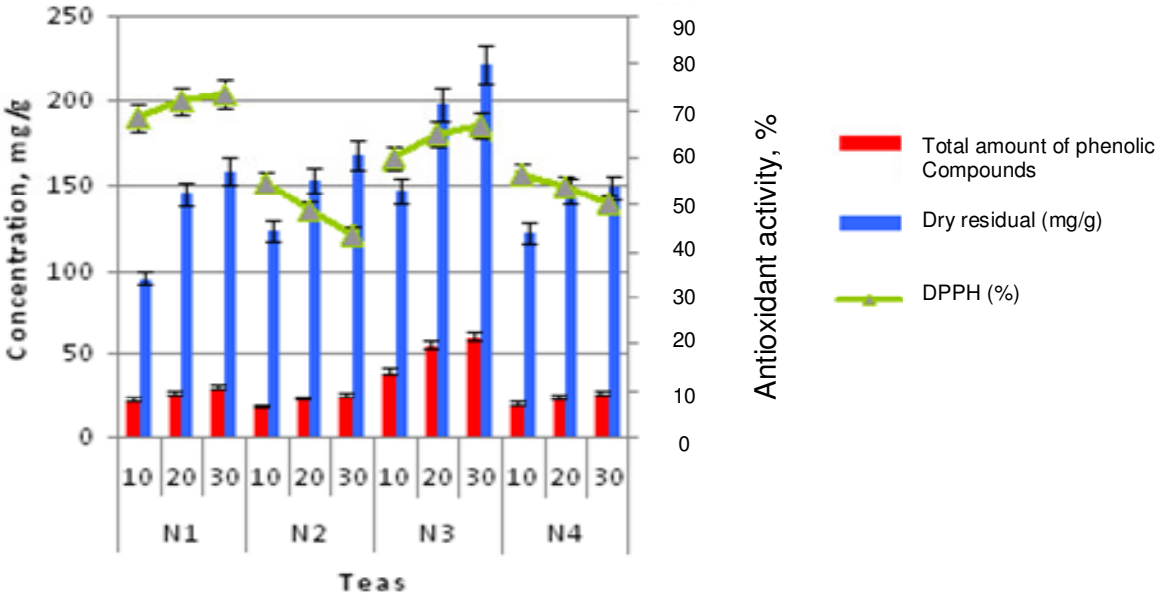

Figure 1. The influence of extraction time on the quality of daily prepared aqueous green tea extracts. Tea specimens: N1 – tea from Japan, N2 - tea from Sri Lanka (first specimen), N3 – tea from China, N4 - tea from Sri Lanka (second specimen).

without the tea preparation from the mass of heated porcelain plate with the content of residues out of green tea preparations (European Pharmacopoeia, 2007).

High-liquid performance chromatography (HPLC) has been used for identification of active compounds (especially phenolic compounds). HPLC analysis has been performed using HPLC system, which mainly consists of mobile phase, reservoirs, pump, injector, C18 column, UV detector, pump, supplying DPPH with reaction noose, computer for registration of data.

Detailed analysis of equipment:(1) Valving syringe pump of periodic action Phoenic 20 CU“ (Carlo Ebra instruments, Italy); degasation equipment “Series 1100“ (Hewlett Packard, Japan), (2) Pump of permanent action “Series 1100“ (Hewlett Packart, Japan), (3) Auto injector “Series 200“ (Prekin Elmer, USA), (4) UV detector “Spectra 200“ (Spectra Physics, USA), (5) Column C18, filled with silica gel sorbent with octadecyl bonded syllanolic groups. Parameters: length - 250 mm, diameter – 3 mm, charged particles diameter 5 µm (Knauer, Germany), (6) UV – visible light detector “Spectra 100“ (Spectra Physics, USA). Data is registered and analyzed with ChromStar program‘s 3.25S version.

Experimental fluid extractions for HPLC have been produced from green tea stock by extraction with methanol (BARTA a CIHLAR, spol. s r.o., Czech Republic). Methanol extractions have been purified by the method of solid-phase extraction with vacuum collector for solid-phase extraction “Supeco Visiprep“ USA and Core “LiChlorout RP – 18“ (Merck, Germany) of 1 ml, filled with adequate sorbent. Solid-phase extraction is used for purification of analyzed compounds, concentration and formation of suitable volume of extract for HPLC analysis (Povilaitytė et al., 2001; Zgorka et al., 2003). Caffeine, gallic acid, rutin and epigallocatechin have been used for identification of active compounds.

