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Original Research

Antioxidant and antibacterial activity of root extracts of Georgian medicinal plants obtained using different extraction methods

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Abstract

This study was designed to investigate the impact of different extracting solvents (methanol and DMSO) on the biological activities of root extract of *Paeonia daurica* subsp. *mlokosewitschii* (Lomakin) D. Y. Hong and *Sempervivum transcaucasicum* Muirhead. *In vitro* assessment of the antioxidant activity of extracts was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH assays), while their antibacterial activity was tested against *Escherichia coli* ATCC 25922 strain. The analysis of the obtained results revealed that the highest anti-DPPH potential and the highest antibacterial activity were achieved in the methanolic root extracts of both plants. The methanolic root extracts of *Paeonia daurica* subsp. *mlokosewitschii* exhibited significantly higher antioxidant and antibacterial activities in comparison with *Sempervivum transcaucasicum* root extracts. The results of the study indicated the possible use of root extracts of *Paeonia daurica* subsp. *mlokosewitschii* in pharmacy.

Keywords: *Paeonia daurica* subsp. *mlokosewitschii* (Lomakin) D. Y. Hong, *Sempervivum transcaucasicum* Muirhead, extraction methods, antioxidant activity, DPPH assay, antibacterial activity, *E. coli*.

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Introduction

The chemical compounds found in medicinal plants are readily accessible and powerful sources of antibacterial and antioxidant substances. These compounds can work alone or in combination to treat illness and enhance well-being. Tannins with antibacterial and antifungal properties, phenolic compounds with antioxidant and other pharmacological effects, and other phytochemicals are typically found in a single plant. [1]. Georgia's diverse temperature zones and landscapes make it a "hot spot" for biodiversity and a unique home for several hundred species of medicinal plants (discussed in [2]). Around 700 plant species are used in Georgian traditional medicine, and the country's official pharmacopoeia includes 200 taxa. [3]. However, despite the centuries-long history of Georgian folk medicine, the chemical composition and the effects of the extracts of traditional medicinal plants remain poorly investigated. The high concentration of various antioxidant compounds and the significant antimicrobial and antifungal potential were documented in many species of Georgian medicinal plants (discussed in [2] and [4]). In the present study, we investigated the antioxidant and antibacterial effect of representatives of two families of Georgian medicinal plants - *Paeonia daurica* subsp. *mlokosewitschii* (Lomakin) D. Y. Hong and *Sempervivum transcaucasicum* Muirhead.

The genus Paeonia includes 52 accepted members (36 species, 15 subspecies, and 1 variety) and 10 species of the genus distributed in Georgia: *Paeonia macrophylla* (Albov) Lomak., *P. steveniana* Kem.-Nath., *P. wittmanniana* Hartwiss ex Lindl., *P. mlokosewitschii* Lomak., *P. ruprechtiana* Kem.-Nath., *P. caucasica* (Schipcz.) Schipcz., *P. lagodechiana* Kem.-Nath., *P. majko* Ketzch., *P. carthalinica* Ketzch., *P. tenuifolia* L. Nine of them are endemic species of narrow distribution of the Georgian flora and *Paeonia tenuifolia* L. is a cosmopolitan plant [5].

The traditional uses of plants of the genus Paeonia are medicinal. In the folk medicine of many cultures, the different Paeonia parts (roots, root bark, flowers, leaves, seeds) of these species are used to treat neurological and infectious diseases, urinary system diseases, inflammation and trauma [6]. The study of the effect of different solvents on the antioxidant and antibacterial potential of leaf extracts of three *Paeonia* species revealed that the ethyl acetate extract of *Paeonia officinalis* L. had the highest levels of phenolic content and antioxidant potential [7]. The antioxidant activity was also demonstrated for essential oil obtained from the roots of 12 Paeonia species and the highest activity was revealed for *Paeonia lactiflora* Pall. [8]. High antioxidant activity was demonstrated for extracts of *Paeonia daurica* ssp. *macrophylla (*Albov) D. Y. Hong roots [9]. The results of our previous study of different drying and extraction methods and solvent polarity on the antioxidant properties of *Paeonia daurica* subsp. *mlokosewitschii* leaves demonstrated the highest antioxidant activity of *Paeonia daurica* subsp. *mlokosewitschii* leaves

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freeze-dried and extracted for 24 h with 80% methanol. [10].

