Comparison of phytochemical composition, antimicrobial, antifungal, and antioxidant activities of lipophilic and ethanolic green tea leaf extracts

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Infection diseases are a worldwide important problem for medicine and pharmacy. Today, there is a high interest in the evolution of new antimicrobial drugs due to the increasing percentage of resistant bacteria and fungi strains. Green tea leaf contains a variety of natural compounds that are possible to apply in the creation of new antimicrobial drugs.

The aim of the work was to compare phytochemical composition, antimicrobial, antifungal activities of lipophilic and ethanolic green tea leaf extracts.

Materials and methods. The object of the research was the lipophilic extract obtained using chloroform, along with two ethanolic extracts of green tea leaves. One of the ethanolic extracts had been previously processed with chloroform, while the other had not. Antioxidant activity was determined by the potentiometric method, while antimicrobial and antifungal activities were assessed by the "wells" method.

Results. The lipophilic extract of green tea leaves contains predominantly caffeine and organic acids, with the lowest amount of phenolic compounds. In contrast, the ethanolic extracts show the opposite pattern, with phenolic compounds predominating and caffeine present in lower quantities. The lipophilic extract exhibits greater inhibition of the growth of *S. aureus*, *E. coli*, *P. vulgaris*, *B. subtilis*, and *C. albicans* compared to the 96 % ethanolic extract processed with chloroform, with inhibition rates of 19 %, 18 %, 12 %, 12 %, 16 %, and 20 % respectively. When comparing antimicrobial activity to the 96 % ethanolic extract without chloroform treatment, the results remained consistent. The antioxidant activity of the lipophilic extract was 58.7 and 60.0 times lower than that of the 96 % ethanolic extract processed with chloroform and the 96 % ethanolic extract without treatment, respectively.

Conclusions. The study revealed that the lipophilic extract exhibited greater inhibition of the growth of both Gram-positive and Gram-negative bacteria as well as fungi compared to the ethanolic extracts. However, it showed a lower level of antioxidant activity. It is hypothesized that caffeine, organic acids, and catechins may interact synergistically to enhance the antimicrobial and antifungal activity of green tea leaf extracts. The lipophilic extract shows promise for further development in the production of antimicrobial and antifungal drugs.

Keywords: green tea, lipophilic extract, caffeine, organic acids, pharmacologic action.

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Порівняння фітохімічного складу, антимікробної, протигрибкової та антиоксидантної активності ліпофільного й етанольного екстракту листя зеленого чаю

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Інфекційні захворювання є актуальною проблемою медицини та фармації в усьому світі. Значний науковий інтерес викликає розроблення нових протимікробних препаратів, що обґрунтовано збільшенням відсотка резистентності штамів бактерій і грибів. Листя зеленого чаю містить різноманітні природні сполуки, що можна використовувати для створення нових протимікробних препаратів.

Мета роботи – порівняти фітохімічний склад, антимікробну, протигрибкову дію ліпофільного та етанольних екстрактів листя зеленого чаю.

Матеріали і методи. Об'єкт дослідження – ліпофільний екстракт, отриманий хлороформом, та два етанольні екстракти листя зеленого чаю (один із них попередньо екстрагували хлороформом). Антиоксидантну активність визначали потенціометричним методом, антимікробну та протигрибкову – методом «колодязів».

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Результати. Кількісний вміст кофеїну й органічних кислот переважає у ліпофільному екстракті листя зеленого чаю, а в етанольних екстрактах домінують фенольні сполуки, катехіни та флавоноїди. Ліпофільний екстракт пригнічує ріст *S. aureus*, *E. coli*, *P. vulgaris*, *B. subtilis* і *C. albicans* на 19 %, 18 %, 12 %, 12 %, 16 % і 20 % краще, ніж 96 % етанольний екстракт після обробки сировини хлороформом. Порівнявши антимікробну активність з 96 % екстрактом без обробки хлороформом, відмінностей результатів не виявили.

