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ANTIOXIDANT ACTIVITY OF GREEN TEA LEAVES (*CAMELLIA SINENSIS* L.) LIQUID EXTRACTS

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Abstract

Nowadays, it has been proved that cardiovascular diseases are caused by free radicals due to imbalance between antioxidant and prooxidant systems. Antioxidants are applied for scavenging free radicals in order to prevent and stop oxidative stress. Leaf of green tea is a source of potent antioxidants of phenolic nature which can be used for elaborating dietary supplements, cosmetology products and medicine with antioxidant activity.

The aim of study was to determine antioxidant activity of green tea leaves (*Camellia sinensis* L.) liquid extracts obtained by the 96, 60, 40, 20% ethanol and distilled water. The antioxidant activity of these extracts was measured by potentiometric method. The results showed that 96% ethanolic extract had the significant value of antioxidant activity compared to the other extracts. The 60% ethanolic extract had the greatest amount of phenolic compounds among extracts, however, it is not possessing a potent antioxidant activity due to presence of carbohydrates, amino acids and organic acids in extract which are not have the antioxidant activity. The very high correlation was found between antioxidant activity and content of phenolic compounds.

Keywords: *green tea leaves, antioxidant activity, analysis, liquid extracts*

Introduction

Tea, from the plant *Camellia sinensis*, is consumed in different parts of the world as green, black, or Oolong tea. Among all of these, however, the most significant effects on human health have been observed with the consumption of green tea [1]. It has estimated that about 2.5 million tons of tea leaves are produced each year throughout the world, with 20% produced as green tea, which is mainly consumed in Asia, some parts of North Africa, the United States, and Europe [2].

The chemical composition of green tea is consisted of phenolic compounds (30% dry weight in leave), 3 – 4% of alkaloids known as methylxanthine such as caffeine, theobromine and theophylline, proteins (15-20% dry weight in leave), carbohydrates (5-7% dry weight in leave) [3]. The main component of green tea is phenolic compounds, which are presented by flavonols, flavandiols, flavonone and phenolic acids. Most of flavonoids are presented by catechins [4]. In our previous research, we have estimated that green tea leaves contain 5 kinds of catechins: catechin (0.61% dry weight in leave), epicatechin (2.95% dry weight in leave), epigallocatechin (8.03% dry weight in leave), epicatechin-3-gallate (8.12% dry weight in leave) and epigallocatechin-3-gallate (10.85% dry weight in leave) [5].

Due to having such a wide variety of phenolic compounds, green tea possesses different pharmacological activities such as antioxidant [6], anti-inflammatory [7], antiviral [8], anti-bacterial [9], anti-tumor [10], anxiolytic [11] activity. Many scientific researches have found that green tea catechins possess a significant antioxidant activity [12].

It is well known that the development of most diseases, such as stress, neurosis, cardiovascular diseases, malignant neoplasms, inflammation of various etiologies, are accompanied by the activation of the process of free radical lipid peroxidation. Diseases of the cardiovascular system are referred to as "diseases of free radicals", since in these diseases the development of oxidative stress is noted and oxidative reactions become the main pathogenetic factor in the development of the disease [13]. So, as a result of the induction of lipid peroxidation in the endothelium and membranes of the smooth muscle elements of the vessels, the permeability to calcium increases and hypoxic damage to the myocardium occurs, which leads to its ischemia. Protection of human tissues and organs from free radicals is provided by the endogenous antioxidant system of the body [14]. However, endogenous antioxidant system is not able to protect a person from the development of oxidative stress under the action of chronic diseases [15]. For this reason, the search for drugs with antioxidant properties for the prevention and treatment of diseases accompanied by an increase in free-radical oxidation reactions is popular among scientists.

The aim of the study was to determine the antioxidant activity of green tea leaves liquid extracts.

Methods

Green tea leaves were the object of the study, which were collected in Anhui province, China at spring season. The pH meter HANNA 2550 (Germany) with a combined platinum electrode EZDO 50 PO were applied to conduct potentiometric measurements. Quantitative analysis of biological active compounds was provided on UV-spectrophotometer UV – 1000 (China) with matched 1 cm quarts cell.

Extraction procedure

The five samples of 10.0 g (exact mass) was taken and separately extracted with 96, 60, 40, 20% ethanol and distilled water within 1 hour on boiling bath with condenser. The suspended mixtures were filtrated and filtrates were collected in a five flasks. The procedure was performed thrice to provide completely extraction of biological active substances (BAS), then the filtrates were united and concentrated concentrated by vacuum evaporator to ratio of extract to raw material 1:2. As result, it was obtained 5 extracts of 96, 60, 40, 20% ethanol and water.

