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Botany

Phenolic Profile and Antimicrobial Activity of the Rind of the Georgian Pomegranate ("Sakerdze" Variety)

Manana Karchava[*], Khatia Tsagareishvili[*], Eka Beselia[*], Khatia Khvadagiani[*], Shota Jinjolia[**], Nana Baratashvili[§], Aleko Kalandia[§§]

[*] *Faculty of Technological Engeneering, Akaki Tsereteli State University, Kutaisi, Georgia*
[**] *Faculty of Exact and Natural Sciences, Akaki Tsereteli State University, Kutaisi, Georgia*
[§] *Levan Sakvarelidze National Center for Disease Control and Public Health, Georgia*
[§§] *Faculty of Natural Sciences and Health, Batumi Shota Rustaveli State University, Batumi*

(Presented by Academy Member Ramaz Gakhokidze)

Abstract. The profile of phenolic compounds of Georgian pomegranate rind and their antioxidant and antimicrobial activity have been studied. The following phenolic compounds have been identified: quininic acid derivatives; punicalagin derivatives; catechin; gallic acid; ellagic acid; ellagic acid hexoside. The total content of phenols was 95.222 mg/g in terms of chlorogenic acid, including phenolic acids (9.822 mg/g), flavonoids (39.48 mg/g), catechins (3.68 mg/g), and leucoanthocyanins (0.523 mg/g). The antimicrobial activity of the phenolic extract of Georgian pomegranate rind was evaluated on: Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa control strains; fungicidal activity was evaluated on laboratory biomass of the fungus Candida (*Candida albicans*). It was established that the diameter of Staphylococcus aureus growth inhibition by the phenolic extract is 13.2 cm. The diameter of *Escherichia coli* growth inhibition is 19.7 cm and the diameter of Pseudomonas aeruginosa growth inhibition is 15.4 cm. The diameter of *Candida albicans* growth inhibition is 23.5 cm, which confirms the high antimicrobial activity of the phenolic compounds of the rind of the Georgian pomegranate. That is why this extract can be successfully used as a biologically active natural ingredient with antimicrobial activity in food technology. © *2026 Bull. Natl. Acad. Sci. Georg.*

Keywords: Georgian pomegranate, phenolic compounds, punicalagin, antibacterial, fungicidal activity, antioxidant

Introduction

Pomegranate (*Punica granatum*) belongs to the Punicaceae family, grows in tropical and subtropical zones and adapts well to changing climatic conditions. In 2024, its production worldwide amounted to about 5 million tons. In Georgia, pomegranate is grown on around 600 hectares. Yields are 20-25 tons per hectare; that is, Georgia produces up to 10,000 tons of pomegranate annually. The edible part of the fruit is about 50% of the whole fruit. (juice – 40%, seeds – 10%), and the rest 50% is in

the form of the rind. The pomegranate varieties common in Georgia are: „Pink Gulosha“, „Kirmiz-Kabukhi“, „Salavaturi“, „Shah-Nari“, „Krakhuna“, „Sakerdze“, “Balamursali” and others. In Imereti region, „Krakhuna“, „Sakerdze“ and „Pink Gulosha“ are prevalent.

Pomegranate and pomegranate products are known for their antimicrobial, antifungal, anticancer, and antiviral properties (including resistance to the SARS-CoV-2 virus), anti-inflammatory, antioxidant, and other beneficial biological activities (Wenjuan et al., 2022; Guo et al., 2021; Mohamed & Mabrok, 2021; Shalini et al., 2014), which are due to their high content of phenolic compounds, such as phenolic acids, catechins, anthocyanins, flavonoids, and especially tannins. Of the tannins, ellagitannins are characterized by special biological activity that are derived from gallotannins. The main ellagitannin in the pomegranate rind is punicalagin and its derivative – punicalin. These compounds, when hydrolyzed, produce ellagic acid, which is also known for its high biological and especially antimicrobial activity. They damage the cell wall of microorganisms, inhibit DNA replication and protein synthesis without damaging the DNA itself, and reduce intracellular acidity and rate of ATP synthesis (Mendoza et al., 2022; Maphetu et al., 2021; Teaiama et al., 2022; Putkaradze et al., 2023).

The research aims to investigate the phenolic profile and antimicrobial (antibacterial and fungicidal) activity of the Georgian pomegranate rind with a view to creating an antimicrobial natural food ingredient.

