SMTCT
Society of Land Measurements and Cadastre from Transylvania

Maslov O *et al*. (2024)
Notulae Scientia Biologicae
Volume 16, Issue 3, Article number 12062
DOI:10.15835/nsb16312062
Research Article

NSB
Notulae Scientia Biologicae

Analysis of chemical composition, antimicrobial, antioxidant activities of pomegranate fruit thick and green tea leaf extracts

Olexander MASLOV[1]*, Mykola KOMISARENKO[2], Dmytro PLIS[1], Svitlana PONOMARENKO[3], Tetiana OSOLODCHENKO[3], Sergii KOLISNYK[1]

*[1]National University of Pharmacy, Department of General Chemistry, 61001, Kharkiv, Ukraine; alexmaslov392@gmail.com (*corresponding author); altoxchemistry@yahoo.com; s_kolesnik@nuph.edu.ua*
[2]National University of Pharmacy, Department of Pharmacognosy and Nutriciology, 61001, Kharkiv, Ukraine; a0503012358@gmail.com
[3]Mechnikov Institute of Microbiology and Immunology of the NAMS of Ukraine, Laboratory of Biochemistry and Biotechnology, 61001, Kharkiv, Ukraine; chemistry1@online.ua; imi_lbb@ukr.net

Abstract

Infection diseases is a worldwide important problem for medicine and pharmacy. The purpose of this work was to study and compare phytochemical composition, antimicrobial, antioxidant potential of pomegranate fruit thick and green tea leaf liquid extracts. The quantification of biologically active substances (BAS) was performed using spectrophotometric, titrimetric, and HPLC analysis methods. Antioxidant activity was measured through a potentiometric method, while antimicrobial and antifungal effects were assessed using the well method and determining the minimum inhibition concentration. The total content of phenolic compounds was 0.40 and 10.10%, organic acids – 5.80 and 1.60% for pomegranate fruit thick and green tea leaf extract. The total content of catechins in the green tea leaf extract was 10500.0 mg/100 g, where epicatechin-3-O-gallate was dominated (3730.0±2.00 mg/100 g). The total content of anthocyanins in the pomegranate fruit thick extract was 107.10 mg/100 g, where cyanidin-3-O-glucoside was dominated (41.74 ± 0.10 mg/100 g), whereas delphinidin-3-O-glucoside had a lowest content (4.99 ± 0.10 mg/100 g). Both extracts possessed a high antioxidant potential, and effective antimicrobial effects. The antioxidant, antimicrobial activity of pomegranate fruit extract was higher than green tea leaf extract. In addition, we assumed that anthocyanins had higher antioxidant, antimicrobial properties than catechins. These findings would promote application of pomegranate fruits extract as pharmaceuticals and nutraceuticals.

Keywords: anthocyanins; antimicrobial effect; antioxidant power; catechins; green tea leaf; pomegranate fruit

Introduction

Nowadays, the problem of bacterial infection is still relevant. According to recent statistical studies, it has been found that every year 13.7 million people per year die from bacterial infections in the world. The

Received: 28 May 2024. Received in revised form: 02 Aug 2024. Accepted: 20 Sep 2024. Published online: 24 Sep 2024.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

mortality rate for all ages was 99.6 deaths per 100.000 population. The 54.9% of the 7.7 million deaths was caused by the Gramm-positive strains such as *Staphylococcus aureus*, and *Gramm-negative strains*: *Klebsiella pneumonia, Escherichia coli, Streptococcus pneumoniae, Pseudomonas aeruginosa,* whereas *S aureus* was associated with than 1.1 million deaths. In 135 countries S. aureus was the problem one in causing the death over 15 years of age (940.000) (Ikuta *et al.*, 2019). In addition, an important threat to human populations is fungal infection. According to the latest statistics, every year 1,433,000 people suffer from systemic candidal infections, of which approximately 611 thousand people die annually (Denning, 2022; Arsene *et al.*, 2022). Thus, this problem affects all areas of application of antibiotics, including the treatment of urinary tract infections."

Today, medical plants that are rich source of flavan-3-ols and anthocyanins have a high attention from scientific community (Maslov *et al.*, 2023). Above all, it relates with fact that some resistance pathogens are more sensitive to natural products, secondly, natural compounds have potent antioxidant effect and moreover, the side effects are rarely happened after application of natural compounds than after synthetic drugs.

The perspective source of anthocyanins was chosen pomegranate fruits, whereas a green tea leaf is the source of catechins. Pomegranate (*Punica granatum* L.) is a fruit-bearing deciduous tree that belongs to the family *Lythraceae*, the cultivation has carried out in the region with a warm climate such as South Caucasus, Transcaucasia, South and Central Asia and in Mediterranean region (Vučić *et al.*, 2019). The chemical composition of peel is represented by ellagotannins, catechins, flavonoids, whereas arils contain mostly anthocyanins and organic acids (Maslov *et al.*, 2023). The composition of green tea leaf (*Camellia sinensis* L.) contains: catechins (epigallocatehin-3-O-gallate, epicatehin, (+)-catechin, epigallocatechin), organic acids (oxalic acid), flavonoids (rutin) and hydroxycinnamic acids (caffeic acid) (Maslov *et al.*, 2021).