Quantitative analysis

The total phenols were measured by the Folin-Ciocaltau assay [16]. The total phenols in extract, expressed as gallic acid was calculated according to the following equation:

$$X(mg/mL) = \frac{C_x \cdot K_{dil} \cdot 1000}{V_{ext}}$$

where, C_x – concentration of gallic acid according to calibration curve, $C \cdot 10^{-6}$, g/mL; V_{ext} – volume of extract, mL; K_{dil} – coefficient of dilution.

The vanillin reagent assay was applied to find out the total catechins [17]. The total catechins in extract, expressed as epigallocatechin-3-O-gallate was calculated according to following equation:

$$X(mg/mL) = \frac{C_x \cdot K_{dil} \cdot 1000}{V_{ext}}$$

where, C_x – concentration of epigallocatechin-3-O-gallate according to calibration curve, $C \cdot 10^{-6}$ g/mL; V_{ext} – volume of extract, mL; K_{dil} – coefficient of dilution.

The total flavonoids were determined using assay of complex formation with $AlCl_3$ [18]. The total flavonoids in extract, expressed as rutin was calculated according to following equation:

$$X(mg/mL) = \frac{A \cdot K_{dil} \cdot 1000}{A_{st} \cdot V_{ext}}$$

where, A – absorbance of analyzed solution, A_{st} – absorbance of standard solution of rutin; V_{ext} – volume of extract, mL; K_{dil} – coefficient of dilution.

The total hydroxycinnamic acid content was measured by assay of complex formation with $NaNO_2$-Na_2MoO_4 [19]. The total hydroxycinnamic acids in extract, expressed as chlorogenic acid was calculated according to following equation:

$$X(mg/mL) = \frac{A \cdot K_{dil} \cdot 1000}{188 \cdot V_{ext}}$$

where, A – absorbance of analyzed solution,188 – specific adsorption coefficient of chlorogenic acid; V_{ext} – volume of extract, mL; K_{dil} – coefficient of dilution.

Antioxidant activity

Antioxidant activity of analysed extracts were performed by potentiometric method [20]. A 2 mmol/L solution of $K_3[Fe(CN)_6]$ was prepared by weighing 0.8232 g into a 25.0 mL volumetric flask, dissolving a compound in a distilled water and filling the flask to volume with the same solvent. A 0.02 mmol/L of $K_4[Fe(CN)_6]$ was prepared by weighing 0.0921 g into a 250.0 mL volumetric flask, dissolving a compound in a distilled water and filling the flask to volume with the same solvent. Than a 5.00 mL aliquot of both prepared solutions was taken and transferred into a 250.0 mL volumetric flask and made up to the mark by 0.067 mol/L phosphate buffer solution. A 50.00 mL of prepared mediator solution was transferred in an electrochemical cell. The initial potential of mediator solution was measured after initial one was established, a 1.00 mL of aliquot of the prepared solutions was added and a final potential was measured. The difference (ΔE) between the initial (E_0) and final (E_1) potentials was found. The shift of potential is explained by the change of ratio of oxidized and reduced forms of the mediator system. Antioxidant activity was calculated according to the following equation and expressed as mmol-eqv./$m_{res\ dryweight}$:

$$AOA = \frac{C_{ox} - \alpha \cdot C_{red}}{1 + \alpha} \cdot K_{dil} \cdot 10^3 \cdot \frac{m_1}{m_2}$$

where, $\alpha = C_{ox}/C_{red} \cdot 10^{(\Delta E - E_{ethanol})nF / 2.3RT}$; C_{ox} – concentration of $K_3[Fe(CN)_6]$, mol/L; C_{red} – concentration of $K_4[Fe(CN)_6]$, mol/L; $E_{ethanol}$ – $0.0546 \cdot C_{\%} - 0.0091$; $C_{\%}$ – concentration of ethanol; ΔE – change of potential; F = 96485.33 C/mol – Faraday constant; n = 1 – number of electrons in electrode reaction; R = 8.314 J/mol · K – universal gas constant; T – 298 K; K_{dil} – coefficient of dilution; m_1 – mass of dry weight residue; m_2 – mass of dry residue in 1.0 mL of extract.