Materials and Methods

The present study investigated the phenolic profile of the rind of Georgian pomegranate, its antioxidant potential, and its antimicrobial activity.

The study of the phenolic profile and antioxidant activity of the pomegranate rind was conducted at the Western Georgia Regional Chromatographic Center (Batumi).

The analysis of the antimicrobial activity of the rind of pomegranate was conducted at Imereti Zonal Diagnostic Laboratory of the National Center for Disease Control and Public Health (Kutaisi).

The 2021-2023 harvest of pomegranates (the most common variety in Western Georgia – „Sakerdze“) collected in the village of Dimi, Baghdati Municipality, Imereti region, with a dry matter content of 15% ÷ 16.9%, acidity of 3.4% ÷ 6.2%, and total sugars of 11.4% ÷ 13.2%, was used for the study. The rind of the pomegranate was dried at a temperature of 36-40°C in a ventilated oven of the POL-EKO-APARATURA SP.J system and then ground in an electric grinder.

For the identification and quantitative analysis (Surmanidze et al., 2024; Boudemagh et al., 2022) of the total and individual phenolic profile of the rind of the pomegranate, 1 g of the sample was extracted in 190 ml of ethanol and filtered. For chromatography, the sample was concentrated under vacuum conditions (40ºC), filtered through a membrane filter and chromatographed. The identification of individual compounds was carried out using commercially available standards by ultra-performance liquid chromatography (UPLC) using photodiode array (PDA) and mass-spectrometer (MS) detectors. Quantitative analysis of individual compounds was performed by the UPLC-PDA-MS method (Waters, UPLC Acquity, QDa Detectore). The chromatographic column used for the separation of compounds was Acquity UPLC BEN C18, 1.7 m, solvent system: 0.3 ml/min, deionized water +0.2% formic acid (solvent A) and acetonitrile (solvent B). Gradient-solvent B: 0-20 min, 5-16%; 20-28 min, 16-40%; 28-32 min, 40-47%; 32-36 min, 70-99%; 36-45 min, 99% and 45-46 min, 99-5%. Injection – 3 µL. Prior to chromatography, samples and eluents were filtered through a 0.45 µm pore filter. Column temperature 35°C, MS scan 100-1200 Da, Probe 500°C, negative (ESI-MS)-, 0.8 kV, capillary 1.5 kV, CV-15. The number of individual compounds was calculated using D-catechin (Sigma-Aldrich).

Total phenols were determined by the Folin-Ciocalteu spectrophotometric method (in terms of gallic acid). The sample taken for analysis was extracted with 80%-ethyl alcohol at a temperature of 70-75ºC. 0.5 or 1 ml of the total extract was placed in a 25 ml volumetric flask, 5 ml of H_2O was added, 1 ml of Folin-Ciocalteu was left for 8 minutes at room temperature, then 10 ml of 7% Na_2CO_3 was added, the flask was filled with H_2O and left for 2 hours in the dark at room temperature. The determination was carried out on a Mettler Toledo uv 5 spectrometer – at 750 nm.

Total flavonoids were quantified using the AlCl3 reagent by the spectral method: the sample taken for analysis was extracted with 80%-ethyl alcohol at a temperature of 70-75°C. 1 ml of the total extract was placed in a 10-ml test tube, 5 ml of H_2O was added, 0.3 ml of 5% $NaNO_2$ was added, and the mixture was left for 5 minutes, then 0.3 ml of 10% $AlCl_3$ was added, and the mixture was left for 6 minutes; then 2 ml of 1N NaOH was added, and the total amount of phenols was determined on a Mettler Toledo UV 5 spectrometer at 510 nm.

Quantitative determination of catechins was carried out by the spectral method: the sample taken for analysis was extracted with 80%-ethyl alcohol at a temperature of 70-75°C. 3 ml of vanillin reagent was added to 1 ml of the total volume of the extract, and 3 minutes later, the optical density of the red-colored sample was determined on a Mettler Toledo uv 5 spectrometer at 500 nm.

For the analysis of phenolic acids, 250 µl of the extract taken from the total volume of the alcoholic extract was added to 250 µl of 0.1% hydrochloric acid and 4.55 ml of 2% hydrochloric acid solution. After vigorous stirring, the mixture was left for 15 minutes, and the total amount of phenolic acids was determined at 320 nm. For the quantitative determination of leucoanthocyanins, the sample taken for analysis was extracted with 80%-ethyl alcohol at a temperature of 70-75ºC. 8 ml of leucoanthocyanidin reagent was added to 1 ml of the total volume of the extract. A parallel sample was prepared for each sample, which was not heated. The second sample was subjected to heating for 40 minutes. After this time, the samples were determined at 550 nm.