Рівень антиоксидантної активності ліпофільного екстракту у 58,7 та 60,0 раза менший, ніж у 96 % екстракту після обробки хлороформом та 96 % екстракту без обробки відповідно.

Висновки. Встановлено, що ліпофільний екстракт активніше пригнічував ріст грампозитивних, грамнегативних бактерій і *C. albicans*, ніж етанольні екстракти, але мав низький рівень антиоксидантної дії. Припустили, що кофеїн, органічні кислоти та катехіни мають пряму синергетичну антимікробну та протигрибкову активність в екстрактах листя зеленого чаю. Ліпофільний екстракт є перспективним для виробництва антимікробних і протигрибкових препаратів.

Ключові слова: листя зеленого чаю, ліпофільний екстракт, кофеїн, органічні кислоти, фармакологічна активність.

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Bacteria and fungi are single-celled organisms and among the earliest forms of life on Earth. While many bacteria and fungi are harmless and even beneficial, aiding in processes such as food digestion in the human body [1]. Approximately 1 % of these microorganisms are opportunistic or pathogenic, capable of causing infectious diseases [2].

According to literature data, every year 7.7 million people die from bacterial infections in the world, which is 13.6 % of all deaths in the world. The main pathogens that cause half of all bacterial deaths are: *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumonia*, and *Pseudomonas aeruginosa* [3]. Meanwhile, nearly a billion people suffer from fungal infections of the skin, nails, and hair, and more than 150 million people suffer from serious fungal diseases that can later be fatal [4]. Consequently, the search for new biologically active substances that will have a high level of antimicrobial and antifungal effects is still relevant today.

In infectious diseases, an excess of free radicals occurs as a result of pathophysiological and biochemical processes caused by the effects of pathogenic bacteria and fungi on human cells [5]. Thus, free radicals play a crucial role in the development of pathogenesis in infectious diseases.

In our opinion, green tea leaf (*Camellia sinensis* L.) are a promising plant that may have antimicrobial, antifungal and antioxidant effects. In our previous research, it was found that the chemical composition of *C. sinensis* leaf is represented by: flavonols (1.38 %), flavonones (0.25 %), phenolcarboxylic acids (1.34 %), flavan-3-ols (20.56 %), as well as organic acids (1.80 %) and caffeine (2.56 %) [6,7]. Due to the presence of flavan-3-ol derivatives, the extracts obtained have powerful antioxidant, anti-inflammatory, antiviral, and antimicrobial effects [8].

Today, scientists pay great attention to the study of the pharmacological activity of extracts obtained with alcohol-water and aqueous-alcohol extractants [9,10,11]. This is of great interest to the scientific community due to the presence of flavan-3-ol derivatives in raw materials, and primarily epigallocatechin-3-O-gallate [12,13,14]. But, at the same time, in the scientometric database Scopus and Web of Science there are no scientific works on the study of the quantitative content of biologically active substances and the pharmacological activity of the lipophilic extract of *C. sinensis* leaf.

Aim

The purpose of our work was to conduct phytochemical researches to study the antimicrobial, antifungal and antioxidant effects of lipophilic and ethanolic *C. sinensis* leaf extracts.

Materials and methods

Plant material. Green tea (*Camellia sinensis* L.) leaf was collected in Anhui province, China (30.634140518993203, 116.33254121482477).

Equipment. 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. Procedure for obtaining lipophilic extract: 25.0 g of crushed leaves were mixed with 500 mL of chloroform. The extraction was carried out for 1 hour in a water bath with a condenser, then repeated twice with a new portion of the solvent. The obtained extracts were then filtered and concentrated using a rotary evaporator to a volume of 50.0 mL.

Procedure for obtaining 96 % ethanol extract after chloroform. The obtained raw material after extraction of chloroform was dried at room temperature. Then the raw material was extracted twice with 96 % ethanol for 1 hour in a water bath with a condenser, then repeated twice with a new portion of the solvent. The obtained extracts were then filtered and concentrated using a rotary evaporator to a volume of 50.0 mL.