There are a lot of numbers of research about investigation pharmacological activity of *P. granatum* fruit and *C. sinensis* extracts. It is known that anthocyanins and catechins from *P. granatum* fruit, and *C. sinensis* leaf possess: antimicrobial, anti-inflammatory, anti-hyperglycemic, immune-modulation, and anticancer effects. Besides, in folk medicine *P. granatum* and *C. sinensis* leaf are traditionally applied to treat fever, urinary and skin infections, diabetes, cancers, liver and diseases (Skrovankova *et al.*, 2015). In our view, the anthocyanins and catechins are perspective for the development of new antimicrobial, and antioxidant pharmaceuticals. Therefore, the aim of investigation was to assess antimicrobial, and antioxidant potential of *P. granatum* fruit thick and *C. sinensis* leaf liquid extracts and provide phytochemical analysis of obtained extracts.

Materials and Methods

Raw material

The study focused on *P. granatum* fruits, which were harvested from areas where they are naturally grown. The collection took place in 2022 following the fruiting season, Azerbaijan (40°72′35″ N, 46°44′10″ E). The study focused on the leaf of *C. sinensis* from the 'Chun Myn' variety, which was gathered as raw material in the Anhui province of China during the months of March through May.

Reagents

Acetonitrile (purchased from "Allchem", Kharkiv), acetic acid (purchased from "Allchem", Kharkiv), phosphoric acid (purchased from "Allchem", Kharkiv), methanol (purchased from "Allchem", Kharkiv) cyanidin-3-O-glucoside (≥98.0%), cyanidin-3,5'-diglucoside (≥98.0%), pelargonidin-3,5-diglucoside (≥98.0%), delphinidin-3,5-diglucoside (≥98.0%), pelargonidin-3-O-glycoside (≥98.0%), delphinidin-3-O-glucoside (≥98.0%), epicatechin (≥98.0%), epigallocatechin-3-O-gallate (≥98.0%), epigallocatechin (≥98.0%), epicatechin-3-O-gallate were purchased in Sigma Aldrich Company, Lublin, Poland.

Technique of extraction

A 100.0 g (exact mass) of *P. granatum* arils was pressed, then it was added of 96% ethanol in a threefold amount to the extraction, after that filtration, then obtained filtrate was concentrated by a vacuum-evaporator at a temperature of 50-60 °C until the humidity of the extract is 25%.

The *C. sinensis* extract was obtained by the following way: the raw material was conducted with 60% ethanol at 80 °C within 1 hour with a condenser, ratio raw material/solvent – 1/20. The extraction technique was completed twice to provide totally extract all BAS, then the filtrates were joint and evaporated by vacuum rotary to ratio of extract to raw material 1:2.

Phytochemical analysis

The total phenolic compounds were quantified using the Folin-Ciocaltau method, with absorbance readings taken at 760 nm (Maslov *et al*., 2022). The total catechins were assessed using the vanillin reagent assay, where absorbance was measured at 505 nm (Maslov *et al.*, 2022). For the quantification of total anthocyanin content, molecular adsorption analysis was utilized, with measurements of absorbance at 546 nm (Maslov *et al.*, 2023). The content of total organic acids was established through acid-base titration, using a potentiometric method to determine the end-point (Maslov *et al.*, 2021).

Antioxidant effect assay

Antioxidant activity of extract was evaluated by potentiometric method (Maslov *et al.*, 2021; Maslov *et al.*, 2021).

The standardized *C. sinensis* leaf liquid extract, which was obtained by 60% ethanol and solution of epigallocatechin-3-O-gallate were used as the reference standards.

HPLC analysis of C. sinensis leaf extract

For the analysis, a Prominence LC-20 Shimadzu liquid chromatography system with a Thermo Scientific Syncronis aQ C18 column (4.6 × 250) was utilized. All analyses were conducted at a temperature of 40 °C. The mobile phases consisted of a methanol aqueous solution (A) and a 1.0% solution of phosphoric acid (B). The gradient protocol started with 20-42% A over the first 15 minutes, shifted to 42-43% A from 15 to 25 minutes, changed to 43-90% A from 25 to 45 minutes, maintained 90% A from 45 to 55 minutes, decreased to 20% A from 55 to 60 minutes, and then held at 20% A from 60 to 70 minutes. Prior to use, the mobile phases were filtered using 25 mm × 0.45 μm Supelco Iso-Disc Filters PTFE 25-4 and degassed. A flow rate of 0.5 mL/min was maintained, and the injection volume of the samples was 5 μL. Detection wavelengths were set at 255, 286, and 350 nm. Chromatographic peaks of analytes were identified by the following similarity indexes, which were calculated between the test substance and the standard according to the formulas:

$$I_T = 1 - |T_{st} - T_u|$$
$$I_{255} = 1 - \left|h_{255_{st}} - h_{255_u}\right|$$
$$I_{286} = 1 - \left|h_{286_{st}} - h_{286_u}\right|$$
$$I_{350} = 1 - \left|h_{350_{st}} - h_{350_u}\right|$$

where, I_T – retention time similarity index, T_{st} – retention time of standard (min), T_u – test substance retention time (min), I_{255}, I_{286} and I_{350} – spectral similarity indices, h_{255st}, h_{286st} and h_{350st} – spectral characteristics of the standard, h_{255u}, h_{286u} и h_{350u} – spectral characteristics of the test substance.