The ethyl acetate extract of *Paeonia officinalis* L. leaves was effective against two Gram-positive bacteria *Listeria monocytogenes* and *Staphylococcus aureus*; two Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*, and a fungus *Candida albicans* [7]. The extract of *Paeonia peregrina* L. petals obtained using 2,2-difenil-1-pixil-hidrazil inhibited the growth of *Klebsiella pneumoniae* 3 times more effectively compared to Erythromycin. [11]. The 95% methanol extract of *Paeonia officinalis* L. roots exhibited significant antibacterial efficacy against methicillin-resistant *Staphylococcus aureus* [12]. The essential oil obtained from the entire *Paeonia mascula* (L) Miller plant showed moderate activity against *Yersinia pseudotuberculosis* and *Bacillus cereus.* [13].

The genus *Sempervivum* unites over 40 species native to the mountains of Europe, Africa, and Asia. [14] . The name "Sempervivum" has its basis in the Latin Semper ("always") and vivus ("living") due to the ability of the plant to store water in leaves allowing them to inhabit rocks in the mountain, alpine and subalpine belts. [14], [15], [16]. The *Sempervivum* plants are used in traditional medicine for the treatment of ear inflammation and wounds, sores, burns, and abscesses [15], [17]. The 80% ethanol extract of the aboveground part of *Sempervivum davisii* Muirhead had potent antioxidant activity, and kaempferol and quercetin derivatives were shown to be sources of potent antioxidant properties of the extract [18]. Water-ethanolic extracts (50%) of *Sempervivum tectorum* L. leaves also exhibited significant antioxidant activity [19]. Methanol extract of *Sempervivum armenum* Boiss.& A.Huet leaves exhibited strong antioxidative and antigenotoxic effects [15]. The study of 50% ethanol leaf extracts of 22 *Sempervivum* species demonstrated that these extracts are excellent sources of trace elements, antioxidants, and phenolic components [20].

Antibacterial activity of the 50% ethanol extract of *Sempervivum tectorum* L. leaves against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was demonstrated [21]. The investigation of antibacterial activity of *Sempervivum tectorum* leaf extracts on four Gram-positive (*Bacillus subtilis, Micrococcus lysodeikticus*, methicillin-resistant *Staphylococcus aureus* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) demonstrated that Gram-negative bacterial strains showed higher sensitivity to extract of this plant [22].

Despite multiple reports on the antioxidant and antibacterial activities of plants of *Paeonia* and *Sempervivum* genera, the properties of representatives of these genera, native to the Caucasus, *Paeonia daurica* subsp. *mlokosewitschii* (Lomakin) D. Y. Hong and *Sempervivum transcaucasicum* Muirhead were not investigated. Herein, we aimed to examine

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The effect of different extraction methods and extraction solvents on the antioxidant and antibacterial activity of root extracts of *Paeonia daurica* subsp. *mlokosewitschii* (Lomakin) D. Y. Hong and *Sempervivum transcaucasicum* Muirhead.

Materials and methods

Plant material

Paeonia daurica subsp. *mlokosewitschii* and *Sempervivum transcaucasicum* plants were collected in July 2024 in the National Botanical Garden of Georgia (Tbilisi). Samples were placed in paper bags and transported to the laboratory within 1 h. Once in the laboratory, roots were detached from the plants. Samples of fresh plant material were frozen in liquid nitrogen and stored at –80 °C.

Drying processes

Roots of both plant species (5 g) were freeze-dried using a DW-10N freeze dryer in a vacuum flask of 500 mL at 10 Pa and a final condenser temperature of -55 °C until the plant material reached a constant weight, determined by measuring dry weight (DW). The drying process took 6 hours on average.

Sample extraction

The extraction efficiencies of different solvents were tested (Fig. 1). Roots of each plant species were extracted in a ratio of 1:10 with either 80% methanol (Fig. 1, A) or 80% DMSO (Fig. 1, B), followed by continuous stirring for 24 h at room temperature using an orbital shaker at 270 rpm. The extracts were centrifuged at 5000g for 15 min. Then DMSO extracts were stored at -80 °C for further analysis. Extracts obtained using 80% methanol were rotary evaporated at 50 °C and residue was dissolved in 80% DMSO and stored at -80 °C for further analysis.