Qualitative analysis. The total content of phenolic compounds was measured by the Folin–Ciocaltau assay, the optical density was measured at 760 nm [15]. 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:

$$X(\%) = \frac{C_x \times K_{dil} \times 100}{V}, \qquad \text{(Eq. 1)}$$

where, C_x is the concentration of gallic acid according to the calibration curve, $C \times 10^{-6}$ g/ml; V is the volume of extract, ml; K_{dil} is the coefficient of dilution.

The vanillin reagent assay was applied to find out the total catechins [16], 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 the extract, expressed as epigallocatechin-3-O-gallate, was calculated according to the following equation:

$$X(\%) = \frac{C_x \times K_{dil} \times 100}{V}, \quad \text{(Eq. 2)}$$

where, C_x is the concentration of epigallocatechin-3-O-gallate according to calibration curve, $C \times 10^{-6}$ g/ml; V is the volume of extract, ml; K_{dil} is the coefficient of dilution.

The total flavonoids were determined by assay of complex formation with $AlCl_3$, the absorbance was measured at 417 nm [17]. The total flavonoid content in the extract, expressed as rutin was calculated according to the following equation:

$$X(\%) = \frac{C_x \times K_{dil} \times 100}{A_{st} \times V}, \quad \text{(Eq. 3)}$$

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

The total hydroxycinnamic acids derivatives content was measured by assay of complex formation with $NaNO_2$–Na_2MoO_4, the absorbance was measured at 505 nm [18]. The total content of hydroxycinnamic acids derivatives in extract, expressed as chlorogenic acid was calculated according to the following equation:

$$X(\%) = \frac{C_x \times K_{dil}}{188 \times V}, \quad \text{(Eq. 4)}$$

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

The total organic acids content was determined by acid-base titration with the fixation end-point by potentiometric method [19]. The total content of organic acids in the extract, expressed as citric acid was calculated according to the following equation:

$$X(\%) = \frac{(V_{equiv} - V_x) \times 0.0032 \times K_{dil} \times K \times 100)}{V}, \quad \text{(Eq. 5)}$$

where, 0.0032 is the amount of citric acid, which is equivalent to 1 ml of sodium hydroxide solution (0.05 mol/l), g; V_{equv} is the volume of sodium hydroxide solution (0.05 mol/l), which was used for titration, ml; Vx is the volume of sodium hydroxide solution (0.05 mol/L), which was spent for titration in a blank experiment, ml; V is the volume of extract, ml; K_{dil} is the coefficient of dilution; K is the correction coefficient for 0.05 mol/l sodium hydroxide solution.

The content of caffeine was assessed by molecular adsorption analysis [19]. The total content of caffeine in the extract was calculated according to the following equation:

$$X(\%) = \frac{A \times K_{dil} \times m_{st} \times 100 \times 100}{A_{st} \times V}, \quad \text{(Eq. 6)}$$

where, A is the absorbance of analyzed solution; A_{st} is the absorbance of standard solution of caffeine; V is the volume of extract, ml; K_{dil} is the coefficient of dilution.

Antioxidant activity assay. The antioxidant activity of the extract was evaluated by the potentiometric method [20,21]. Antioxidant activity was calculated according to the following equation and expressed as mmol-equiv./$m_{dry\ res.}$:

$$AOA = \frac{C_{OX} - \alpha \times C_{red}}{1 + \alpha} \times Kdil \times 103 \times \frac{m_1}{m_2}, \quad \text{(Eq. 6)}$$

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

Epigallocatechin-3-O-gallate, 60 % extract of *C. sinensis* leaf was used as the standard.

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

Antimicrobial activity assay. In our study, we used the extract solution, the solvent of which was 60 % ethanol. The method of diffusion of the drug into agar was carried out by the "wells" method [22]. Studies of antibacterial activity were performed by the "wells" method. Preparation of microorganisms suspensions with determined concentrations of microorganisms (optical density) was carried out by the standard of turbidity (0.5 units according to the scale of McFarland) with equipment of Densi-La-Meter (Czech, wavelength 540 nm). Suspensions were prepared according to the equipment and information list. The colony forming unit was 107 microorganisms at 1 ml of growth medium and determined by the standard of McFarland). On solidified agar, by a pipette under sterile conditions in Petri dishes 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 were 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 at an angle of 45° (accounting for reflected light). The diameter of the growth retardation zones was measured by the caliper [23].

