

GALLANT SOLDIER – A PROMISING RAW MATERIAL WITH ANTIBACTERIAL PROPERTIES

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Galinsoga parviflora Cav. (Asteraceae family) is a segetal-ruderal species, introduced from Peru and spread throughout the territory of Ukraine, the European and other continents. In our country, it is a dangerous invasive species that litters crops and natural biomes. Simultaneously, *G. parviflora* is an edible plant that is used in traditional medicine in some countries, and there are some results of scientific research that testify to the effectiveness of its use as a medicinal plant material with anti-inflammatory, wound-healing, hypotensive, hemostatic, cytotoxic properties and others. Since the *G. parviflora* species is morphologically variable, there is a need to study its chemical composition and properties before using it, in particular as a medicinal plant raw material or for other purposes. The phytochemical investigation of aqueous and aqueous ethanolic extracts of *G. parviflora* herb resulted in the identification of several groups of biologically active compounds. Most of them are polyphenols, flavonoids, carotenoids – have high antioxidant activity, and therefore can cause antibacterial effects. The aim of the study was to collect *G. parviflora* plants, prepare extracts from the above-ground part (grass) and determine the content of polyphenolic compounds, flavonoids, carotenoids, as well as the antibacterial activity of the prepared extracts. In extracts of the *G. parviflora* herb collected in the vicinity of Lviv, the content of polyphenols was determined at the level of 4.38–7.71 mg·g⁻¹ of dry mass (DM) in terms of gallic acid, the content of flavonoids at the level of 3.56–7.27 mg·g⁻¹ of DM in terms of quercetin, and carotenoids – $10.95 \pm 0.5 \mu\text{g} \cdot \text{g}^{-1}$ of DM. Studies of the antibacterial activity of ethanolic and aqueous-ethanolic extracts of the herb *G. parviflora* against test cultures of *Escherichia coli* and *Bacillus brevis*, conducted using the well method, allowed to establish that the extract prepared with 20 % aqueous ethanol most strongly inhibits the growth of the studied cultures. Strong statistically significant relationships were established between the zones of inhibition of *E. coli* and flavonoids – $p < 0.001$.

Key words: *Galinsoga parviflora* Cav., aqueous and aqueous-ethanolic extracts, antibacterial activity.

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Незбутниця дрібноцвіта – перспективна сировина з протибактеріальними властивостями Воробець Н.¹, Яворська Г.²

Galinsoga parviflora Cav. (Родина айстрових) сегетально-рудеральний вид, інтродукований з Перу і поширений по всій території України, Європейського та інших континентів. У нашій країні це небезпечний інвазивний вид, який засмічує посіви та природні біоми. Водночас *G. parviflora* є їстівною рослиною, яку використовують в народній медицині деяких країн, і є результати наукових досліджень, якими підтверджують ефективність її використання як лікарської рослинної сировини з протизапальними, ранозагоювальними, гіпотензивними, кровоспинними, цитотоксичними властивостями та ін. Оскільки вид є морфологічно мінливим, виникає необхідність вивчення його хімічного складу та властивостей перш ніж використовувати, зокрема як лікарської рослинної сировини або з іншою метою. Фітохімічне дослідження водного та водно-етанольного екстрактів надземної частини (трави) *G. parviflora* Cav. (Asteraceae) дало змогу ідентифікувати вміст кількох груп біологічно активних сполук. Більшість з них – поліфеноли, флавоноїди, каротиноїди – мають високу антиоксидантну активність, а відтак, можуть зумовлювати антибактерійну дію. Метою дослідження було визначити вміст поліфенольних сполук, флавоноїдів, каротиноїдів, а також дослідити антибактеріальну активність водного та водно-етанольного екстрактів надземної частини (трави) *G. parviflora*. В екстрактах трави *G. parviflora*, зібраної в околицях м. Львів, визначено вміст поліфенолів, що становить 4,38–7,71 мг · г⁻¹ сухої маси в перерахунку на галову кислоту, вміст флавоноїдів – 3,56–7,27 мг · г⁻¹ сухої маси в перерахунку на кверцетин, каротиноїдів – $10,95 \pm 0,5 \text{ мкг} \cdot \text{г}^{-1}$ сухої маси. Дослідження антибактеріальної активності етанольних та водно-етанольних екстрактів трави *G. parviflora* проти тест-культур *Escherichia coli* та *Bacillus brevis*, проведені за допомогою методу лунок,

дозволили встановити, що екстракт виготовлений з 20 % водним етанолом найсильніше пригнічує ріст досліджуваних культур. Було встановлено сильні статистично значущі зв'язки між зонами інгібування *E. coli* та флавоноїдами – $p < 0,001$.

