IN VITRO ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF GREEN TEA LEAVES (*CAMELLA SINENSIS* L.) LIQUID EXTRACTS

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Introduction

There is no secret that even today, herbal medicines play an important role in the health care of 80% of the world population and is estimated that more than half of the drugs under clinical use at present owe their origin to plants [1]. The high interest to phytomedicenes is explained by less side effects, commonly seen with products containing synthetic agents [2].

Nowadays, there is great problem with microbial mutation that caused by uncontrolled prescription, mismanagement and maladministration of antibiotics. This process has led to the developing drug – resistant strains. As result, antibiotics that have applied to cure infectious diseases have lost their effectiveness. Therefore, the search for new antimicrobial drugs from natural sources is warranted.

The cells of living organisms generate free radicals as a result of pathophysiological and biochemical process in response to factors such as chronic disease, a bad environment conditions [3].

Free radicals are critically important role in the pathogenesis of different diseases such as neurodegenerative disorders, diabetes, cancer, cardiovascular diseases [4, 5].

Green tea was taken for present study as a potential source of phytomedicines that possess antioxidant and antibacterial activities. It contains catechins (25 – 35%) [6], flavanols (1 – 2.5%), flavanones (1.5 – 3%), phenolic acids (2 – 5%); caffeine (1.5 – 2.5%), amino acids (1 – 5.5%), organic acids (1 – 1.8%) in green tea leaves [7].

The aim of the study was determined the antioxidant and antibacterial activity of green tea leaves ethanolic and aqueous liquid extracts.

Materials and methods

Green tea leaves of spices Chun Myn were the object of the study, the raw material was collected in Anhui province (China) from March to May. The green tea leaves were standardized according to European Pharmacopeia 9.0 [8]. All solvent and other chemical used in the study were of analytical grade.

The pH meter HANNA 2550 (Germany) with a combined platinum electrode EZDO 50 PO (Taiwan) was applied for potentiometric measurements. Quantitative analysis of biological active compounds was carried out on UV-spectrophotometer UV – 1000 (China) with matched 1 cm quarts cells.

Weighing was carried out using digital analytical balance AN100 (AXIS, Poland) with d = 0.0001 g.

Extraction procedure

10.0 g of the grinded leaves was mixed with 200 mL of 96% ethanol and distilled water. Extraction was carried out within 1 hour on water bath with a condenser, then repeated two times with a new portion of the solvent. After that the obtained extracts were filtrated and concentrated using rotary evaporator to 20 mL.

Quantitative analysis

The total content of phenolic compounds was measured by the Folin-Ciocaltau assay, the optical density was measured at 760 nm [9]. The calibration curve was plotted with interval concentrations 1.0 – 5.0 μg/mL, the calibration equation Y = 0.1055X + 0.1745 (R^2=0.9951). Expressed as gallic acid and calculated according to the following equation:

$$X(mg/mL) = \frac{C_x \times K_{dil} \times 1000}{V}$$

where, C_x – concentration of gallic acid according to the calibration curve, $C\times10^{-6}$, g/mL; V – volume of extract, mL; K_{dil} – coefficient of dilution.

The vanillin reagent assay was applied to find out the total catechins [10], the absorbance was measured at 505 nm. The calibration curve was plotted with interval concentrations 100 – 400 × 10^{-6} g/mL, the calibration equation Y = 0.0025X – 0.0851 (R^2 = 0.9951). The total catechins content in extracts, expressed as epigallocatechin-3-O-gallate, was calculated according to the following equation:

$$X(mg/mL) = \frac{C_x \times K_{dil} \times 1000}{V}$$

where, C_x – concentration of epigallocatechin-3-O-gallate according to calibration curve, $C\times10^{-6}$ g/mL; V – volume of extract, mL; K_{dil} – coefficient of dilution.