Gentamicin and fluconazole were used as reference drugs for assessing antimicrobial and anti-fungal activity.

Table 1. Results of determination of total content of phenolic compounds, catechins, flavonoids, hydroxycinnamic acids, organic acids, and caffeine in *C. sinensis* extracts

Sample	Total phenolic compounds, % ± SD	Total catechins, % ± SD	Total flavonoids, % ± SD	Total hydroxy-cinnamic acids, % ± SD	Caffeine, % ± SD	Total organic acids, % ± SD
Lipophilic extract	0.43 ± 0.02	–	–	–	1.21 ± 0.01	0.80 ± 0.01
96 % extract after chloroform	8.20 ± 0.20	8.39 ± 0.20	0.51 ± 0.02	0.77 ± 0.02	0.10 ± 0.01	0.53 ± 0.01
96 % extract	8.67 ± 0.20	8.40 ± 0.20	0.51 ± 0.02	0.78 ± 0.02	0.31 ± 0.01	1.32 ± 0.01

SD: standard deviation, n = 3.

Table 2. Results of antimicrobial and anti-fungi of *C. sinensis* extracts

Sample	Concentra-tionmmol/L, (expressed in total poly-phenols as gallic acid)	Diameter of the growth retardation zone, mm ± SD					
		Gramm-positive		Gramm-negative			Fungi
		Staphylo-coccus aureus	*Bacillus subtilis*	*Escherichia coli*	*Proteus vulgaris*	*Pseudo-monas aeruginosa*	*Candida albicans*
Lipophilic extract	0.008	32.33 ± 0.33	30.67 ± 0.20	29.00 ± 0.20	24.33 ± 0.33	25.67 ± 0.20	24.00 ± 0.20
96 % extract after chloroform	0.14	26.33 ± 0.33	25.67 ± 0.20	23.67 ± 0.67	21.33 ± 0.33	22.67 ± 0.20	20.00 ± 0.20
96 % extract	0.15	32.00 ± 0.20	31.00 ± 0.20	28.33 ± 0.33	24.33 ± 0.33	25.00 ± 0.20	24.00 ± 0.20
96 % ethanol	–	14.00 ± 0.20	14.00 ± 0.20	13.00 ± 0.20	13.00 ± 0.20	13.00 ± 0.20	13.00 ± 0.20
Gentamycin	0.003[a]	22.00 ± 0.20	24.00 ± 0.20	25.33 ± 0.33	25.00 ± 0.20	25.67 ± 0.67	12.00 ± 0.20
Fluconazole	0.003[b]	18.00 ± 0.20	12.00 ± 0.20	14.33 ± 0.33	12.33 ± 0.33	10.00 ± 0.20	20.00 ± 0.50

a: molar concentration of gentamycin; **b:** molar concertation of fluconazole; **SD:** standard deviation, n = 3.

Results

The lipophilic extract from *C. sinensis* leaf, as well as two ethanolic extracts, one with pre-treatment of the raw material with chloroform and the other without, were obtained. According to the results shown in *Table 1*, it was found that the content of phenolic compounds was 0.43 ± 0.02 % in the 96 % extract after treatment with chloroform and 8.20 ± 0.02 % in the 96 % extract without chloroform treatment. The difference in the content of phenolic compounds between the two 96 % extracts was 0.47 %, which practically corresponds to the content of phenolic compounds in the lipophilic extract.

