The phytochemical profile and antibacterial activity of fluid extracts of *Galium verum* L. herb

**Aim.** To obtain and study the qualitative composition and the content of the main groups of biologically active substances (BAS) of fluid extracts (FEs) of *Galium verum* L. herb and to assess their antibacterial activity.

**Materials and methods.** FEs of *Galium verum* L. herb were obtained by three-fold water extraction or ethanol (20 %, 60 %, 96 %) extraction of the raw material when heating, followed by the concentration of the combined extracts. Phenolic compounds of FEs were studied by the methods of paper and thin-layer chromatography, and spectrophotometry. The content of polysaccharides was determined gravimetrically. The antibacterial activity of FEs was determined *in vitro* by the agar diffusion method.

**Results and discussion.** The chromatographic study of FEs of *Galium verum* L. herb revealed the presence of chlorogenic acid and rutin in all FEs, the fluid 96 % ethanol extract contains chlorogenic acid, cyanoside, quercetin and rutin. Hydroxycinnamic acids, flavonoids and the amount of phenolic compounds were quantified in all extracts, and polysaccharides were determined in the aqueous extract and 20 % ethanol extract. When studying the antimicrobial activity of FEs it has been found that the fluid 96 % ethanol extract exhibits the highest activity. *Bacillus subtilis* was the most susceptible to all the extracts under study. *Proteus vulgaris* showed insignificant sensitivity to the test concentration of fluid extracts obtained with water and 60 % ethanol. The rest of the microorganism test strains used were sufficiently sensitive to the FEs under study.

**Conclusions.** The results obtained indicate the prospects of further in-depth study of the antimicrobial activity of fluid extracts of *Galium verum* L. herb in order to develop antibacterial agents on their basis.

**Key words:** *Galium verum* L.; fluid extracts; antibacterial activity
Материалы и методы. ЭЖ травы подмаренника настоящего получены путем трехкратной экстракции сырья водой или 20 %, 60 %, 96 % этанолом при нагревании с последующим концентрированием объединенных извлечений. Фенольные соединения ЭЖ исследовали методами бумажной и тонкослойной хроматографии и спектрофотометрии. Содержание полисахаридов определяли гравиметрическим методом. Антибактериальную активность ЭЖ устанавливали методом «колоцев» in vitro.

Результаты и их обсуждение. При хроматографическом изучении ЭЖ травы подмаренника настоящего установлено, что все ЭЖ содержат хлорогеновую кислоту и рутин. ЭЖ с использованием в качестве экстрагента 96 % этанола содержит хлорогеновую кислоту, цинарозид, кверцетин и рутин. Установлено содержание гидроксикоричных кислот, флавонидов и суммы фенольных соединений во всех экстрактах: содержание полисахаридов – в водном и спиртовом (20 % этанол) экстрактах. При изучении антибактериальной активности ЭЖ установлено, что наиболее активным является экстракт, полученный с использованием 96 % этанола. Наиболее чувствительным ко всем исследованным экстрактам оказался Bacillus subtilis. Незначительную чувствительность к испытуемой концентрации ЭЖ, полученных с использованием воды и 60 % этанола, показал Proteus vulgaris. Остальные использованные тест-штаммы микроорганизмов оказались достаточно чувствительными к исследованным ЭЖ.

Выводы. Полученные результаты свидетельствуют о перспективности дальнейшего углубленного изучения антибактериальной активности жидких экстрактов из травы подмаренника настоящего с целью создания на их основе антибактериальных средств.

Ключевые слова: подмаренник настоящий; экстракты жидкие; антибактериальная активность

The search for the antibacterial and antifungal agents of the plant origin is among the topical issues facing pharmacy.

In the folk medicine Bedstraw species (Galium L.) of Madder family (Rubiacae Juss.) are widely used in the treatment of skin diseases, respiratory and urogenital disorders, and sepsis [1]. The numerous experimental studies conducted worldwide demonstrate the antimicrobial properties of Galium species [2-5].