The least among the three similarity index values of spectral characteristics dictates the similarity level (IL) between substances and standards based on these traits. A higher IL value increases the probability of more precise identification of the substance. Substances whose similarity index with the catechin standard was at least 0.7, and whose peaks on the chromatogram appeared between the catechin peak and the earliest flavonoid peak, were classified as catechins (Khodakov, 2013).

HPLC analysis of P. granatum fruit thick extract

The anthocyanins content was determined using a Perkin-Elmer Series 400 HPLC and a semi-preparative Dynamax Rainin Model SD-300 Liquid Chromatograph, which was outfitted with a Hewlett-Packard 1040A photodiode array detector. The analytical HPLC system employed a 250 × 4.6-mm inner diameter Prodigy 5 ODS 3 column from Phenomenex. The mobile phases were arranged into a binary solvent system comprising: (A) 100% acetonitrile and (B) a mixture of 5% acetonitrile, 10% acetic acid, and 1% phosphoric acid. The gradient used for the solvents was as follows: 0-5 min at 100% B, 5-20 min transitioning from 20% to 80% A/B, 20-25 min at 40-60% A/B, and 25-30 min back to 100% B. The flow rate was set at 1 mL/min with an injection volume of 50 μL. Samples were filtered using a 0.45-μm Millipore filter type HA (Millipore Corp., Bedford, Mass., U.S.A.). Detection occurred at 520 nm, with the quantification of individual peaks expressed as a percentage of the total peak area.

Test organisms

S. aureus ATCC 25923, E. coli ATCC 25922, B. subtilis ATCC 6538, C. albicans ATCC 885/653, P. vulgaris NTCS 4636, and *P. aeruginosa ATCC 27853* were employed following established guidelines for evaluating the antimicrobial efficacy of pharmaceuticals.

Screening antimicrobial activity of extracts

The method of diffusion of the drug into agar carried out using the method of "wells" (Volyanskiy *et al*., 2006; Volyanskiy *et al*., 2004) (Table 1).

Table 1. Interpretation criteria for microbial sensitivity

Microbial sensitivity	Diameter of the growth retention zone, mm
High sensitivity	>25
Sensitive	15-25
Low sensitivity	10-15
Not sensitivity	<10

Assay of determination of minimum inhibitory concentration (MIC)

MIC is defined as the smallest concentration of an antibacterial agent that entirely prevents bacterial growth. The MIC for various extracts was determined through the broth microdilution technique (Mbarga *et al*., 2021).

Results

Phytochemical analysis of BAS

According to obtained results shown in Table 2 the green tea leaf extract (10.10 ± 0.25%) had higher content of phenolic compounds, than in *P. granatum* thick fruit extract (0.40 ± 0.02%).

Table 3 demonstrates that the total content of anthocyanin in *P. granatum* thick fruit extract was 0.11 ± 0.002%, whereas in green tea leaf extract anthocyanin was not presence. The percentage of anthocyanin out of total polyphenols was 28% in *P. granatum* extract.

The highest amount of organic acids was determined in *P. granatum* thick fruits extract (5.80 ± 0.50%), whereas in the green tea leaf extract it was lower 72% (1.60 ± 0.10%). In *P. granatum* extract, the total organic acids were in 14.5 times higher than polyphenols, whereas in the green tea leaf, the total organic acids were in 6.3 times lower than polyphenols (Table 3).

Table 2. Quantitative content (in %, as mean ± SD) of total phenolic compounds, anthocyanin and organic acids

Sample	Total phenolic compounds	Total anthocyanin	Total catechin	Total of organic acids
P. granatum thick extract	0.40 ± 0.02	0.11 ± 0.002	-	5.80 ± 0.50
C. sinensis leaf extract	10.10 ± 0.25	-	10.47 ± 0.25	1.60 ± 0.10

*Note: SD – standard deviation, n=3, $p<0.05$

The HPLC method was used to carry out a qualitative and quantitative analysis of catechins and anthocyanins in the obtained extracts of *C. sinensis* leaf and *P. granatum* fruits extract. According to the results of the study, 5 catechins were identified in *C. sinensis* leaf extract, whereas in *P. granatum* fruits extract 6 anthocyanins (Figures 1 and 2).

The total content of catechins in the obtained *C. sinensis* leaf extract was 10500.0 mg/100 g. Among catechins, epigallocatechin-3-O-gallate dominates – 3730.0±74.6 mg/100 g (35.52% out of the total catechins), whereas the lowest content was (+)-catechin 210 mg/100 g (2.00% out of the total catechins). (Table 3)

As shown in Table 4, cyanidin-3-O-glucoside dominated among all anthocyanins (38.98% out of the total anthocyanins), pelargonidin-3,5-diglucoside (19.49% out of the total anthocyanins) was in second place, and the lowest content was delphinidin-3-O-glucoside (4.66% out of the total anthocyanins).