DPPH free radical scavenging activity assay

The free radical scavenging activity was measured using by 2,2′-diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described earlier [10].

The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol. The working solution was obtained by diluting the DPPH solution with methanol to attain an absorbance of about 0.98±0.02 at 517 nm using the spectrophotometer. A 3 ml aliquot of the working solution was mixed with 100 µl of the sample at various concentrations (10 - 500 µg/ml). The reaction mixture was shaken well and incubated in the dark for 30 min at room temperature. Then the absorbance was taken at 517 nm. A typical blank contained 3 ml of the working solution and the appropriate volume of the corresponding solvent and was incubated under the same conditions. Ascorbic acid was used as standard. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

Scavenging effect (%) = [(control absorbance–sample absorbance)/(control absorbance)]×100

The concentrations of the sample required for 50 % inhibition (IC_{50}) were calculated to determine the concentration of the sample required to inhibit 50% of radical. The lower the IC_{50} value, the higher the antioxidant activity of the samples. IC_{50} was

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calculated by plotting the concentration of extract versus inhibition of DPPH (%) and data were fit with a straight line (linear regression). IC_{50} value was estimated using the fitted line, i.e.:

$Y = a * X + b,$

$IC_{50} = (50 - b)/a.$

Antibacterial assay

The agar-disc diffusion method was employed for antibacterial activity screening. The *Escherichia coli* ATCC 25922 strain was used in the study. The bacteria were grown in LB medium for 16-18 h at 37 °C (10^9-10^{10} CFU/ml). Sterile blank discs with 6 mm diameter were individually placed on a nutrient agar plate covered with 300 µl of the bacteria strain. Different concentrations of plant extract extracted with either 80% methanol or 80% DMSO were put into the sterile blank disc. These plates were incubated at 37 °C for 24 h. The antimicrobial activity was determined in triplicate by measuring the diameter of the inhibition zone (mm). Amoxicillin (20 and 30 µg/disk) was used as the positive control. Dimethyl sulfoxide (80 %) was used as negative control.

Statistical analysis

All the procedures for extraction and antioxidant studies were repeated in triplicate. The results were expressed as means± standard deviation of three parallel replicates. All data of the DPPH assay were analyzed statistically by one-way analysis of variance (ANOVA) using Microsoft Excel. A *p-value* of less than 0.05 was considered statistically significant.

Results and discussion

Solvent screening

The most critical steps of the extraction of bioactive compounds in phytochemical research are the drying temperature of plant material and solvent selection [23]. The effect of different factors, such as solvent polarity, temperature, and time on the extraction efficiency can be independent or coupled [24]. For different drying methods, our previous studies [10] and research made by other authors demonstrated that freeze-drying may avoid the loss of valuable chemical components when compared to other conventional methods [25]. In this study, the effect of different solvents, methanol and DMSO, used for the extraction in the same concentration of 80%, on antioxidant activity and antibacterial activity of plant root extract was assessed (see Fig. 1).