The amount of catechins expressed as epigallocatechin-3-O-gallate was 8.40 ± 0.20 % in the 96 % extract, and 8.39 ± 0.02% in the 96 % extract after treatment with chloroform, in turn, in the lipophilic no catechins were detected in the extract. The quantitative content of the sum of flavonoids expressed as rutin in the two ethanolic extracts was 0.51 ± 0.02 and was not found in the lipophilic extract *(Table 1).*

Table 1 shows that the content of the total hydroxycinnamic acids in the 96 % ethanolic extract without treatment with chloroform was 0.78 ± 0.02 %, and in the extract after treatment with chloroform it was 0.77 ± 0.02 %. No hydroxycinnamic acids were found in the lipophilic extract.

The highest caffeine content was determined in the lipophilic extract (1.21 ± 0.02 %), and the lowest in the 96 % extract after treatment with chloroform (0.10 ± 0.01 %). The content of organic acids expressed as citric acid in the lipophilic extract of *C. sinensis* was 0.80 ± 0.01 %, in the 96 % ethanolic extract it was 1.32 ± 0.01 %, and in the 96 % extract after treatment with chloroform, it was 0.53 ± 0.01 %. The difference between the two ethanolic extracts was 0.79 ± 0.01 %, corresponding to the content of organic acids in the lipophilic extract (*Table 1*).

The lipophilic extract of green tea leaves contains higher levels of caffeine and organic acids, while phenolic compounds are presented in lesser amounts. In contrast, the ethanolic extracts show the opposite trend, with phenolic compounds dominating but lower levels of caffeine.

Table 2 illustrates the significant antimicrobial and antifungal activity exhibited by all the extracts obtained. The lipophilic extract demonstrates superior inhibition of growth compared to the 96 % ethanolic extract treated with chloroform, with respective improvements of 19 %, 18 %, 12 %, 12 %, 16 %, and 20 % against *S. aureus, E. coli, P. vulgaris*, *B. subtilis*, and *C. albicans*. Furthermore, the antimicrobial activity remains unchanged compared to the 96 % extract without chloroform treatment.

Comparing the obtained results with the reference standard gentamicin, it was observed that *S. aureus*, *B. subtilis*, and *E. coli* exhibited less sensitivity to gentamicin compared to the lipophilic extract, the 96 % extract after chloroform treatment, and the 96 % ethanolic extract of *C. sinensis* leaf. Conversely, *P. vulgaris* and *P. aeruginosa* were more sensitive to gentamicin. In terms of antifungal activity against *C. albicans*, it was found that the lipophilic extract, the 96 %

Table 3. Results of antioxidant activity of *C. sinensis* extracts

Samples	Antioxidant activity, mmol-eqv./$m_{dry\ res}$	Conditional term of antioxidant activity
Lipophilic extract	10.30 ± 0.10	Lower Medium
96 % extract after chloroform	605.00 ± 6.00	Very high
96 % extract	617.29 ± 6.00	Very high

Table 4. Level of antioxidant activity of *C. sinensis* leaf extracts and standard: epigallocatechin-3-O-gallate at concentration 0.03 mol/L

Sample	Concentration, mol/L	Antioxidant activity, mmol-eqv./$m_{dry\ res.}$ ± SD
Lipophilic extract	0.03	10.30 ± 0.10
96 % extract after chloroform		37.81 ± 2.00
96 % extract		37.80 ± 2.00
Epigallocatechin-3-O-gallate		30.78 ± 2.00

extract after chloroform treatment, and the 96 % ethanolic extract of *C. sinensis* leaf were more effective at inhibiting fungal growth than fluconazole *(Table 2)*.

The level of antioxidant activity of the obtained extracts was studied by the potentiometric method. According to the research results, it was found that the level of antioxidant activity of the lipophilic extract was 58.7 and 60.0 times less than the 96 % extract after treatment with chloroform and 96 % extract without treatment, respectively. Although, according to the developed classification of the "strength" of antioxidant activity according to Maslov, the lipophilic extract has a level of antioxidant activity that is below average, 96 % extract after treatment with chloroform is very high, and 96 % extract without treatment is very high *(Table 3)*.