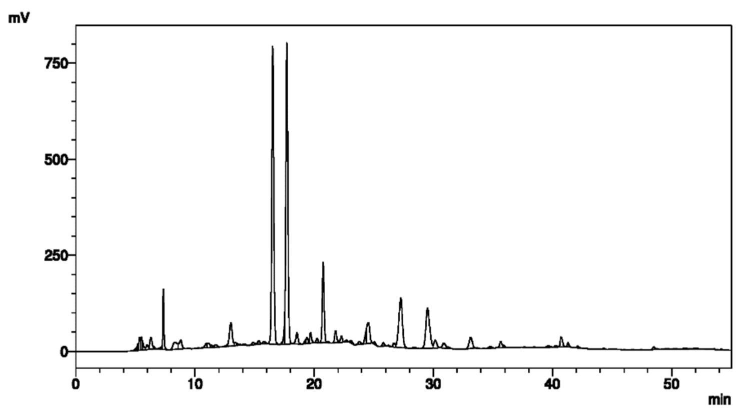

Figure 1. HPLC fingerprint (255 nm) of the *C. sinensis* leaf extract

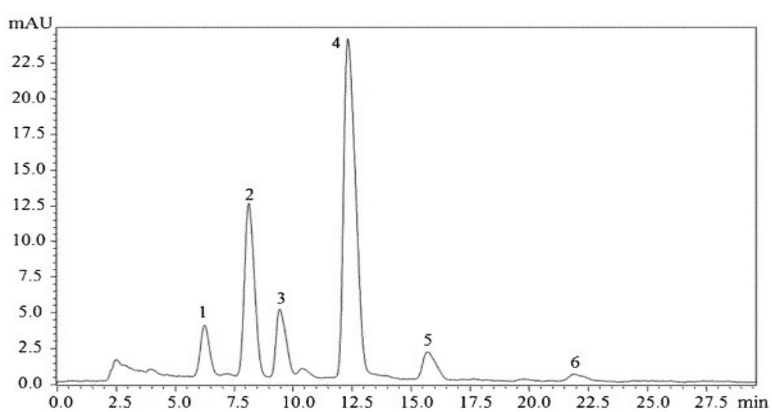

Figure 2. HPLC fingerprint (520 nm) of the *P. granatum* fruit thick extract

Table 3. Chemical composition of catechins in *C. sinensis* leaf extract by HPLC-UV analysis

№	Catechins	Retention time (min)	Similarity index (IL)	Catechins (mg/100 g of extract ± SD)	% out of total catechins
1	Epigallocatechin	13.013	0.875	2760.0±55.2	26.29
2	(+)-catechin	13.780	0.996	210.0±4.2	2.00
3	Epicatechin	16.494	0.851	1010.0±20.2	9.62
4	Epigallocatechin-3-O-gallate	17.686	0.990	3730.0±74.6	35.52
5	Epicatechin-3-O-gallate	20.754	0.814	2788.0±55.8	26.57
	Total catechins			10500.0	

*Note: SD – standard deviation, n=3, $p<0.05$

Table 4. Chemical composition of anthocyanins in *P. granatum* fruit thick extract by HPLC analysis

№	Anthocyanins	Retention time (min)	Anthocyanins (mg/100 g of extract ± SD)	% out of total anthocyanins
1	Cyanidin-3,5'-diglucoside	6.250	12.70±0.25	11.86
2	Pelargonidin-3,5-diglucoside	7.650	20.87±0.42	19.49
3	Delphinidin-3,5-diglucoside	9.320	19.06±0.38	17.80
4	Cyanidin-3-O-glycoside	12.470	41.74±0.83	38.98
5	Pelargonidin-3-O-glycoside	16.120	7.71±0.15	7.20
6	Delphinidin-3-O-glucoside	21.700	4.99±0.10	4.66
	Total anthocyanins		107.10	

*Note: SD – standard deviation, n=3, $p<0.05$

Antioxidant activity

A potentiometric method for determining antioxidant activity was used to evaluate the effect of the obtained extracts. Table 5 shows that the level of antioxidant activity of *P. granatum* fruits extract significantly interferer to the *C. sinensis* leaf extract. The antioxidant activity of *C. sinensis* leaf higher in 8.4 times of *P. granatum* extract.

In light of the data obtained, it can be established that the *C. sinensis* leaf extract has the highest level of antioxidant activity. According to the modern classification of antioxidant activity, which was previously developed by us (Maslov *et al.*, 2021), it was found that all extracts obtained have a high level of antioxidant activity. Moreover, a comparative analysis of the "strength" of antioxidant activity was carried out with the gold standard *C. sinensis* leaf. Further, it was prepared solutions (in terms of the amount of polyphenols expressed as gallic acid) of extracts with 0.03 M concentration of *P. granatum* thick fruits extract, *C. sinensis* leaf and epigallocatechin-3-O-gallate. As a result of the study, it was found that the level of antioxidant activity of *P. granatum* extract was higher 64% of *C. sinensis* leaf extract and 61% epigallocatechin-3-O-gallate (Table 6).