Previously, the antimicrobial and antifungal properties of certain Galium species were found [6]. Among them Galium verum L. should be mentioned, its lipophilic fractions exhibit a wide range of the antimicrobial and antifungal activity [7-9].

The aim of this work was to study the composition and antibacterial activity of fluid extracts of Galium verum L. herb obtained with different extragents, namely water, 96 % ethanol and water-alcoholic mixtures.

Materials and methods

Galium verum herb was harvested at full flowering stage in the Kharkiv region in June of 2016. The objects of the research were FEs of Galium verum L. herb obtained with water (EP-w) or 20 % (EP-20), 60 % (EP-60), 96 % (EP-96) three-fold extraction (30 min each) of the air-dried raw material in the ratio of the raw material: extractant of 1 : 10 when heating with the subsequent concentration of the combined three extracts to the ratio of the raw material – finished product of 1 : 10.

Phenolic compounds of FEs were studied by paper chromatography (PC) and thin-layer chromatography (TLC). The conditions for PC were as follows: Filtrac chromatographic paper (FN-12); the solvent system of ethyl acetate – formic acid – water (10 : 2 : 3 , v/v/v); the temperature – 20 ºC; UV light (λ = 354 nm); developers – ammonia vapors; 3 % KOH alcohol solution. The conditions for TLC were as follows: Sorbil and Silufol plates; UFS-254/365 irradiator; the solvent system of ethyl acetate – formic acid – water (10 : 2 : 3 , v/v/v); developers – 5 % AlCl3 alcohol solution, 3 % KOH alcohol solution; 2 % ZrOCl2 alcohol solution with citric acid.

As reference standards 0.1 % solutions of authentic samples of chlorogenic acid, rutin, quercetin, kaempferol, apigenin, luteolin, cyanoside were used. Compounds were identified by coloring after the reaction with chromogenic developers, by the character of fluorescence in UV light and the Rf value compared to the authentic samples.

In the fluid extracts the content of phenolic compounds was determined using an Evolution 60 S UV-Visible (Thermo scientific) spectrophotometer: hydroxycinnamic acids at the wavelength λ = 327 nm (as chlorogenic acid), flavonoids at the wavelength λ = 410 nm (as rutin), the amount of polyphenolic compounds at the wavelength λ = 270 nm (as gallic acid).

According to the WHO recommendations for the study of the antibacterial activity of drug products to assess the antibacterial activity of FEs the test-strains of Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Proteus vulgaris ATCC 4636, Bacillus subtilis ATCC 6633, Candida albicans 885-663 were used.

In the study 0.3 ml of FEs were used.

The antibacterial activity of FEs was studied in vitro by the agar diffusion method (agar well method) [10]. The microbial load was 107 microbial cells per 1 ml of the medium; it was determined visually according to McFarland turbidity standard.

To determine the antimicrobial activity the microorganism cultures were grown on meat-peptone agar at t = 37 ºC. Microorganisms were cultured for 24 hours. To determine the antifungal activity of FEs the Sabouraud medium was used. The degree of sensitivity of microorganisms in relation to the FEs under study was estimated by the size of their growth inhibition zones.

The sensitivity of microorganisms to the FEs under study was measured as a radius of the inhibition zone. The assessment of the sensitivity of microorganisms was performed according to the following criteria:

• no growth inhibition zones, as well as the inhibition zone to 10 mm showed that the microorganism was not sensitive to the substance studied or the selected concentration of the substance;
The composition of fluid extracts of *Galium verum* L. herb

| Extragent      | Polysaccharides (%) | Hydroxycinnamic acids (%) | Flavonoids (%) | The amount of phenolic compounds |
|----------------|---------------------|---------------------------|----------------|-------------------------------|
| Water          | 6.27                | 4.24                      | 0.40           | 3.66                          |
| Ethanol 20 %   | 2.40                | 3.10                      | 0.24           | 2.90                          |
| Ethanol 60 %   | 3.10                | 1.63                      | 0.16           | 3.84                          |
| Ethanol 96 %   | 2.70                | 0.18                      |                | 2.70                          |

Note. * – no polysaccharides.