Ключові слова: *Galinsoga parviflora* Cav., водний і водно-етанольні екстракти, антибактерійна активність.

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Introduction

The species *G. parviflora* is distributed worldwide as a native (to South and North America) or introduced to various countries (in Asia, Africa, and Australia) with temperate and subtropical climates (Damalas et al. 2008; Bharathi et al. 2021). Various biologically active substances have been identified in the aboveground and underground parts of *G. parviflora*, including quercetin, beta-sitosterol, gallic acid, kaempferol, etc. (Mostafa et al. 2013; Shabasy 2019). The presence of these substances gives *G. parviflora* potential pharmacological properties, in particular, antibacterial, antimalarial, antidiabetic and anti-inflammatory (Studzińska-Sroka et al. 2018; Katiyar et al. 2020). These properties are primarily associated with the antioxidant effect of phenolic compounds in plant extracts (Studzińska-Sroka et al. 2018). Some relevant reports on *G. parviflora* from different localities have revealed variations in its chemical constituents (Mostafa et al. 2013).

Galinsoga parviflora has significant antibacterial activity against various bacteria. For example, extracts of *G. parviflora* were weak effective against *B. subtilis*, and had antibacterial potential against *Klebsiella pneumoniae* and *Salmonella typhimurium*, and significant effects were observed against *E. coli* and *Pseudomonas aeruginosa*, compared to standard cefotaxime (Mostafa et al. 2013). There are reports of antibacterial activity against gram-positive bacteria of the leaf oil of *G. parviflora* (Pino et al. 2010). Several compounds of this oil have been shown as mosquito larvicide properties (Govindarajan et al. 2018).

However, the effect of *G. parviflora* extracts on many other species and strains of bacteria remains undiscovered, although there is a need to discover new antibacterial plant extracts that could be used as an alternative to antibiotics against resistant bacteria.

Bacillus brevis (or *Brevibacillus brevis* or *Aneurinibacillus migulanus*) is an aerobic, spore-forming Gram-positive bacteria that secrete various secondary metabolites – agents for combating phytopathogens (Yang, Yousef 2018). In certain cases, these bacteria themselves become the causative agents of the disease (Parvez et al. 2009; Wenbo et al. 2023).

Escherichia coli belongs not only to commensal strains, but also to pathogenic ones, which cause

various human diseases, leading to more than 2 million deaths each year (Kaper et al. 2004; Tenaillon et al. 2010). Commensal and pathogenic strains of *E. coli* demonstrate diverse phenotypic and genotypic variants, with more than 700 serotypes identified. Strains are classified into several phylogenetic groups: A, B1, B2, and D (Clermont et al. 2013). The first registration of the analysis of *E. coli* genome sequences took place in 1997, and since then more than 4800 genomes of these bacteria have been sequenced. The rapid growth of *E. coli* makes them important for studying the evolution of microorganisms, particularly during long-term experimental studies (Tenaillon et al. 2016).

It is believed that *E. coli* mainly inhabits the lower parts of the intestinal tract of warm-blooded animals and humans, and enters the environment through feces (Berthe et al. 2013). The use of *E. coli* as a bacterial host has led to the development of a variety of gene manipulation systems, which have facilitated the production of numerous industrial products. The strain *Escherichia coli* ATCC 25922 was originally isolated from a clinical specimen in Seattle, Washington (1946), its complete genome is known, and the strain is often used in quality control testing (Minogue et al. 2014).

The aim of this study was to evaluate the utility of aqueous and aqueous-ethanolic extracts of the *Galinsoga parviflora* herb in terms of antibacterial activity against *Escherichia coli* and *Bacillus brevis*, and to relate this to the phytochemical profile of these extracts.