The total flavonoids were determined using assay of complex formation with $AlCl_3$, the absorbance was measured at 417 nm [11]. The total flavonoids content in extracts, expressed as rutin was calculated according to the following equation:

$$X(mg/mL) = \frac{A \times K_{dil} \times 1000}{A_{st} \times V}$$

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

The total hydroxycinnamic acids content was measured by assay of complex formation with $NaNO_2$-Na_2MoO_4, the absorbance was measured at 505 nm [12]. The total content of hydroxycinnamic acids in extracts, expressed as chlorogenic acid was calculated according to the following equation:

$$X(mg/mL) = \frac{A \times K_{dil} \times 1000}{188 \times V}$$

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

Antioxidant activity of extracts was evaluated by potentiometric method [13]. Antioxidant activity was calculated according to the following equation and expressed as mmol-equiv./$m_{dry\ res.}$:

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

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where, $\alpha = C_{ox}/C_{red} \times 10^{(\Delta E - E\text{ethanol})nF/2.3RT}$; 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/molK – universal gas constant; T – 298 K; K_{dil}– coefficient of dilution; m_1 – mass of dry residue; m_2 – mass of dry residue in 1.0 mL of extract.

Test organisms

Museum strains of *Staphylococcus aureus ATCC 25923*, *Staphylococcus aureus 6538 ATCC*, *Escherichia coli ATCC 25922*, *Proteus vulgaris NTCS 4636*, *Pseudomonas aeruginosa ATCC 27853* and *Candida albicans 885/653 ATCC* were used in accordance with the recommendations for the assessment of antimicrobial activity of drugs.

Antimicrobial activity

In our study, we used 1% solutions of extracts, the solvents of which were water and 96% ethanol. The method of diffusion of the drug into agar carried out using the method of "wells" [14]. Studies of antibacterial activity performed using the method of wells. Preparation of microorganisms suspensions with determined concentrations of microorganisms (optical density) was carried out by the standard of turbidity (0.5 units according to scale of McFarland) with using of equipment of Densi-La-Meter (Czech, wavelength 540 nm). Suspensions were prepared according to equipment and information list [15]. Colony forming unit was 10^7 microorganisms at 1 mL of growth medium and determined by standard of McFarland). On solidified agar, using a pipette under sterile conditions in Petri dishes made 1 mL of a suspension of microorganisms. After uniform distribution of microorganisms over the entire surface of the agar, the plates were incubated at room temperature for 15-20 minutes Next, wells with a diameter of 6 mm were made in the cups, into which solutions of the test substances were introduced. The samples incubated at 37° C for 16-24 hours. After incubation, the plates were placed upside down on a dark matte surface so that light fell on them at an angle of 45° (accounting in reflected light). The diameter of the growth retardation zones measured using a caliper. Chlorophyllipt spray manufactured by the State Scientific Center of Drugs (DNCLZ) with concentration 1% in 96% ethanol was used as the reference drug.

Statistical analysis

For all the experiments, two samples were analyzed 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 and discussion

Total phenolic compounds were determined by Folin-Ciocalteu method and expressed as gallic acid equivalent. As shown in Table 1, the amount of phenolic compounds in ethanolic extract was greater in 45.33% than in aqueous extract.

Total catechins was expressed in epigallocatechin gallate equivalent. Table 1 represents that content of catechins in ethanolic extract (96.89%) higher than in aqueous extract (110%). The percentage of catechins out phenolic compounds was 96.89% and 110% for ethanolic and aqueous extract, respectively.

Total amount of flavonoids was measured by the $AlCl_3$ complex formation assay. The amount of flavonoids in ethanolic extract was higher in 0.78%. It means that both extract showed approximately the same amount of flavonoids. The percentage of flavonoids out phenolic compounds 5.93 and 10.72% for ethanolic and aqueous extract, respectively.