The mobile phase consisted of methanol (A) and solvent (B) (pH=3 phosphoric acid H_3PO_4 (Reachim, Russia) in the water). The elution profile was: 0 min 27% A in B, 20 min 40% A in B, 45 min 70% A in B, 50 min 80% A in B, 51 min 27% A in B. Statistical analysis was performed using statistical software package Statistica 5.5. The data are presented as mean ± SD (standard deviation), standard errors (SE), and percentage CV (coefficient of variation). Statistical analysis was performed using Student’s *t* test, and $P<0.05$ was used as the level of significance. All samples were prepared in triplicate.

RESULTS AND DISCUSSION

Test results have indicated that the amount of dry residue (which represents total amount of extractive compounds) is dependant on the extraction time (Figure 1). As it has been shown in the Figure 1 the most materials are extracted from the bulk in first ten minutes. After that during 20 minutes the extraction of substances decreases. Estimation of total amount of phenolic compounds has indicated the most intensive extraction takes pace in the first 10 min (in this period highest amounts of phenolic compounds are indicated). Later on the extraction of phenolic compounds decreases (Figure 1). Statistically significant difference ($P=0.0062$) has been indicated in N3 tea extract while evaluating the amounts of phenolic compounds after 10 and 30 min of extraction.

Test results indicate that quality of green tea extracts depends on the stock characteristics. Results of the analysis have shown that the highest amount (60.00 mg/g) of phenolic compounds is in the tea from China (N3), respectively it (29.41 mg/g) decreases in teas from Japan (N1) and (25.01 and 25.73 mg/g) is the least in the teas from Sri Lanka (N2 and N3), after 30 min extraction. Dry residue (221.67 mg/g) is the highest in the tea N3 and the least (148.67 mg/g) in the tea N4 after 30 min of extraction. Antioxidant activity is the most intensive of tea

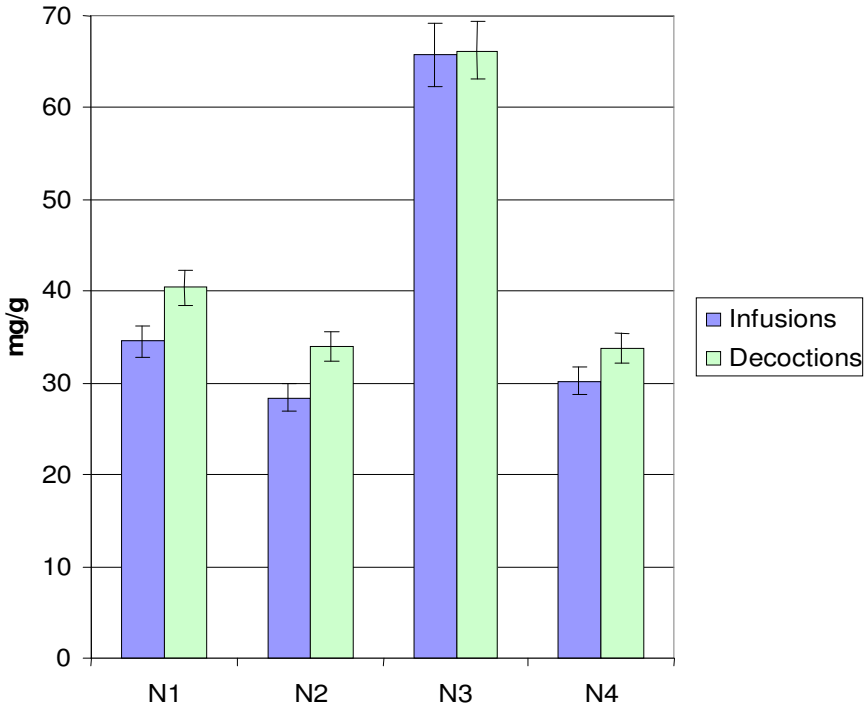

Figure 2. Comparison of total amount of phenolic compounds in infusions and decoctions.Tea specimens: N1 – tea from Japan, N2 - tea from Sri Lanka (first specimen), N3 – tea from China, N4 - tea from Sri Lanka (second specimen).