To determine the antioxidant activity, the radical bonding capacity was determined by the DPPH (2,2-Diphenyl-1-picrylhydrazil) method using the stable radical of 2,2-diphenyl-1-picrylhydrazil. To 1 ml of the analyzed extract, we added 3 ml of an alcoholic solution of DPPH (0.1 mM DPPH – 0.004 g/100 ml of ethyl alcohol), and 30 minutes later, we determined the absorption of the test sample at 515 nm. The reference solution was a DPPH solution, and the background was 96%-ethyl alcohol. The antioxidant activity by 50%-inhibition of stable free radical (DPPH) is calculated by the following formula: In % = (AC – AS)/AC*100 (1), where In % is a 50%-inhibition of 0.1 mM DPPH; AC – is the absorption of 0.1 mM DPPH alcohol solution, and AS is the absorption of the test extract and 0.1 mM DPPH alcohol solution. To determine the antioxidant activity of the product directly, we used the following formula: 2) C = m/V*VIn%, where C is mg of the sample that inhibits 0.1 mM DPPH by 50%; m – the mass of the sample taken in milligrams; V– volume of the test extract (ml); F is the dilution factor; and VIn % is the sample amount for 50% inhibition of 0.1 mM DPPH.

To evaluate the antimicrobial activity of the pomegranate rind, the reference strains of microorganisms taken from the repository of the Lugar Public Health Research Center of the National Center for Disease Control and Public Health (*Staphylococcus aureus* – ATCC 25923; *Escherichia coli* – ATCC 25922; *Pseudomonas aeruginosa* – ATCC 27853) were used, while the reference preparation of *Candida albicans* was taken from laboratory biomass. Solid nutrient media used for the cultivation of microorganisms (Mueller Hinton Agar; Sabouraud Chloramphenicol Dextrose Agar) and other consumables were taken from Imereti Zonal Diagnostic Laboratory of the National Center for Disease Control and Public Health.

To determine the antimicrobial activity of the pomegranate powder, 200 g of powder was added to 200 ml of 30%-ethanol aqueous solution for the extraction of phenolic compounds from the rind powder and placed in an ultrasonic extractor-homogenizer (US SOLID ULTRASONIC PROCESSOR) for 15 minutes. The extract was squeezed, filtered, and then concentrated first on a rotary distiller to 21% dry matter and then in a freeze-dryer to 55% dry matter. We evaluated the antimicrobial activity of the obtained concentrated extract on 3 types of bacteria (*Staphylococcus aureus* – ATCC 25923; *Escherichia coli* – ATCC 25922; *Pseudomonas aeruginosa* – ATCC 27853) and the fungicidal activity on a laboratory biomass of the fungus Candida (*candida albicans*). All three bacteria were scattered separately on Mueller Hinton Agar, while Candida was scattered on Sabouraud Chloramphenicol Dextrose Agar.

Bacteria were incubated on Mueller Hinton agar for 48 hours at 37 degrees Celsius and on Sabouraud agar for 48 hours at room temperature. The concentrated pomegranate rind extracts were applied to the cups of cultivated microbes (bacteria and fungi) with a special loop, and the diameter of the inhibition of microbial growth was recorded. Counting was taken 2 hours after the application of the pomegranate rind phenolic extracts to the prepared cups.

The statistical analysis was conducted by calculating the standard error for each set of data utilizing the Microsoft Excel software. A confidence level was established, with a significance threshold set at $p \leq 0.05$.

Results and Discussion

The analysis of phenolic compounds revealed that the total content of phenols in the pomegranate rind extracts was 95.222 mg/gram in terms of chlorogenic acid. Four classes of phenolic compounds were identified: 1. Phenolcarboxylic acids (9.822 mg/gram), including quininic acid derivatives, and gallic and ellagic acids; 2. Flavonoids (39.48 mg/gram), including punicalagin derivative 1 and punicalagin derivative 2; 3. Catechins (3.68 mg/gram); 4. Leucoanthocyanins (0.523 mg/gram) (Table 1).