Table 5. Level of antioxidant activity of *P. granatum* thick extract, *C. sinensis* leaf extract

Sample	Antioxidant activity (mmol-eqv. / $m_{dry\ res.}$ ± SD)	Conditional term of antioxidant level
P. granatum thick fruits extract	65.01 ± 1.00	High level
C. sinensis leaf extract	548.79 ± 10.98	Very high level

*Note: SD – standard deviation, n=3, $p<0.05$

Table 6. Level of antioxidant activity of *P. granatum* thick extract, *C. sinensis* leaf extract and standard: epigallocatechin-3-O-gallate at concentration 0.03 mol/L

Sample	Concentration (mol/L)	Antioxidant activity (mmol-eqv./$m_{dry\ res.}$ ± SD)
P. granatum thick fruits extract	0.03[a]	78.40 ± 1.00
C. sinensis leaf extract		27.49 ± 1.00
Epigallocatechin-3-O-gallate		30.78 ± 1.00

*Note: SD – standard deviation, n=3, p<0.05, a – molar concentration of pomegranate thick extract and green tea leaf extract was calculated as total phenolic compounds expressed as gallic acid

Antimicrobial activity

In this research work, the antimicrobial and antifungal activity of the obtained *P. granatum* thick fruits and *C. sinensis* leaf extract was investigated against the following strains of *S. aureus*, *B. subtilis*, *E. coli*, *P. vulgaris*, *P. aeruginosa*, as well as a strain of the fungus *C. albicans*. According to the obtained results, extracts obtained from the *P. granatum* fruit and *C. sinensis* leaf had an effective antimicrobial and antifungal effect.

Among pathogens strains, *P. granatum* fruits extract was the most inhibits *B. subtilis* strains (34.00 ± 0.20 mm), whereas at the second place were *S. aureus* (30.00±0.20 mm) and *B. subtilis* (31.00 ± 0.20 mm), Gramm-positive strains *P. vulgaris* was the most resistance bacteria to the action of *P. granatum* fruits extract (20.00 ± 0.40 mm). Comparing results with *C. sinensis* leaf extract, it was determined that *P. granatum* fruits extract was 3.3, 23.5, 32 better inhibit bacterial strains of *S. aureus*, *B. subtilis, E. coli* than *C. sinensis* leaf extract, respectively. Comparing obtained results with reference standard gentamycin, it was found that *S. aureus*, *B. subtilis* and *E. coli* were 27, 29 and 18% more sensitive to *P. granatum* fruits extract than gentamycin. Whereas, *P. vulgaris* and *P. aeruginosa* was 20 and 3% more sensitive to gentamycin.

Anti-fungal investigation against *C. albicans* showed that *P. granatum* fruits extract 13 and 17% more actively inhibit the growth of fungi than *C. sinensis* leaf and fluconazole, respectively (Table 7).

Table 7. Retardation zone (mm) resulting from the screening of antimicrobial activity of *P. granatum* thick extract, *C. sinensis* leaf extract and standards: gentamycin, fluconazole

Sample	Concen-tration (mM)	Diameter of the growth retardation zone (mm ± SD)					
		Gramm-positive		Gramm-negative			Fungi
		S. aureus ATCC 25923	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922	*P. vulgaris* ATCC 4636	*P. aeruginosa* ATCC 27853	*C. albicans* ATCC 653/885
P. granatum thick extract	0.007[a]	30.00 ± 0.20	34.00 ± 0.20	31.00 ± 0.20	20.00 ± 0.40	25.00 ± 0.30	24.00 ± 0.30
C. sinensis leaf extract	0.009[a]	29.00 ± 0.20	26.00 ± 0.30	21.00 ± 0.40	24.00 ± 0.30	28.00 ± 0.20	21.00 ± 0.40
Gentamycin	0.003	22.00 ± 0.40	24.00 ± 0.30	25.33 ± 0.33	25.00 ± 0.30	25.67 ± 0.30	12.00 ± 0.80
Fluconazole	0.003	18.00 ± 0.50	12.00 ± 0.80	14.33 ± 0.70	12.33 ± 0.80	10.00 ± 0.80	20.00 ± 0.50

*Note: SD – standard deviation, n=3, a – molar concentration of pomegranate thick extract and green tea leaf extract was calculated as total phenolic compounds expressed as gallic acid

Minimum inhibitory concentrations

The investigated extracts significantly inhibit the bacterial and fungi strains with MIC. In the previously above conducted antimicrobial study, the extract of *P. granatum* fruits was the most active independently of the tested strains. Table 8 shows, *S. aureus* among all Gram-positive strains was the most sensitive against the *P. granatum* fruits extract with MIC value of 0.22 μM, whereas among Gram-negative strains *E. coli* was the most active. *C. sinensis* leaf extract MIC values was 80% lower. The highest MIC value of *C. sinensis* leaf extract was against fungi pathogens *C. albicans*. The MIC value of *P. granatum* fruits extract against pathogens *S.*

aureus, *E. coli*, *P. vulgaris, P. aeruginosa* and *B. subtilis* was significantly lower than in the case of *C. sinensis* leaf extract.