N1 and it respectively decreases in teas N3, N4 and N2 (Figure 1). Also experiments affirmed literature references, indicating that green tea has antioxidant effect (Horzig et al., 2009; Milašienė et al., 2007).

Infusions and decoctions of green tea have been produced for the experiments. Their quality has been estimated upon total amount of phenolic compounds. Highest levels of phenolic compounds are demonstrated in Chinese tea (N3) in both cases of infusion and decoction (respectively 65.71 and 66.22 mg/g of phenolic compounds). Test result have shown that although there is a difference between the amount of phenolic compounds in infusions and decoctions, but it is statistically insignificant. Also the extraction process is effective only during first 10 to 15 min. Therefore, longer extraction is not expedient. Figure 2 shows that total amount of phenolic compounds in infusions and decoctions depends not only on the preparation form, but as well on the kind of tea stock as well. Decoctions and infusions in comparison to daily prepared teas are of a higher quality (quality is characterized by concentration of phenolic compounds). The superiority of decoctions in comparison to infusions is based on the duration of boiling (30 min). The superiority of decoctions and infusions in comparison to daily prepared tea aqueous extractions is based on the special equipment used for preparation of infusions and decoctions and optimal boiling/cooling relation.

Analysis has shown that the correlation between dry residues and phenolic compounds is always direct and strong (K=0.997) in extracts N1 and N3. On the other hand, reverse correlation (K=0.982) has been indicated in extracts N2 and N4 while evaluating antioxidant activity and amount of phenolic compounds. Also reverse correlation (K=0.922) in extracts N2 and N4 is obvious while evaluating antioxidant activity and dry residue. The correlation between both antioxidant activity and phenolic compounds, either between dry residue may be direct as reverse as well as strong or very strong. It is obvious that as long as the correlation between antioxidant activity and amount of phenolic compounds is direct/adverse, it stays direct/adverse in the case of correlation between antioxidant activity/dry residue. All these correlations are direct for two teas – N1 and N3. Common correlation is the highest in tea N3. Teas N2 and N4 (teas from Sri Lanka) have adverse strong correlations between antioxidant activity/phenolic compounds and antioxidant activity/dry residue. This means that antioxidant activity decreases as dry residue and amount of phenolic compounds increases. Correlation of extraction time, phenolic compounds and dry residue is direct (the longer period of time of extraction (it endures 30 min), the higher quantities of dry residue and phenolic compounds). On the contrary, time of extraction and antioxidant activity may not always be in direct proportion (elongation of extraction time results in loss of antioxidant activity in teas N2 and N4). Therefore, the conclusion that the quantity of phenolic compounds is not always correlated

Table 1. Correlation coefficients of analyzed tea parameters calculated for each tea specimen.

Tea specimen	Correlation between these dimensions	r
N1*	Antioxidant activity/phenolic compounds	0.966
	Antioxidant activity/dry residue	0.996
	Phenolic compounds/dry residue	0.938
N2	Antioxidant activity/phenolic compounds	-0.982
	Antioxidant activity/dry residue	-0.988
	Phenolic compounds/dry residue	1
N3	Antioxidant activity/phenolic compounds	1
	Antioxidant activity/dry residue	0.997
	Phenolic compounds/dry residue	0.996
N4	Antioxidant activity/phenolic compounds	-0.988
	Antioxidant activity/dry residue	-0.856
	Phenolic compounds/dry residue	0.927

* – Tea specimens: N1 – tea from Japan, N2 - tea from Sri Lanka (first specimen), N3 – tea from China, N4 - tea from Sri Lanka (second specimen).