Total hydrocinnamic acids content was determined by $NaNO_2$–$NaMoO_4$ and expressed as chlorogenic acid. The greater content of hydroxycinnamic acids was observed in case of ethanolic extract. The difference between extracts was 2.96 mg/mL. The percentage of hydroxycinnamic acids was 8.93 and 10.11% for ethanolic and aqueous extract, respectively.

According to obtained results of quantitative analysis of biological active compounds in extracts, it was found that the main component of phenolic compounds were catechins. Although, the amount of catechins in aqueous extract were even more than total phenolic compounds, it is can be related with uncompleted reaction between Folin-Ciocalteu`s reagent and phenolic compounds.

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

Sample	Total phenolic compounds, (mg/mL)	Total catechins, (mg/mL)	Total flavonoids, (mg/mL)	Total hydroxycinnamic acids, (mg/mL)
Ethanolic extract	86.70±1.73	84.00±1.68	5.14±0.10	7.75±0.16
Aqueous extract	47.40±0.95	52.50±1.05	5.08±0.10	4.79±0.10

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. In our previous study, we developed and proposed the method of determination the total antioxidant activity of raw material. This method can be applied for needs in order to understand antioxidant capacity of it and further apply obtained results in developing drug, dietary supplements and cosmetologically products. The total antioxidant activity of green tea leaves was 660.00 mmol-equiv./$m_{res.\ dry}$ [16]. The Table 2 represents that antioxidant activity of ethanolic extract higher in 63.87%. The percentage of ethanolic

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extract out the value of the total antioxidant activity was 93.33% whereas aqueous extract – 34.33%. Regarding to results below, the ethanolic extract possessed more potent antioxidant activity.

Table 2. Results of antioxidant activity of green tea leaves ethanolic and aqueous extracts

Sample	Antioxidant activity, mmol-equiv./$m_{res.\ dry}$	Percentage out total antioxidant activity, %
Ethanolic extract	617.29±12.35	93.33
Aqueous extract	226.60±4.52	34.33

The study of the antibacterial activity of the extracts was carried out by the agar diffusion method in the Laboratory of Biochemistry and Biotechnology, Mechnikov Institute of Microbiology and Immunology of the NAMS of Ukraine under the supervision of Candidate of Biology (Ph.D.) T. P. Osolodchenko.

Analysed extracts showed the antimicrobial and antifungal against *Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Proteus vulgaris ATCC 4636, Pseudomonas aeruginosa ATCC 27853, Bacillus subtilis ATCC 6633, Candida albicans ATCC 653/885олдюю*. According to the conducted research, it was found that ethanolic extract strongly inhibited the growth of *Staphylococcus aureus* (26.33±0.5 mm). In the case of Gram – negative bacteria, it was found that ethanolic and aqueous extract strongly inhibited the growth of *Escherichia coli* (23.67±0.5 and 22.67±0.5 mm, respectively). The most resistant strains among bacteria turned out to be *Proteus vulgaris*. *Candida albica* was sensitive for both extracts (20.00±0.0 and 19.67±0.5 mm). Moreover, the obtained results showed that obtained extracts possessed higher antimicrobial effect against Gram – positive bacteria than Gram – negative. Comparing results of investigated extracts and reference drug – «Chlorophyllipt» (DNCLZ) it can be pointed out that reference drug inferiors of antibacterial activity

Table 3. Antimicrobial and antifungal activity of green tea leaves extracts and reference drug

Extracts	Diameter of the growth retardation zone, mm					
	Staphylococcus aureus ATCC 25923,	*Escherichia coli ATCC 25922,*	*Proteus vulgaris ATCC 4636,*	*Pseudomonas aeruginosa ATCC 27853,*	*Bacillus subtilis ATCC 6633,*	*Candida albicans ATCC 653/885,*
Ethanolic extract	26.33 ± 0.50	23.67± 0.50	21.33 ± 0.50	22.67 ± 0.50	25.67 ± 0.50	20.00 ± 0.50
Aqueous extract	26.00 ± 0.50	22.67± 0.50	19.67± 0.50	20.67 ± 0.50	25.33 ± 0.50	19.67 ± 0.50
Chlorophyllipt (DNCLZ)	19.33 ± 0.50	19.33 ± 0.50	17.67 ± 0.50	17.67 ± 0.50	19.33 ± 0.50	19.00 ± 0.50