Statistical analysis

For all the experiments, five samples were analysed and all the assays were carried out in 5 times. The results were expressed as mean values with confident interval. The MS EXCEL 7.0 and STATISTIKA 6.0 were used to provide statistical analysis.

Results

Total phenolic compounds were determined by Folin-Ciocalteu method and expressed as gallic acid equivalent. As shown in Table 1, the highest amount of phenolic compounds was observed in 60% ethanolic extract (100.10 mg/mL), followed by 96% ethanolic extract (87.40 mg/mL), 40% ethanolic extract (67.20 mg/mL), 20% ethanolic extract (63.80 mg/mL) and aqueous extract (48.10 mg/mL).

Total catechins was expressed in epigallocatechin gallate equivalent. Table 1 represents that amount of catechins in 60% ethanolic extract (104.70 mg/mL) greater than in 96% ethanolic extract (84.70 mg/mL), 40% ethanolic extract (67.40 mg/mL), 20% ethanolic extract (55.90 mg/mL) and aqueous extract (53.30 mg/mL).

Total amount of flavonoids was expressed in rutin equivalent. It was established that both ethanolic and aqueous extract had the highest amount of flavonoids, whereas the 40% ethanolic extract had lowest content. It seems that 96% ethanolic extract contained more aglycones of flavonoids, whereas aqueous extract – glycosides of flavonoids.

Total hydrocinnamic acids content was determined by $NaNO_2$–$NaMoO_4$ and expressed as chlorogenic acid. The highest content of hydroxycinnamic acids was observed in 96% ethanolic extract (7.75 mg/mL), followed by 40% ethanolic extract (4.79 mg/mL) as well as 20% ethanolic extract had the lowest amount of hydroxycinnamic acids.

According to obtain results of quantitative analysis of biological active compounds in extracts. It was found that 60% ethanol is the most optimal extractor for extraction phenolic compounds. Many studies have represented that phenolic compounds are responsible for antioxidant activity in the plants as they possess a high donor`s hydrogen ability. The antioxidant activity of green tea leaves extracts is summarized in Table 2. The antioxidant activity increasing in the following order aqueous extract (229.61 mmol-eqv./$m_{res\ dry\ weight}$) < 20% ethanolic extract (310.41 mmol-eqv./$m_{res\ dry\ weight}$) < 40% ethanolic extract (341.06 mmol-eqv./$m_{res\ dry\ weight}$) < 60% ethanolic extract (548.79 mmol-eqv./$m_{res\ dry\ weight}$) < 96% ethanolic extract (622.44 mmol-eqv./$m_{res\ dry\ weight}$). Ethanolic extract was shown the greatest value of antioxidant activity than other extracts. However, according to obtained results of quantitative analysis of BAS the 60% ethanolic extract was supposed to be possessed the highest value of antioxidant activity as extract contained the highest amount of phenolic compounds. In our view, it can be explained by the fact that 60% ethanolic extract may contained carbohydrates which increase the mass of residue of extract as result decreasing its antioxidant activity.

In order to evaluate the correlation between antioxidant activity and the content of BAS in green tea extracts, linear regression analysis and Pearson's coefficient (R) were used. As a result, it was found that there is a very high correlation between antioxidant activity and the amount of phenolic compounds (R = 0.9255), between antioxidant activity and the amount of catechins - a high correlation (R = 0.8710), between antioxidant activity and the amount of flavonoids - not a significant correlation (R = 0.2528), between antioxidant activity and the amount of hydroxycinnamic acids - moderate correlation (R = 0.5748). The results are shown in Fig. 1, 2, 3, 4

Conclusion

The antioxidant activity of green tea leaves extracts has been evaluated by potentiometric method. The 96% ethanolic extract has characterized by a potent antioxidant activity among other extracts. The research has revealed that 60% ethanolic extract has contained the highest amount of phenolic and catechins. It has been found the very high correlation between antioxidant activity and content of phenolic compounds. In conclusions, 96% ethanolic extract of green tea leaves can be used can in elaborating dietary supplements, cosmetology products and medicine with antioxidant activity.

Acknowledgments

The authors declare that there are no conflicts of interest.