The antimicrobial activity of extracts of *Galium verum* L. herb

| Extragent      | Staphylococcus aureus (M + m) | Escherichia coli (M + m) | Pseudomonas aeruginosa (M + m) | Bacillus subtilis (M + m) | Proteus vulgaris (M + m) | Candida albicans (M + m) |
|----------------|-------------------------------|--------------------------|-------------------------------|--------------------------|--------------------------|--------------------------|
| Water          | 17.7 ± 0.4                    | 17.3 ± 0.3               | 16.3 ± 0.3                    | 18.7 ± 0.3               | 15.0 ± 0.2               | 15.6 ± 0.2                |
| Ethanol 20 %   | 20.0 ± 0.2                    | 19.7 ± 0.2               | 15.7 ± 0.4                    | 18.3 ± 0.2               | 15.3 ± 0.3               | 16.7 ± 0.3                |
| Ethanol 60 %   | 18.3 ± 0.3                    | 17.3 ± 0.3               | 17.3 ± 0.3                    | 21.0 ± 0.2               | 14.3 ± 0.3               | 16.4 ± 0.3                |
| Ethanol 96 %   | 20.7 ± 0.4                    | 20.3 ± 0.3               | 18.7 ± 0.4                    | 21.7 ± 0.3               | 18.7 ± 0.3               | 19.3 ± 0.4                |

- inhibition zones with the diameter of 10-15 mm indicated low sensitivity of the microorganism to the concentration of the test substance;
- inhibition zones with the diameter of 15-25 mm were regarded as an indicator of sufficient sensitivity of the microorganism to the test substance;
- inhibition zones with the diameter exceeding 25 mm showed a high sensitivity of microorganisms to the substances studied.

The statistical processing of the results was performed according to Glants S. [11].

**Results and discussion**

The chromatographic study of FEs of *Galium verum* L. herb revealed the presence of chlorogenic acid and rutin in all FEs, the fluid 96 % ethanol extract contained chlorogenic acid, cyanoside, quercetin and rutin.

The results of the BAS content determination showed that the highest quantity of polysaccharides, hydroxycinnamic acids and flavonoids was in the aqueous extract – FE-w (Tab. 1). The lowest quantity of phenolic compounds was in FE-96.

When studying the antimicrobial activity of FEs it was found that all extracts under study exhibited the antimicrobial activity (Tab. 2).

*Proteus vulgaris* was highly susceptible to FE-96 and unsusceptible to FE-60.

Extracts FE-20 and FE-96 exhibited the highest activity in relation to *Staphylococcus aureus* and *Escherichia coli*.

FE-96 exhibited the highest activity in relation to *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Candida albicans*.

*bacillus subtilis* was highly susceptible to FE-96 and FE-60.

On average, FE-96 exhibited the highest antimicrobial activity. Among the test strains used *bacillus subtilis* was the most susceptible to all extracts under study.

**CONCLUSIONS**

1. For the first time the antibacterial and antifungal activities of fluid extracts of *Galium verum* L. herb obtained with water and ethanol (20 %, 60 % and 96 %) extraction and differed in the content of BAS have been studied.
2. It has been found that all fluid extracts exhibit the antimicrobial activity. The fluid 96 % ethanol extract exhibits the highest activity. *bacillus subtilis* was the most susceptible to all the extracts under study.
3. The results obtained indicate the prospects of further in-depth study of the antimicrobial activity of fluid extracts of *Galium verum* L. herb in order to develop antibacterial agents on their basis.

**Conflict of Interests:** authors have no conflict of interests to declare.

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