Table 8. Minimal inhibitory concentration of the different *P. granatum* thick extract, and *C. sinensis* leaf extract against the 6 references pathogens

Sample	MIC (μM)					
	S. aureus **ATCC 25923**	***B. subtilis*** **ATCC 6633**	***E. coli*** **ATCC 25922**	***P. vulgaris*** **ATCC 25922**	***P. aeruginosa*** **ATCC 27853**	***C. albicans*** **ATCC 653/885**
P. granatum thick extract	0.22	0.22	0.22	0.88	0.44	0.44
Green tea leaf extract	0.70	0.70	2.80	2.80	2.80	5.60

Discussion

Phytochemical analysis of BAS

The content of BAS in *P. granatum* fruit extracts was quantified by spectrophotometric, titrimetric and HPLC methods of analysis. The organic acids were present in both extracts, where the highest content of organic acids was determined in *P. granatum* fruits extract than in *C. sinensis* leaf extract. In our view, it relates with different purpose of accumulation organic acids. The organic acids are precursor for biosynthesis of sugars in fruits, whereas in leaf, organic acids only play a role in photosynthesis as result there is no purpose of high accumulation organic acids in leaf (Arena *et al.*, 2023). Russo (2018) investigated anthocyanin content of *P. granatum* fruit 50% methanol extract by HPLC method. According to their results, it was detected following anthocyanins (mg/100 g per extract): cyanidin-3-O-glucoside (2.80 mg/100 g), cyanidin-3-O-rutinoside (4.60 mg/100 g), cyanidin-3-O-xyloside (4.20 mg/100 g), pelargonidin-3-O-glucoside (1.70 mg/100 g) and delphinidin-3-O-glucoside (1.10 mg/100 g). Compering with our results, the content of anthocyanins in our research was lower, but cyaniding-3-O-glucoside was dominated in both extracts. The chemical composition of fruit is changed during fruits development, its ripening, and different cultivars.

Antioxidant activity

At first glance, the *C. sinensis* leaf extract had significantly higher antioxidant potential than *P. granatum* fruit extract. However, comparing extracts at the same molar concentration, it was found that *P. granatum* fruit extract had 2 times higher the level of antioxidant activity than *C. sinensis* leaf extract. In our view, it relates with the fact that anthocyanins more potent antioxidants than catechins. Lapidot (1999) determined antioxidant activity of malvidin-3-glucoside, catechin, malvidin and resveratrol by the method of oxidation myoglobin with H_2O_2. It was shown that inhibition efficiency of the antioxidant decreased in following order: malvidin-3-glucoside > catechin > malvidin > resveratrol. In the research of Muselik (2007), it was carried out evaluation the level of antioxidant activity of derivatives of catechins: epicatechin, (+)-catechin, epicatehin, epicatechin-3-O-gallate, gallocatechin; and anthocyanins: cyanidin-galactoside, malvidin-3-glucoside and delphinidin-3-glucoside by ferric reducing antioxidant power assay. It was found the level of antioxidant activity decreased in the following order: epicatechin-3-O-gallate > delphinidin-3-glucoside > cyanidin-galactoside > gallocatechin > malvidin-3-glucoside > epicatechin > catechin. The antioxidant activity of epicatechin-3-O-gallate had the highest antioxidant power whereas the catechin – the lowest one, where cyanidin-3-galactoside interfere to epicatechin-3-O-gallate, but greater than other derivatives of catehins. The major part of composition of *C. sinensis* leaf is presented by epicatechin-3-O-gallate and low amount – epicatechin and (+)-catechin. However, it is quite difficult to evaluate the contribution of each compound on total antioxidant power of extract as well as it is unknown whether catechins interact by synergistic way or antagonistic one. Thus, the level of antioxidant activity of extract depends not only on composition of extract, but also, on ration and interaction of compounds.

Antimicrobial activity

The analyzed *P. granatum* fruit and *C. sinensis* leaf extracts showed high antimicrobial and antifungal activity against the following strains of *S. aureus*, *P. aeruginosa*, *P. vulgaris, B. subtillis* and *C. albicans*. According to the obtained data, at first glance it can be considered that the antimicrobial and antifungal activity of *P. granatum* fruit and *C. sinensis* leaf extracts is significantly inferior to the action of gentamicin and fluconazole, because their concentration of solutions was in 3 times lower than the content of polyphenols in the extract. However, we would like to note that gentamicin has serious toxicity to the auditory nerve, kidneys and liver, which can lead to serious complications of the disease. Comparing the antifungal effects of fluconazole and *P. granatum* fruit and *C. sinensis* leaf extracts, it was found that they inhibited the growth of the fungal strain at the same level, while the concentration of fluconazole was also lower, like gentamicin. We can declare that fluconazole is a leader as anti-fungi medicine, but at the same time it weakly inhibits the growth of gram-negative and gram-positive bacteria, but to *P. granatum* fruit and *C. sinensis* leaf extracts both strains of bacteria and fungus are sensitive. Thus, *P. granatum* fruit and *C. sinensis* leaf extracts is a combined pharmaceutical that affects different mechanisms of vital activity of bacteria and fungi, thereby having a wide spectrum of action against different strains of bacteria and fungi, and at the same time not possessing serious toxicity.