Materials and methods

Galinsoga parviflora Cav. plants were collected during the flowering stage in the vicinity of Lviv (Western Ukraine), the above-ground part was separated and dried in the shade at room temperature to an air-dry state. Fresh plant samples were used for macroscopic analysis and identification. The dry raw material was stored in hermetically sealed boxes until use. Before preparing the extracts, the dry raw material was ground by an electric mill (for grinding coffee beans) from Bosch to obtain a powder. The raw material obtained in powder form was used to prepare aqueous and aqueous-ethanol (AE) extracts with 20 %, 60 % and 96 % ethyl alcohol. The extracts were prepared according to the

requirements of the State Pharmacopoeia of Ukraine: ratio of sample: extractant = 1:10 or 1:20 (weight, g/volume, ml). Aqueous extracts and AE extracts made with 20-, 60-, 96 % AE in the same ratio were prepared on a slowly boiling water bath for 30 min under reflux. After cooling, the extracts were filtered through a Whatman paper filter and used in the experiment.

The content of phenolic compounds and flavonoids in the extracts was determined spectrophotometrically according to the methods described in Yavorska et al. (2023). The total carotenoid content in the extracts prepared with 96 % ethanol was determined by the method described in Natividal, Rafael (2014). The results of three studies are presented. In the quantitative analysis of various BAS, extinction determination was performed on a Ulab 102, 102 UV spectrophotometer.

To study antibacterial activity, one-day cultures of bacteria were used as test cultures: *Bacillus brevis* VKM B-503 (ATCC 8246) and *Escherichia coli* ATCC 25922 from the culture museum of the Department of Microbiology of the Ivan Franko National University of Lviv. Test cultures of *E. coli* and *B. brevis* bacteria were grown on non-selective trypticasein soy agar (TSA) during 24 h. Ciprofloxacin, 0.3 % (1 drop/well), was used as a control for bacterial cultures, as well as solvents – 20-, 60- and 96 % ethanol. The antimicrobial effect of the extracts was determined by diffusion into a dense agar medium, in which 0.2 ml of aqueous or aqueous-ethanolic extract from the wells diffused into the medium seeded with suspensions of the studied microorganisms (standardized to 0.5 McFarland, which is approximately 10^8 – 10^9 cells/ml). The seeded plates were placed in a thermostat for one or two days at a temperature of 28 ± 1 °C. After cultivation, the diameter of the zone of inhibition (ZI) was measured with a ruler. The criteria described in Cappelli et al. (2021) were used to assess antimicrobial activity.

Statistical analysis of the results was performed using the Microsoft Office Excel 2016 software package. Correlation analysis was performed in Jamovi 2.3.21 using Pearson's linear pairwise correlation.

Results

The results of the determination of the content of polyphenols, flavonoids and carotenoids in the herb *G. parviflora* are presented in Table 1.

The highest phenolics content was in the extract prepared with 20 % AE, followed by prepared with 60 % AE, prepared with 96 % AE, and aqueous, respectively. Extract prepared with 60 % AE attained the highest flavonoids content, followed by with 20 % AE, with 96 % AE and aqueous, respectively (Table 1).

Comparing our observed results with Studzińska-Sroka's et al. (2018) report on the total content of polyphenols in the herb *G. parviflora* shows that the values of this indicator obtained by us are lower, although the total content of flavonoids is commensurate with ours.

The flavonoid content which was determined by Bazylo et al. (2015) in the ethanolic and aqueous extracts of *G. parviflora* was higher than the values obtained by us. The proportionality of the content when similar extraction conditions are used confirms the expediency of using the amount of flavonoids for the standardization of raw *G. parviflora* materials (Bazylo et al. 2015). Certain differences in the quantitative value of polyphenols and flavonoids may be caused by the different growth conditions of *G. parviflora*: climate, as well as the different extractants and extraction conditions used by different authors. However, our results also indicate a high content of phenolic compounds and flavonoids, in the herb of *G. parviflora*.

It has been established that these components can be used in medicine for the treatment of various diseases, such as Alzheimer's and Parkinson's diseases (Shabani et al. 2020); cancer (Keyvani-Ghamsari et al. 2023), and as an anti-arthritic and antiplatelet agent (Katiyar et al. 2020), and plant raw materials can be used as an ingredient in cosmetics, food and medicines (Soni et al. 2005).