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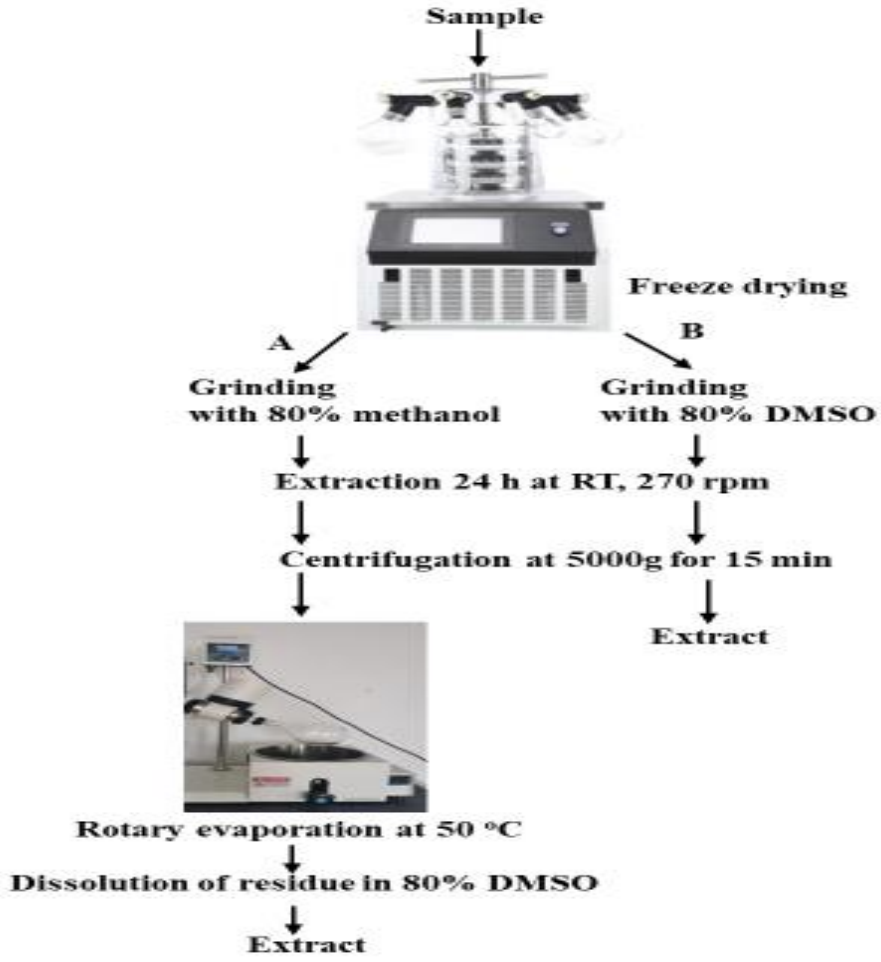

Fig. 1. Two extraction schemes, used in the study. A – extraction of plant material with 80% methanol, B - extraction of plant material with 80% DMSO.

Antioxidant assay -DPPH free radicals scavenging effect.

In the study for the determination of the antioxidant activity of root extracts DPPH assay, which is a rapid and efficient method used for the evaluation of the free radical scavenging activity of medicinal plants [26] was used. Analysis of plant samples against DPPH free radicals revealed that the polarity of the extracting solvent affects DPPH scavenging activity in *Paeonia daurica* subsp. *mlokosewitschii* and *Sempervivum transcaucasicum* roots extract. The comparison of DPPH scavenging activity demonstrated that DPPH scavenging activity was higher in roots of both species, extracted with 80% methanol in comparison with samples extracted with DMSO (Fig. 2 A and B). The *Paeonia daurica* subsp. *mlokosewitschii* roots extracted with 80% methanol were most effective causing 40.01 ± 1.8 % scavenging at 10 mg/ml concentration (Fig. 2 A). At the same concentration *Paeonia daurica* subsp. *mlokosewitschii* roots extracted with 80% DMSO caused 18.50 ± 0.92 % scavenging (Fig. 2 A). The DPPH scavenging ability of *Sempervivum*