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Table 1. The total content of phenolic, catechin, flavonoid and hydroxycinnamic acid compounds in green tea leaves extracts

Sample	Total phenols, (mg/mL)	Total catechins, (mg/mL)	Total flavonoids, (mg/mL)	Total hydroxycinnamic acids, (mg/mL)
96% ethanolic extract	87.40±1.92	84.70±2.54	5.54±0.17	7.81±0.23
60% ethanolic extract	100.10±2.50	104.70±3.49	3.10±0.09	5.62±0.17
40% ethanolic extract	67.20±1.68	67.40±2.02	1.69±0.05	7.08±0.21
20% ethanolic extract	63.80±1.67	55.90±1.68	2.75±0.09	2.15±0.06
Aqueous extract	48.10±1.35	53.30±1.60	5.16±0.15	4.86±0.16

Table 2. Results of antioxidant activity of green tea leaves extracts

Sample	Antioxidant activity, mmol-eqv./$m_{res.\ dry\ weight}$
96% ethanolic extract	622.44±12.45
60% ethanolic extract	548.79±10.98
40% ethanolic extract	341.06±6.82
20% ethanolic extract	310.41±6.21
Aqueous extract	226.601±5.67

Figure 1. Correlation between antioxidant activity and total phenolic compounds content in extracts of green tea leaves

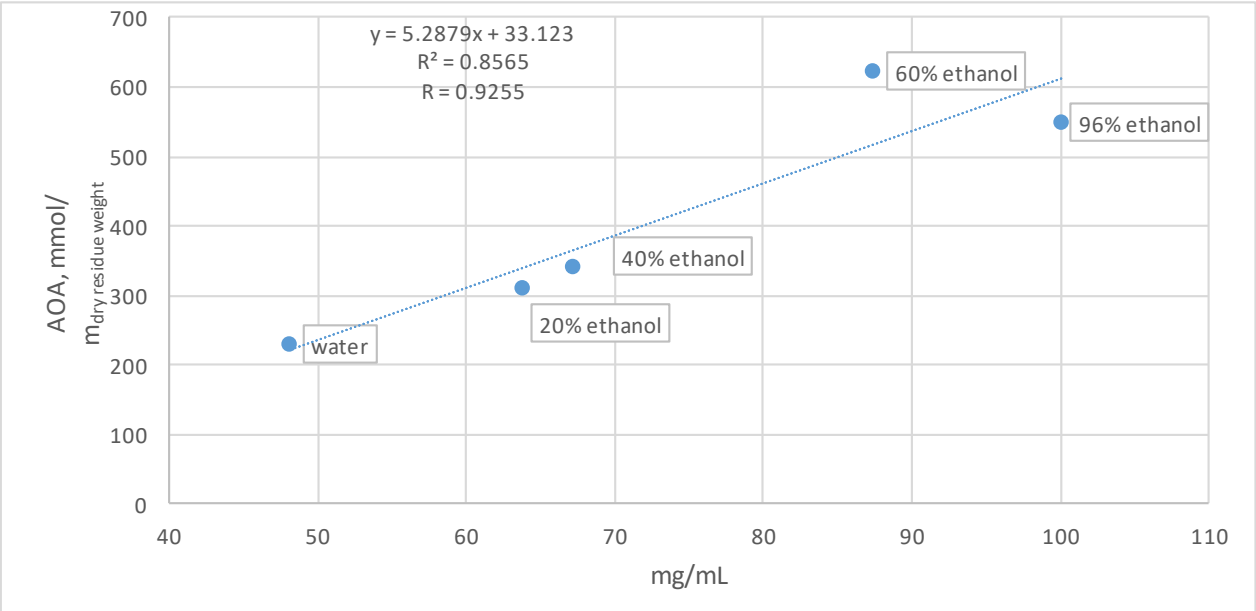

Figure 2. Correlation between antioxidant activity and total catechins compounds content in extracts of green tea leaves