Based on conducted research, it was demonstrated that ethanolic extract showed higher antibacterial activity compared to the aqueous extract. In our view, it is related to the high content of phenolic compounds in the ethanolic extract than in the water extract, as well as to the high level of antioxidant activity. According to the obtained results, catechins take a large part of phenolic compounds than other biologically active compounds in the studied extracts.

In our recent study [6], it was found that epigallocatechin-3-O-gallate (10.85%), epicatechin-3-O-gallate (8.12%) and epigallocatechin (8.03%) are the main components among phenolic compounds in the Chun Ming species of green tea leaves. We believe that catechins are responsible for the antibacterial effect of green tea extracts. According to the literature sources, the mechanism of antibacterial activity of catechins is underlying in inhibition the formation of biofilm [17], dehydrofolate reductase [18], and DNA gyrase [19], which in turn leads to a change in cell wall permeability, denaturation of proteins present in microbial cells and ultimately the death of bacteria [20].

Conclusion

The present work showed that ethanolic extract of green tea leaves possess remarkable antioxidant activity as compared to its aqueous extract. The green tea leaves extracts have a quite high antimicrobial activity in relation to all strains while the greatest effect to *Staphylococcus aureus.* Thus, green tea leaves extracts can prove beneficial in food and pharmaceutical industry as today, there is high demand of antioxidant and antimicrobial drugs.

In vitro antioxidant and antibacterial activities of green tea leaves (*Camella sinensis* l.) liquid extracts

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Introduction. Nowadays, there is high demand of phytochemical extracts with antioxidant and antibacterial properties due to increasing growth of drug-resistant pathogenies. Therefore, study antibacterial and antioxidant activities of green tea extracts is a topical and perspective topic for today. **The aim of the work** was determined the antioxidant and antibacterial activities of green tea leaves ethanolic and aqueous liquid extracts.

Materials & methods. The object of the study was dry green tea leaves of spices Chun Myn were the object of the study, the raw material was collected in Anhui province (China) from March to May. The spectrophotometry was used for the quantitative

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determination of phenolic compounds, catechins, flavonoids and hydroxycinnamic acids; antioxidant activity was determined by potentiometric method. **Results** & **discussion.** Total content of phenolic compounds was 86.70±1.73 and 47.40±0.95 mg/mL, catechins – 84.00±1.68 and 52.50±1.05 mg/mL, flavonoids – 5.14±0.10 and 5.08±0.10 mg/mL and hydroxycinnamic acids – 7.75±0.16 and 4.79±0.10 mg/mL for ethanolic and aqueous extract, respectively. The antioxidant activity was 617.29 and 226.60 mmol-equiv./$m_{res\ dry}$ for ethanolic and aqueous extracts, respectively. *Staphylococcus aureus* bacteria was the most sensitive to the ethanolic and aqueous extracts (26.33±0.5 and 26±0.5 mm) whereas *Proteus vulgaris* was the most resistant to the ethanolic and aqueous extracts (21.33±0.5 and 19.67±0.5 mm). Obtained results showed that ethanolic extract had higher antioxidant and antibacterial activities than aqueous extract. **Conclusions.** In this study, we found that green tea leaves possess the high antioxidant and antibacterial activities. The results showed that ethanol is more appropriate solvent for obtaining the extract. Thus, green tea leaves extracts can prove beneficial in food and pharmaceutical industry as today, there is high demand of antioxidant and antimicrobial drugs.

Key words: green tea leaves, antioxidant activity, antibacterial activity, analysis, liquid extracts

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