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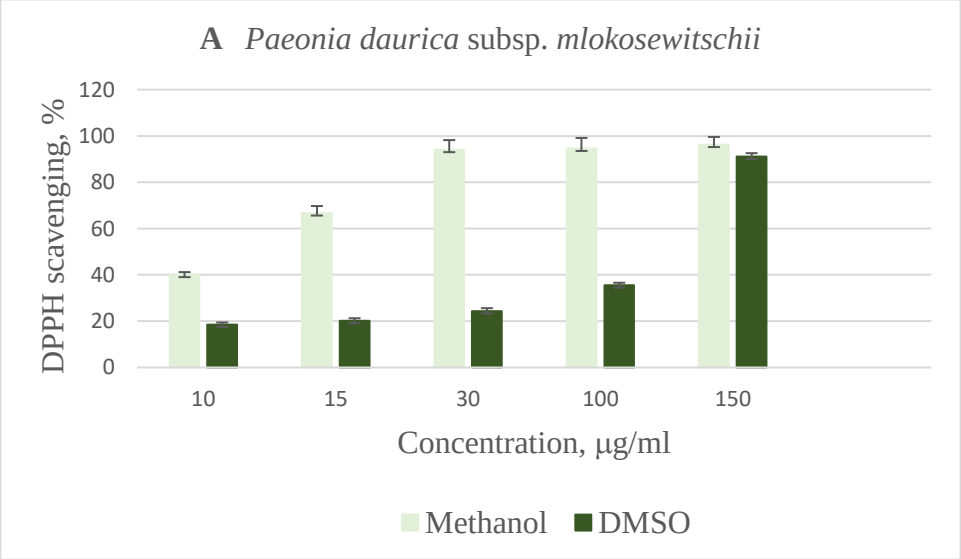
transcaucasicum roots was significantly lower: 5.10 ± 0.44 % scavenging at 15 mg/ml concentration for roots extracted with 80% methanol and 2.11 ± 0.01 % scavenging at 15 mg/ml concentration for roots extracted with 80% DMSO (Fig. 2 B). Median inhibitory concentrations (IC_{50}) were 10.34 ± 0.75 and 30.14 ± 1.37 µg/ml for *Paeonia daurica* subsp. *mlokosewitschii* roots extracted with 80% methanol and 80% DMSO, respectively (Fig. 3C). The IC_{50} values were significantly higher for *Sempervivum transcaucasicum* roots extracted with 80% methanol and 80% DMSO and comprised 161.71 ± 2.58 and 367.21 ± 35.86 µg/ml, respectively.

One-way analysis of variance (ANOVA) of the results obtained for the methanol and ethanol extracts showed that DPPH scavenging activity of *Paeonia daurica* subsp. *mlokosewitschii* and *Sempervivum transcaucasicum* roots were significantly influenced ($p<0.05$) by increasing the polarity of extracting solvent.

Our findings are consistent with previously published results, demonstrating that high-polarity solvents such as ethanol, water, acetone, and methanol are extensively used to extract antioxidant compounds [23], [27], providing a high yield of polar molecules like phenolic and flavonoid components in plant extracts [28]. The abundance of phenolic compounds and their exact positions also have a considerable impact on antioxidant activity [29]. The concentration of phenolic compounds was correlated with the DPPH and the ferric-reducing antioxidant power (FRAP) assay [30], [31]. Based on the results of our study, it can be suggested that extraction with methanol provides efficient recovery of phenolic compounds, contributing to the high antioxidant activity of extracts.



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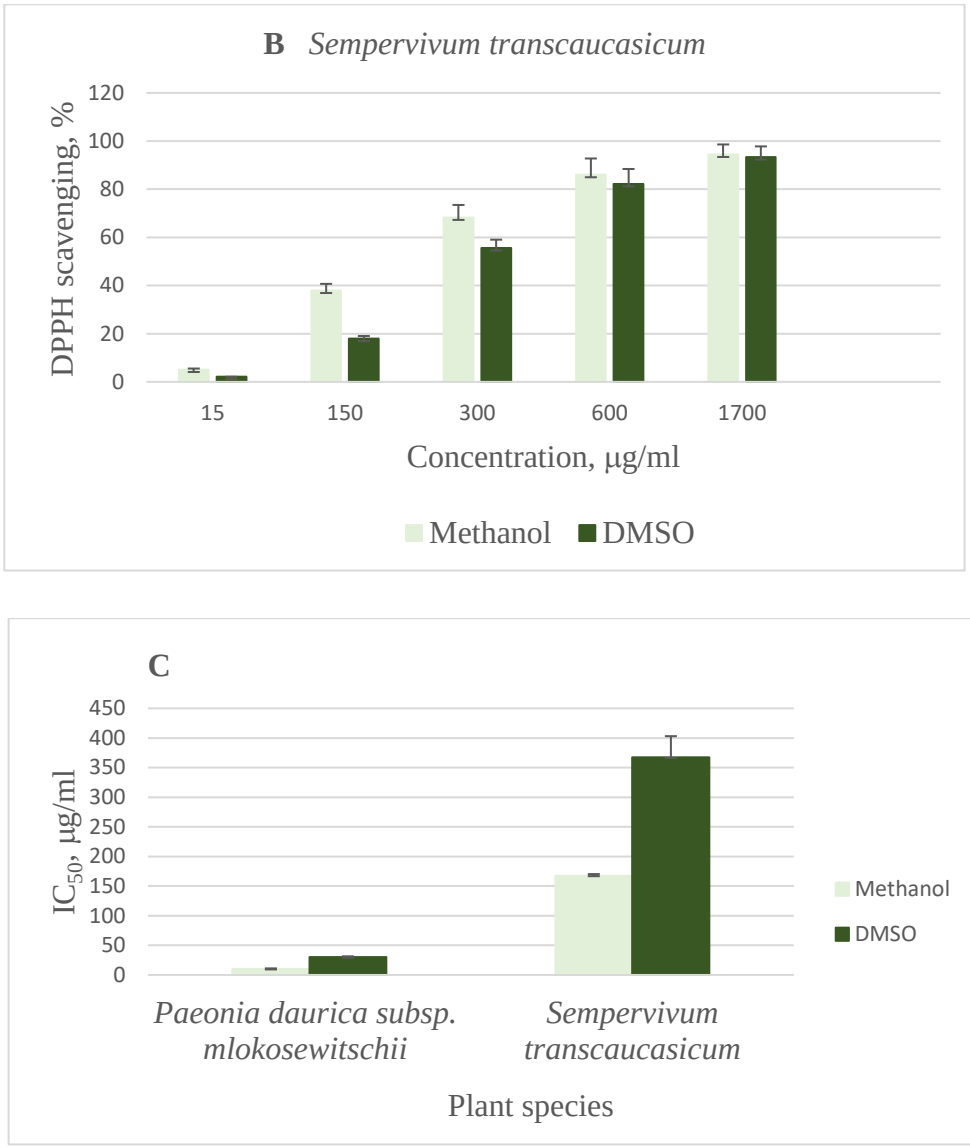