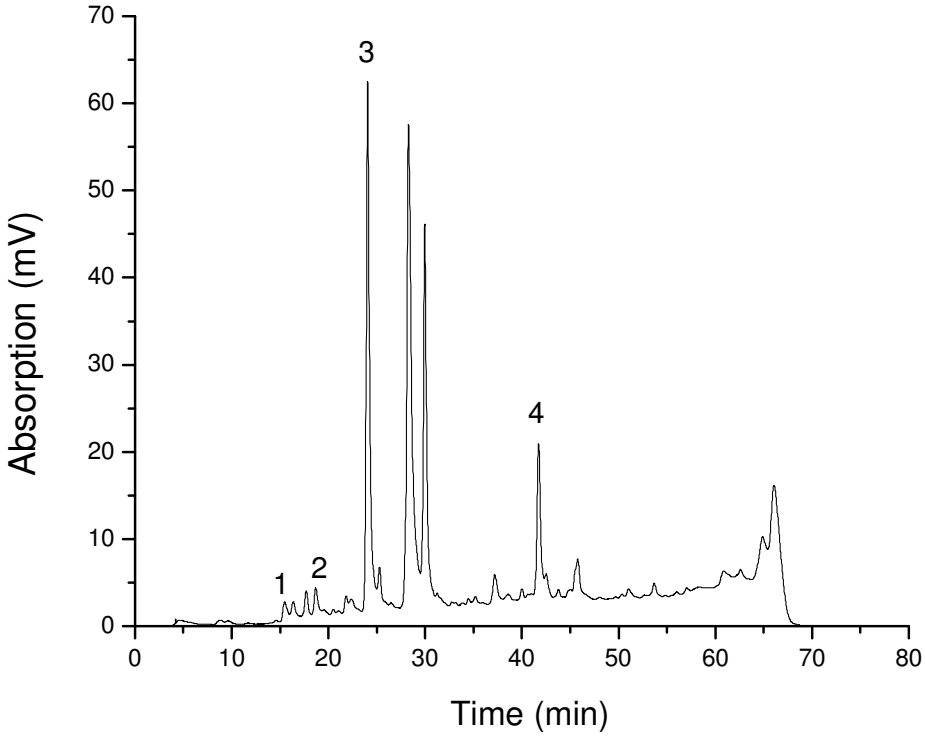

Figure 3. The chromatogram of the identification of the active compounds in green tea N1 from Japan aqueous extraction after 30 min. using HPLC technique, X axis represents time (min.), Y axis represents absorption (measured by mV). 1 - gallic acid, 2 - epigallocatechin, 3 - caffeine, 4 - rutin.

to their quality is obvious (Table 1).

After optimization of HPLC methodically, these biologically active compounds have been identified (after 30 min of extraction): gallic acid (15,793 min), epigallocatechin (18,953 min, caffeine (24,324 min), rutin (42,523 min) (Figure 3).

Conclusion

Aqueous extractions of green tea have been prepared and their quality has been evaluated. It has been determined that the amount of extractive compounds (which are later evaluated as dry residue) depends on the green tea stock and the region where it is from. Studies have shown that the highest amount of dry residue and total amount of phenolic compounds has been found in N3 tea from China. Antioxidant activity has been highest in tea N1 (Japan) in both DPPH* and ABTS* studies, although it's amount of phenolic compounds has been less twice the amount of tea N3. Therefore, the hypothesis which stipulates that the quality of phenolic compounds does not always correlate directly with its anitioxidant activity can be established. Also this hypothesis is proved by reverse correlations between phenolic compounds and antioxidant activity in Teas N2 and N4 (Sri Lanka) – though the amount of phenolic compounds increases, they lose their antioxidant activity.

As the time of extraction lengthens, the amount of extractive compounds increases (the amount of these compounds has been evaluated with method of dry residue in daily extractions after 10, 20 and 30 min. The highest extraction rate of extractive compounds as well as phenolic compounds has been observed in the first 10 min of extraction; afterwards the extraction rate has decreased. In relation to that the amount of extractive compounds has increased in the time of 20 min only by 25 to 40%. It has been evaluated that standardized methods of green tea preparations (infusion, decoctions) are slightly superior to daily preparation of green tea, although the difference is statistically insignificant. After experiments with HPLC 4 compounds of green tea have been indicated (gallic acid, epigallocatechin, caffeine, rutin). In addition, it has been determined that gallic acid and epigallocatechin may be among the major indicators of quality while standardizing green tea as pharmaceutical staple.

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