Further, it was prepared solutions (in terms of the amount of polyphenols expressed as gallic acid) of extracts with 0.03 M concentration of *C. sinensis* leaf extracts and epigallocatechin-3-O-gallate. As a result of the study, it was found that the level of antioxidant activity of 96 % extract, 96 % extract after chloroform *C. sinensis* leaf were higher of standard epigallocatechin-3-O-gallate, whereas the level of antioxidant activity of lipophilic extract was lower *(Table 4)*.

Discussion

The analyzed extracts from *C. sinensis* leaves exhibited high antimicrobial and antifungal activity against strains of *S. aureus*, *P. aeruginosa*, *P. vulgaris*, *B. subtillis*, and *C. albicans*. While the concentration of polyphenols in the extracts was three times lower than that of gentamicin and fluconazole, it might initially appear that the antimicrobial and antifungal activity of the *C. sinensis* leaf extracts is significantly inferior to that of gentamicin and fluconazole. However, it is important to note that gentamicin carries serious toxicity risks to the auditory nerve, kidneys, and liver, potentially leading to severe complications. In contrast, despite the lower concentration, fluconazole and *C. sinensis* leaf extracts exhibited comparable antifungal effects. Fluconazole is recognized as a leading antifungal agent, albeit with weaker activity against gram-negative and gram-positive bacteria. In contrast, *C. sinensis* leaf extracts demonstrated sensitivity against bacterial and fungal strains. Therefore, *C. sinensis* leaf extracts represent a promising pharmaceutical option with a wide spectrum of action against various strains of bacteria and fungi, while also possessing reduced toxicity.

Many studies show that the antimicrobial and antifungal effect is associated with the presence of catechin derivatives. We set out to disprove this by producing three *C. sinensis* leaf extracts: a lipophilic extract, a 96 % extract without chloroform treatment, and a 96 % extract after chloroform treatment. As a result of three different extractions, the biologically active substances of the *C. sinensis* leaf were separated by polarity. Thus, derivatives of organic acids, caffeine, as well as some phenolic compounds were extracted into the lipophilic extract; we assume this may be the presence of gallic acid. At the same time, 96 % of the extract was extracted after treatment with chloroform, phenolic compounds, catechins, flavonoids, hydroxycinnamic acids, and some parts of organic acids and caffeine. Meanwhile, in the 96 % extract without treatment with chloroform, all the main biologically active substances were extracted from the raw material, phenolic compounds, caffeine, and organic acids.

Based on the above results of the antimicrobial and antifungal activity of the extracts, it was found that the 96 % extract after treatment with chloroform is an order of magnitude inferior to the lipophilic and 96 % extract without treatment with chloroform. Thus, we can say that organic acids and caffeine also have antimicrobial and antifungal effects, thereby potentiating the activity of catechins and other phenolic compounds. At the same time, the level of antioxidant activity of the lipophilic extract is significantly lower than ethanol extracts, which suggests that caffeine and organic acids in no way have an antioxidant effect. Meanwhile, 96 % of the extracts have a high level of antioxidant activity.

Hence, we hypothesize that caffeine, organic acids, and catechins interact synergistically to enhance antimicrobial and antifungal activity, while caffeine and organic acids individually do not exhibit antioxidant effects. As a result, the extract, tincture, and infusion constitute complex preparations wherein biologically active substances interact synergistical-

ly, operating through multiple mechanisms [24]. In our view, it is inaccurate to attribute the pharmacological activity solely to one group of biologically active substances.

Conclusions

1. We conducted a study to quantify the content of phenolic compounds, catechins, flavonoids, hydroxycinnamic and organic acids, and caffeine in lipophilic and two ethanolic extracts of green tea leaves.

2. The lipophilic extract demonstrated potent inhibition of Gram-positive and Gram-negative bacteria as well as fungi, albeit with a low level of antioxidant activity.

3. Our findings suggest that caffeine and organic acids act as catechin agonists, enhancing the antimicrobial and antifungal effects of *C. sinensis* leaf extracts. Therefore, the lipophilic extract holds promise for the development of future antimicrobial and antifungal drugs.

Conflicts of interest: authors have no conflict of interest to declare.
Конфлікт інтересів: відсутній.

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