Fig. 2. Antioxidant potential of *Paeonia daurica* subsp. *mlokosewitschii* and *Sempervivum transcaucasicum* root extracts using DPPH assay.

A. *Paeonia daurica* subsp. *mlokosewitschii* roots extracted with 80% methanol or 80% DMSO. **B.** *Sempervivum transcaucasicum* roots extracted with 80% methanol or 80% DMSO. **C.** IC_{50} values of *Paeonia daurica* subsp. *mlokosewitschii* and *Sempervivum transcaucasicum* roots extracted with 80% methanol or 80% DMSO. Values represent % radical scavenging (mean ± SD) from three independent experiments.

Antibacterial activity. The *in vitro* antibacterial activity of the studied *Paeonia daurica* subsp. *mlokosewitschii* and *Sempervivum transcaucasicum* root extracts against *E. coli* ATCC 25922 were determined using disk diffusion assay (Fig. 3, Table 1). At the lowest concentration (100 mg/disk) of extracts used, the highest mean of inhibition zone was recorded for *Paeonia daurica* subsp. *mlokosewitschii* root samples, extracted using 80% methanol (15.66 ± 0.58 mm), followed by *Paeonia*

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daurica subsp. *mlokosewitschii* root samples, extracted using 80% DMSO (12.33 ± 0.58 mm) (Fig. 3A, Table 1). At a concentration of 100 mg/disk, the same lowest mean diameter inhibition zones were recorded for *Sempervivum transcaucasicum* root extracts obtained using 80% methanol or DMSO (6.66 ± 0.58 mm) (Fig. 3B, Table 1).

The same pattern of antibacterial effect was observed for a concentration of 150 mg/disk, the highest mean of inhibition zone was recorded for *Paeonia daurica* subsp. *mlokosewitschii* root samples, extracted using 80% methanol (16.66 ± 0.58 mm), followed by *Paeonia daurica* subsp. *mlokosewitschii* root samples, extracted using 80% DMSO (15.33 ± 0.58 mm). Simila Similarly with a concentration of 100 mg/ disk, the concentration of 150 mg/disk resulted in the lowest mean diameter inhibition zone revealed for *Sempervivum transcaucasicum* root extracts obtained using 80% methanol or DMSO (6.66 ± 0.58 mm).