The results obtained indicate that the content of flavonoids is predominant in phenolic compounds in the Georgian pomegranate rind. They account for 41% of phenolic compounds. Phenolcarbonic acids account for 10% of the total number of phenols. Catechins are 0.03%, leucoanthocyanins are 0.006%.

The study of the antioxidant activity of phenolic compounds revealed that the 50%-inhibition of DPPH radical by the sample is 0.085 mg/kg and, which confirms the high antioxidant activity of phenolic compounds (Table 1).

Table 1. Total phenolic compounds and antioxidant activity of the pomegranate rind

Phenolic compounds	Total phenols mg/g (in terms of chlorogenic acid)	Phenolcarbonic acids mg/g (in terms of caffeic acid)	Total flavonoids mg/g (in terms of quercetin)	Catechins mg/g (in terms of D-catechin)	Leucoanthocyanins mg/g (in terms of leucoanthocyanidin)	Antioxidant activity - 50%-inhibition of DPPH radical by mg sample
Content	95.222	9.882	39.118	2.618	0.523	0.085

Table 2. Antimicrobial activity of the rind of the Georgian pomegranate

Test sample	Solid nutrient medium	Microbial growth inhibition diameter, cm
Staphylococcus aureus – ATCC 25923	MUELLER HINTON Agar	13.2
Escherichia coli – ATCC 25922	MUELLER HINTON Agar	19.7
Pseudomanas aeroginosa – ATCC 27853	MUELLER HINTON Agar	15.4
Candida albicans	Sabouraud Cloramphenicol Dextrose Agar	23.5

The identification of individual phenolic substances using commercially available standards revealed the presence of the following phenolic compounds in the rind of the pomegranate: quininic acid derivative 1; quininic acid derivative 2 gallic acid 0.81 mg/g; catechin 3.68 mg/ml; punicalagin derivative 1; punicalagin derivative 2; ellagic acid hexoside; ellagic acid.

From literary sources, it is known that individual phenolic substances identified from the phenolic complex of Georgian pomegranate are each characterized by strong antioxidant, antimicrobial, fungicidal, anticarcinogenic, anti-inflammatory, and other biological activities. This ensures the versatile (including antibacterial and fungicidal) activity of the Georgian pomegranate phenolic concentrate (Abid et al., 2022; Gigliobianco et al., 2022; Salim et al., 2023; Alexandre et al., 2019; Mphahlele et al., 2016). This is completely confirmed by the analysis of the antimicrobial activity of the drug.

As a result of the study of the antimicrobial activity of phenolic extracts of the rind of Georgian pomegranate (Table 2), it was established that of the three bacteria involved in the research, the phenolic extract of pomegranate rind had the highest activity; that is, it is characterized by the largest diameter of inhibition of microbial growth towards *Escherichia coli*, and with the least activity towards *Staphylococcus aureus*. The activity level of the pomegranate rind towards *Pseudomonas aeroginosa* is medium. The activity of the test material towards *Escherichia coli* is 28% higher than the activity towards *Pseudomonas aeroginosa*, and almost 50% higher than the activity towards *Staphylococcus aureus*. It has also been established that phenolic extracts of the pomegranate rind have a significant negative effect on the growth of *Candida albicans* – the diameter of inhibition is 23.5 cm, which is 19.2% higher than the activity towards *Escherichia coli*. The presented data confirm the high antibacterial and fungicidal properties of phenolic compounds of the pomegranate rind, which is why the concentrate of phenolic compounds obtained from the pomegranate rind can be successfully used as a natural biologically active ingredient with antimicrobial activity in food technology.

Conclusion

The total amount of phenolic compounds in the rind of Georgian pomegranate is 95.222 mg/g in terms of as chlorogenic acid. Four classes of phenolic compounds have been identified: phenolcarbonic acids (9.822 mg/g), flavonoids (39.48 mg/g), catechins (3.68 mg/g), and leucoanthocyanins (0.523 mg/g).

Phenolic extracts of the pomegranate rind are characterized by high antimicrobial activity. The diameter of inhibition of growth of *Staphylococcus aureus* by phenolic extract is 13.2 cm, the diameter of inhibition of growth of *Escherichia coli* is 19.7 cm, the diameter of inhibition of growth of *Pseudomonas aeroginosa* is 15.4 cm, and the diameter of inhibition of growth of *Candida albicans* is 23.5 cm.

The Georgian pomegranate (“Sakerdze” variety) rind powder can be used as a natural ingredient with antimicrobial activity in food technology.