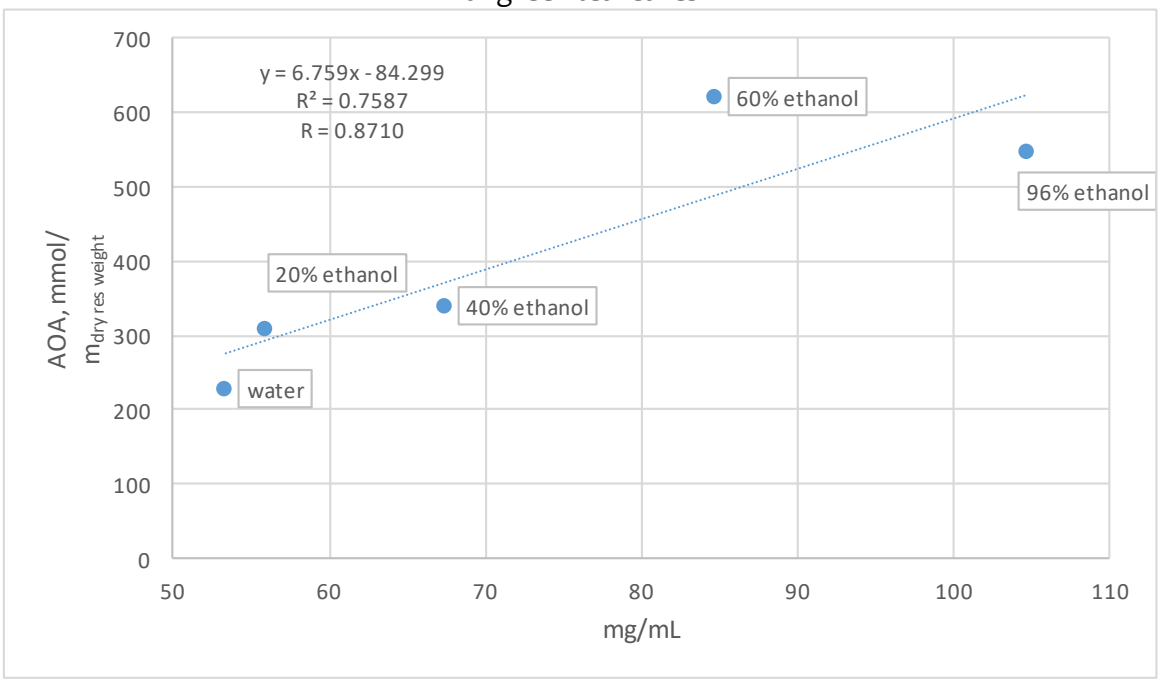

Figure 3. Correlation between antioxidant activity and total flavonoids content in extracts of green tea leaves

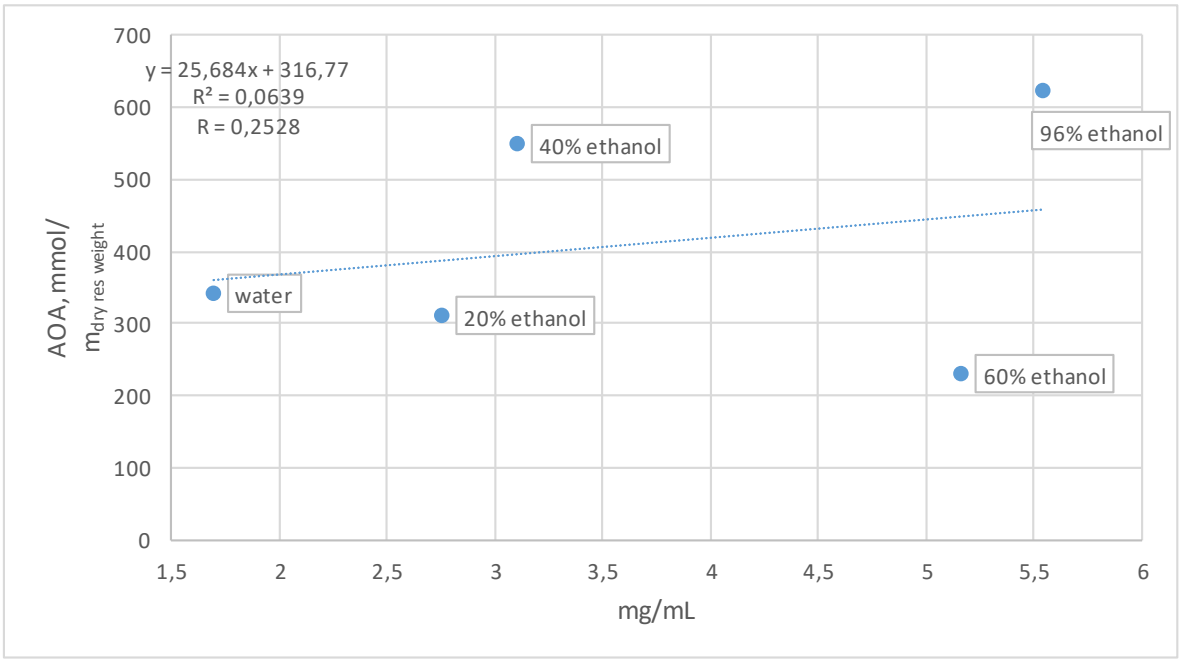

Figure 4. Correlation between antioxidant activity and total hydroxycinnamic acids content in extracts of green tea leaves