At the highest extract concentration of 200 mg/disk, the highest mean diameter was recorded for *Paeonia daurica* subsp. *mlokosewitschii* root samples, extracted using 80% methanol (17.33 ± 0.58 mm), followed by *Paeonia daurica* subsp. *mlokosewitschii* root samples, extracted using 80% DMSO (16.33 ± 1.53 mm) and *Sempervivum transcaucasicum* root extracts obtained using 80% methanol (16.00 ± 1.00 mm) and DMSO (11.66 ± 0.58 mm). The antimicrobial activity of studied root extracts against *Escherichia coli* ATCC 25922 was significant in all experimental groups compared to 0 mg/mL ($p < 0.05$).

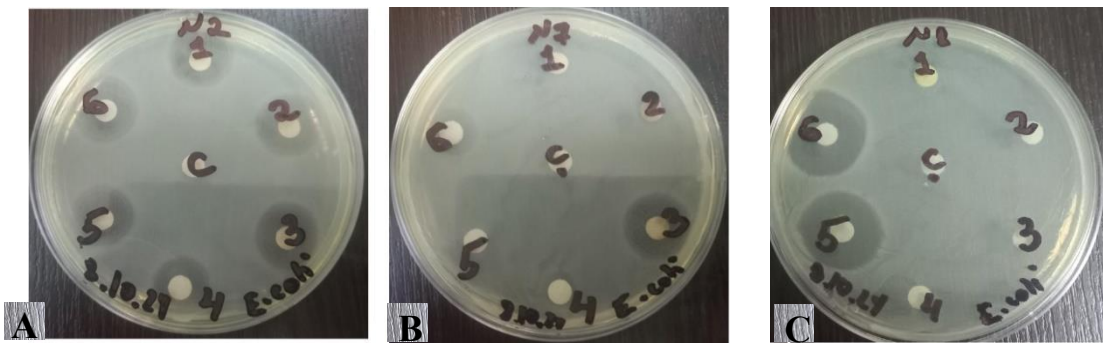

Fig. 3. Inhibition zone test results

A. Extracts of *Paeonia daurica* subsp. *mlokosewitschii* roots, 1 - 100 mg/disk extract in 80% methanol, 2 - 150 mg/disk extract in 80% methanol, 3 - 200 mg/disk extract in 80% methanol, 4 - 100 mg/disk extract in 80% DMSO, 5 - 150 mg/disk 6 - 100 mg/disk extract in 80% DMSO, c - control, 5 ml of 80% DMSO.

B. Extracts of *Sempervivum transcaucasicum* roots, 1- 100 mg/disk extract in 80% methanol, 2 - 150 mg/disk extract in 80% methanol, 3 - 200 mg/disk extract in 80% methanol, 4 - 100 mg/disk extract in 80% DMSO, 5 - 150 mg/disk

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extract in 80% DMSO, 6 - 100 mg/disk extract in 80% DMSO, c – control, 5 ml of 80% DMSO.

C. Control, 1- 10 ml of 80% DMSO, 2- 15 ml of 80% DMSO, 3- 17.5 ml of 80%DMSO, 4 - 20 ml of 80% DMSO, 5 -20 mg/ mg/disk Amoxicillin, 6 - 30 mg/disk Amoxicillin, c – control, 5 ml of 80% DMSO.

Table 1. Antibacterial efficiency of extracts of *Paeonia daurica* subsp. *mlokosewitschii* and *Sempervivum transcaucasicum* roots against *Escherichia coli* ATCC 25922.

Plant species/controls	Extraction solvent	Concentration	IZ diameter, mm
Paeonia daurica subsp. *mlokosewitschii*	80 % methanol	100 mg/disk	15.66 ± 0.58
		150 mg/disk	16.66 ± 0.58
		200 mg/disk	17.33 ± 0.58
	80% DMSO	100 mg/disk	12.33 ± 0.58
		150 mg/disk	15.33 ± 0.58
		200 mg/disk	16.33 ± 1.53
Sempervivum transcaucasicum	80 % methanol	100 mg/disk	6.66 ± 0.58
		150 mg/disk	6.66 ± 0.58
		200 mg/disk	16.00 ± 1.00
	80% DMSO	100 mg/disk	6.66 ± 0.58
		150 mg/disk	6.66 ± 0.58
		200 mg/disk	11.66 ± 0.58
Positive control	Amoxicillin	10 mg/disk	20.67 ± 0.57
		20 mg/disk	25.17 ± 0.29
Negative control	80% DMSO		0