The *P. granatum* fruit is a rich source of anthocyanins, whereas *C. sinensis* leaf of catechins. It is well known that anthocyanins biosynthesis pathway is based on chemical conversion of catechins. Li (2022) declared that at the beginning of ripping period the content of anthocyanin starts increasing, whereas the content of catechins decreasing. However, a question which of this group of flavonoids possess higher antimicrobial activity is still actually for today. In our research, it was comparing antimicrobial potential of *P. granatum* fruit and *C. sinensis* leaf extracts. In the antimicrobial tests, which carried out by method of well, it was shown the *P. granatum* fruit extract was more active against pathogens *P. aeruginosa, E. coli*, *B. subtilis*, *C. albicans* whereas *P. vulgaris* were sensitive to both extracts practically at the same level. Furthermore, it was determined MIC values for both extracts, as result *P. granatum* fruit extract had better results than *C. sinensis* leaf extract. Therefore, based on mentioned results, it was assumed that antimicrobial activity of anthocyanins higher than catechins. However, both extracts had a high content of organic acids and its presence should not be neglected. Further in our research, we planned to answer on question whether impact organic acids on antimicrobial potential or not.

Conclusions

It was found that total phenolic compounds were higher in *C. sinensis* leaf extract, whereas total organic acids were in *P. granatum* fruit thick extract. Both extracts possessed a high antioxidant potential, and effective antimicrobial effects. Although we assumed that anthocyanins had higher antioxidant, antimicrobial properties than catechins. In future studies, the hypothesized impact of organic acids on antimicrobial effects should be verified by isolation of organic acids from both extracts. These findings would promote application of P. granatum fruits extract as pharmaceuticals and nutraceuticals.

Authors' Contributions

Conceptualization: OM, MK, SP; Data curation: OM, MK, SP; Formal analysis: OM, MK; Funding acquisition: DP; Investigation: OM, DP, TP; Methodology: TP, MK; Project administration: SK, TP% Resources; Software; Supervision: SK, TO; Writing - original draft: OM; Writing - review and editing: SK, TO. Please add at the end: All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Aaby K, Ekeberg D, Skrede G (2007). Characterization of phenolic compounds in strawberry (*Fragaria ananassa*) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity. Journal of Agricultural and Food Chemistry 55(11):4395-406. *https://doi.org/10.1021/jf0702592*

Aaby K, Grimmer S, Holtung L (2013). Extraction of phenolic compounds from bilberry (*Vaccinium myrtillus* L.) press residue: Effects on phenolic composition and cell proliferation. LWT - Food Science and Technology 54(1):257-264. *https://doi.org/10.1016/j.lwt.2013.05.031*

Arena ME, Povilonis IS, Borroni V, Pérez E, Pellegrino N, Cacciatore C, Radice S (2023). Changes in carbohydrates, organic acids, and minerals at different development stages of hexachlamys edulis fruit, a wild South American species with horticultural potential. Horticulturae 9(3):314. *https://doi.org/10.3390/horticulturae9030314*

Arsene MM, Viktorovna PI, Davares AK, Parfait K, Andreevna SL, Mouafo HT, … Sergueïevna DM (2022). Antimicrobial and antibiotic-resistance reversal activity of some medicinal plants from Cameroon against selected resistant and non-resistant uropathogenic bacteria. Frontiers in Bioscience-Elite 14(4):25. *https://doi.org/10.31083/j.fbe1404025*

Denning D (2022). Global incidence and mortality of severe fungal disease. Lancet 400:1-21. *http://dx.doi.org/10.2139/ssrn.4560971*

Ikuta KS, Swetschinski LR, Robles AG (2019). Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 400:2221-2248. *https://doi.org/10.1016/s0140-6736(22)02185-7*

Khodakov IV (2013). Method for the identification of polyphenols in plant extracts using HPLC. Determination of the composition of soy isoflavones. Methods Objection Chemistry in Analysis 8:(2):132-142.