Aqueous and AE extracts of *G. parviflora* were found to have antibacterial properties. The 20 % aqueous-ethanolic extract was the most effective (Table 2). *E. coli* was highly sensitive to extracts prepared with 20 % and 96 % AE, *B. brevis* was significantly affected by the aqueous extract.

Table 1. The total content of polyphenols, flavonoids and carotenoids in *Galinsoga parviflora* extracts, $M \pm m$

Extract type	Total polyphenol content, mg · g ⁻¹ of DW of raw material in terms of gallic acid	Total content of flavonoids, mg · g ⁻¹ DW of raw material in terms of quercetin	Total carotenoid content, µg · g ⁻¹ in terms of DW
Aqueous, 1:10, m: V	4.38 ± 0.08	3.56 ± 0.17	–
Prepared with 20% AE, 1:20/ m: V	7.71 ± 0.14	7.17 ± 0.02	–
Prepared with 60% AE, 1:20/ m: V	7.16 ± 0.88	7.27 ± 0.08	–
Prepared with 96% AE, 1:20/ m: V	6.77 ± 0.10	7.09 ± 0.44	10.95 ± 0.50

Table 2. The effect of extracts from the *Galinsoga parviflora* herb on bacterial culture

Type of extract	Diameter of the zone of inhibition test cultures, mm, $M \pm m$	
	<i>Escherichia coli</i>	<i>Bacillus brevis</i>
Control, ciprofloxacin, 0.3 % (0.2/well)	50.0 $\pm$ 0.5	50.1 $\pm$ 0.5
Control: 20 % AE	5.7 $\pm$ 0.3	5.7 $\pm$ 0.3
Control: 60 % AE	6.3 $\pm$ 0.3	6.3 $\pm$ 0.3
Control: 96 % Ethanol	8.0 $\pm$ 0.3	6.3 $\pm$ 1.5
Aqueous, 1:10, m: V	9.3 $\pm$ 0.3	21.0 $\pm$ 0.5
Prepared with 20 % AE, 1:20/ m: V	28.6 $\pm$ 0.3	24.0 $\pm$ 0.3
Prepared with 60 % AE, 1:20/ m: V	10.0 $\pm$ 0.5	16.0 $\pm$ 0.3
Prepared with 96 % AE, 1:20/ m: V	15.0 $\pm$ 0.5	12.0 $\pm$ 0.3

Note: AE – aqueous ethanol

Regarding the aqueous-ethanolic extracts, *B. brevis* was moderately sensitive to the aqueous-ethanolic extract prepared with 96 % AE.

The ability of some plant secondary metabolites, particularly polyphenols, to act as resistance-modifying agents is a promising area in mitigating the spread of bacterial resistance. The presence of polyphenols, flavonoids, as well as the high content of carotenoids as constituents of *G. parviflora* extracts may contribute to the antibacterial effects.

Correlation analysis of the relationships between the zones of inhibition of *E. coli* and *B. brevis* and the content of polyphenols and flavonoids in the extract with 20 % aqueous ethanol made it possible to establish strong statistically significant relationships between the zones of inhibition of *E. coli* and flavonoids – $p < 0.001$.

Escherichia coli are Gram-negative rod-shaped bacteria that are both commensal strains of the intestinal microbiota of humans, mammals, and birds, as well as important pathogens that often cause various diseases and increase deaths every year (Clements et al. 2012; Wirth et al. 2006). Because *E.*

coli lives in the human gut, it can develop antibiotic resistance with frequent antibiotic abuse, which later worsens patient outcomes, so it is important to find ways to prevent the spread of antibiotic resistance in these bacteria (Jang et al. 2017).

The high sensitivity of *B. brevis* and *E. coli* bacteria to *G. parviflora* extracts indicates the potential for their further study in order to create a remedy for the treatment of diseases of the urinary tract (prostatitis and pelvic inflammatory disease) and central nervous system caused by these pathogens (Mueller, Tainter 2025; Parmar et al. 2020).

Conclusions

As a result, the studied extracts of *G. parviflora* may be used in further research as antibacterial herbal agents. The plant extract obtained using 20 % aqueous ethanol demonstrated the strongest inhibitory effect on the growth of the tested bacterial cultures *Escherichia coli* and *Bacillus brevis*.

Declaration of interest

The authors report no conflicts of interest.

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