The obtained results for the first time demonstrate the antibacterial activity of DMSO and methanol extracts of *Paeonia daurica* subsp. *mlokosewitschii* and *Sempervivum transcaucasicum* roots. It should be noted that the results are consistent with findings, demonstrating significant antibacterial activity of other *Paeonia* species. Thus, *Paeonia emodi* whole plant extracts prepared using different extraction solvents (hexane, ethyl acetate, chlorophorm) inhibited the growth of *E. coli* [32]. The extracts of *Paeonia wendlboi* roots obtained by Soxhlet extraction method also exhibited significant activity against *E. coli* [33]. The information on the antibacterial activity of *Sempervivum transcaucasicum* is not available in the literature. The methanolic root extracts of both plants exhibited higher antibacterial activity, than DMSO extracts.

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Conclusions

This study for the first time evaluated the biological activities of root extracts of Georgian medicinal plants *Paeonia daurica* subsp. *mlokosewitschii* and *Sempervivum transcaucasicum* obtained by different extraction methods. According to the obtained results, the activities of the extracts varied depending on the employed methods of extraction. The highest antioxidant activity accessed based on anti-DPPH potential was achieved in the methanolic root extracts of both plants, which were rotary evaporated and dissolved in 80% DMSO. The analysis of antibacterial activity against *E. coli* revealed that the highest potential to inhibit bacterial growth was also achieved in the methanolic root extracts of both plants. The methanolic root extracts of *Paeonia daurica* subsp. *mlokosewitschii* exhibited significantly higher antioxidant and antibacterial activities in comparison with *Sempervivum transcaucasicum* root extracts. The results of the study suggest that root extracts of *Paeonia daurica* subsp. *mlokosewitschii* could be used as effective functional ingredients of pharmaceutical products, as they possess prominent antioxidant and antibacterial activities.

სხვადასხვა ექსტრაქციის მეთოდით მიღებული ქართული სამკურნალო მცენარეების ფესვის ექსტრაქტების ანტიოქსიდანტური და ანტიბაქტერიული მოქმედება

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აბსტრაქტი

კვლევა ჩატარებულია სხვადასხვა ექსტრაქციის გამხსნელების (მეთანოლი და DMSO) გავლენის შესასწავლად *Paeonia daurica subsp*-ის ფესვის ექსტრაქტის ბიოლოგიურ აქტივობაზე. *mlokosewitschii (Lomakin) D. Y. Hong* და *Sempervivum transcaucasicum Muirhead.* ექსტრაქტების ანტიოქსიდანტური აქტივობის *In vitro*

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შეფასება განხორციელდა 2,2-დიფენილ-1-პიკრილჰიდრაზილის გამოყენებით (DPPH ანალიზები), ხოლო მათი ანტიბაქტერიული აქტივობა შემოწმდა Escherichia coli ATCC 25922 შტამის წინააღმდეგ. მიღებული შედეგების ანალიზმა აჩვენა, რომ ყველაზე მაღალი ანტი-DPPH პოტენციალი და უმაღლესი ანტიბაქტერიული აქტივობა მიღწეულია ორივე მცენარის მეთანოლის ფესვის *ექსტრაქტებში. Paeonia daurica subsp-*ის მეთანოლის ფესვების ექსტრაქტები. *mlokosewitschii*-მ აჩვენა, რომ მათში მნიშვნელოვნად მაღალია ანტიოქსიდანტური და ანტიბაქტერიული აქტივობა *Sempervivum transcaucasicum* ფესვის ექსტრაქტებთან შედარებით. კვლევის შედეგებმა მიუთითა *Paeonia daurica subsp.mlokosewitschii*-ის ფესვის ექსტრაქტების მიზანშეწონილ გამოყენებაზე ფარმაციაში.

საკვანძო სიტყვები: *Paeonia daurica subsp. mlokosewitschii (Lomakin) D. Y. Hong, Sempervivum transcaucasicum* Muirhead, ექსტრაქციის მეთოდები, ანტიოქსიდანტური აქტივობა, DPPH ანალიზი, ანტიბაქტერიული აქტივობა, E. coli.

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