Lapidot T, Harel S, Akiri B, Granit R, Kanner J (1999). pH-dependent forms of red wine anthocyanins as antioxidants†. Journal of Agricultural and Food Chemistry 47(1):67-70. *https://doi.org/10.1021/jf980704g*

Li J, Shi C, Shen D, Han T, Wu W, Lyu L, Li W (2022). Composition and antioxidant activity of anthocyanins and non-anthocyanin flavonoids in blackberry from different growth stages. Foods 11(18):2902. *https://doi.org/10.3390/foods11182902*

Maslov O, Kolesnik S, Komisarenko M, Altukhov A, Dynnyk K, Kostina T (2021). Development and validation of a titrimetric method for quantitative determination of free organic acids in green tea leaves. Pharmakeftiki 33(4):304-311. *https://doi.org/10.5281/zenodo.7813135*

Maslov OY, Kolisnyk SV, Komissarenko NA, Kostina TA (2021). Development and validation potentiometric method for determination of antioxidant activity of epigallocatechin-3-O-gallate. Pharmacologyonline 2:35-42. *https://doi.org/10.5281/zenodo.7813098*

Maslov O, Kolisnyk S, Komisarenko M, Golik M (2022). Study of total antioxidant activity of green tea leaves (*Camellia sinensis* L.). Herba Polonica 68(1):1-9. *https://doi.org/10.2478/hepo-2022-0003*

Maslov O, Komisarenko M, Kolisnyk S, Tkachenko O, Akhmedov E, Poluain S, Kostina T, Kolisnyk O (2023). Study of qualitative composition and quantitative content of free organic acids in lingberry leaves. Fitoterapia (1):77-82. *https://doi.org/10.32782/2522-9680-2023-1-77*

Maslov O, Komisarenko M, Ponomarenko S, Horopashna D, Osolodchenko T, Kolisnyk S, Derymedvid L, Shovkova Z, Akhmedov E (2022). Investigation the influence of biologically active compounds on the antioxidant, antibacterial and anti-inflammatory activities of red raspberry (*Rubus idaeous* l.) leaf extract. Current Issues in Pharmacy and Medicine: Science and Practice 35(4):229-235 *https://doi.org/10.2478/cipms-2022-0040*

Maslov OY, Kolisnyk SV, Komisarenko MA, Akhmedov EY, Poluian SM, Shovkova ZV (2021). Study of flavonoids and phenolic acids in green tea leaves. Current Issues in Pharmacy and Medicine: Science and Practice 14(3):287-291. *https://doi.org/10.14739/2409-2932.2021.3.240287*

Maslov OY, Kolisnyk SV, Komisarenko MA, Altukhov AA, Dynnyk KV, Stepanenko VI (2021). Study and evaluation antioxidant activity of dietary supplements with green tea extract Current Issues in Pharmacy and Medicine: Science and Practice 14(2):215-219.: *https://doi.org/10.14739/2409-2932.2021.2.233306*

Maslov OY, Kolisnyk SV, Komisarenko MA, Kolisnyk OV, Ponomarenko SV (2021). Antioxidant activity of green tea leaves (*Camellia sinensis* L.) liquid extracts. Pharmacologyonline (3):291-298. *https://doi.org/10.5281/zenodo.7813115*

Maslov OY, Komisarenko MA, Golik MY, Kolisnyk SV, Altukhov AA, Baiurka SV, Karpushina SA, Tkachenko O, Iuliia K (2023). Study of total antioxidant capacity of red raspberry (*Rubus idaeous* L.) shoots. Vitae 30(1):1-9 *https://doi.org/10.17533/udea.vitae.v30n1a351486*

Maslov OY, Komisarenko MA, Kolisnyk SV, Golik MY, Doroshenko SR, Tkachenko OV, Akhmedov EY, Karpova SP (2023). The study of some quality parameters and the antioxidant activity of dietary supplements with the pomegranate extract (*Punica granatum* L.). News of Pharmacy 106(2):5-12. *https://doi.org/10.24959/nphj.23.119*

Mbarga MJ, Podoprigora IV, Volina EG, Ermolaev AV, Smolyakova LA (2021). Evaluation of changes induced in the probiotic *Escherichia coli* M17 following recurrent exposure to antimicrobials. Journal of Pharmaceutical Research International 158-167.*https://doi.org/10.9734/jpri/2021/v33i29b31601*

Muselík J, García-Alonso M, Martín-López M, Žemlička M, Rivas-Gonzalo J (2007). Measurement of antioxidant activity of wine catechins, procyanidins, anthocyanins and pyranoanthocyanins. International Journal of Molecular Sciences 8(8):797-809. *https://doi.org/10.3390/i8080797*

Russo M, Fanali C, Tripodo G, Dugo P, Muleo R, Dugo L, De Gara L, Mondello L (2018). Analysis of phenolic compounds in different parts of pomegranate (*Punica granatum*) fruit by HPLC-PDA-ESI/MS and evaluation of their antioxidant activity: application to different Italian varieties. Analytical and Bioanalytical Chemistry 410(15):3507-3520. *https://doi.org/10.1007/s00216-018-0854-8*

Skrovankova S, Sumczynski D, Mlcek J, Jurikova T, Sochor J (2015). Bioactive compounds and antioxidant activity in different types of berries. International Journal of Molecular Sciences 16(10):24673-24706. *https://doi.org/10.3390/ijms161024673*

Vučić V, Grabež M, Trchounian A, Arsić A (2019). Composition and potential health benefits of pomegranate: A Review. Current Pharmaceutical Design 25(16):1817-1827. *https://doi.org/10.2174/1381612825666190708183941*