ბოტანიკა

ქართული ბროწეულის („საკერძე“) ქერქის ფენოლური პროფილი და ანტიმიკრობული აქტივობა

მ. ქარჩავა*, ხ. ცაგარეიშვილი*, ე. ბესელია*, ხ. ხვადაგიანი*, შ. ჯინჯოლია**, ნ. ბარათაშვილი§, ა. კალანდია§§

* *აკაკი წერეთლის სახელმწიფო უნივერსიტეტი, საინჟინრო ტექნოლოგიური ფაკულტეტი, ქუთაისი, საქართველო*
** *აკაკი წერეთლის სახელმწიფო უნივერსიტეტი, ზუსტ და საბუნებისმეტყველო მეცნიერებათა ფაკულტეტი, ქუთაისი, საქართველო*
§ *ლევან საყვარელიძის სახ. დაავადებათა კონტროლისა და საზოგადოებრივი ჯანმრთელობის ეროვნული ცენტრი, საქართველო*
§§ *ბათუმის შოთა რუსთაველის უნივერსიტეტი, საბუნებისმეტყველო მეცნიერებათა და ჯანდაცვის ფაკულტეტი, ბათუმი, საქართველო*

(წარმოდგენილია აკადემიის წევრის რ. გახოკიძის მიერ)

ნაშრომში წარმოდგენილია ქართული ბროწეულის ქერქის ფენოლურ ნაერთთა პროფილი, მათი ანტიოქსიდანტური პოტენციალი და ანტიმიკრობული აქტივობა. კვლევისათვის გამოყენებული იყო იმერეთის რეგიონის ბაღდათის მუნიციპალიტეტის სოფელ დიმში აღებული ბროწეულის (დასავლეთ საქართველოში ყველაზე გავრცელებული ჯიში – „საკერძე“) 2021-2023 წლების მოსავალი. ბროწეულის ქერქის ფენოლურ ნაერთთა ანალიზის შედეგად დადგინდა, რომ მასში ფენოლების საერთო შემცველობა ქლოროგენის მჟავაზე გადაანგარიშებით 95,222 მგ/გრამია. იდენტიფიცირებულია ფენოლურ ნაერთთა 4 კლასი: ფენოლკარბონმჟავები 9.822 მგ/გრამი; ფლავანოიდები 39,48 მგ/გრამი; კატექინები 3,68 მგ/გრამი; ლეიკოანტოციანები 0,523 მგ/გრამი. ბროწეულის ქერქის ანტიოქსიდანტური აქტივობა შეადგენს 0,085 მგ-ს. ბროწეულის ქერქის ფხვნილის ანტიმიკრობულ აქტივობას ვაფასებდით 3 სახის ბაქტერიაზე (Staphylococcus aureus – ATCC 25923; Escherichia coli – ATCC 25922; Pseudomonas aeruginosa – ATCC 27853), ხოლო ფუნგიციდურ აქტივობას – სოკო კანდიდას (Candida albicans) ლაბორატორიულ ბიომასაზე. ქართული ბროწეულის ქერქის ფენოლური ექსტრაქტების ანტიმიკრობული აქტივობის კვლევის შედეგად დადგენილია, რომ ქართული ბროწეულის ქერქისაგან მიღებული კონცენტრირებული ფენოლური ექსტრაქტის მიერ მიკრობის – Staphylococcus aureus – ზრდის შეფერხების დიამეტრი არის 13,2 სმ-ს. Escherichia coli – ზრდის შეფერხების დიამეტრი – 19,7 სმ, ხოლო Pseudomanas aeroginosa – ზრდის შეფერხების დიამეტრი 15,4 სმ. ასევე დადგენილია ბროწეულის ქერქის ფენოლური ექსტრაქტების მნიშვნელოვანი უარყოფითი გავლენა candida albicans-ის ზრდაზე – შეფერხების დიამეტრი – 23,5 სმ, რაც ადასტურებს ბროწეულის ქერქის ფენოლურ ნაერთთა მაღალ ანტიბაქტერიულ და ფუნგიციდურ თვისებებს, რის გამოც ბროწეულის ქერქიდან მიღებული ფენოლურ ნაერთთა კონცენტრატი შეიძლება წარმატებით იქნეს გამოყენებული როგორც ანტიმიკრობული მოქმედების ნატურალური ბიოლოგიურად აქტიური ინგრედიენტი საკვები პროდუქტების ტექნოლოგიებში.

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