Phenolic compounds of the liquid extract from cleavers herb (Galium aparine L.)

**Aim.** To study the qualitative composition and the quantitative content of phenolic compounds of the liquid extract obtained from cleavers (Galium aparine L.) herb.

**Materials and methods.** The extract of cleavers herb was obtained by triple extraction of the raw material with 60% ethanol when heating, followed by the concentration of the combined extracts to the ratio of the raw material : finished product of 1 : 1. The content of extractives was determined by the gravimetric method. Phenolic compounds were studied by the methods of high-performance liquid chromatography (HPLC) and UV-spectrophotometry.

**Results and discussion.** In the chromatographic study of the extract from cleavers herb 11 compounds of phenolic nature were reliably identified, including 5 hydroxycinnamic acids – caffeic, chlorogenic, neochlorogenic, 3,5- and 4,5-dicaffeoylquinic acids; and 6 flavonoids – catechin, quercetin, quercitrin, isoquercitrin, rutin and hyperoside. Rutin (1.89 mg/ml) and chlorogenic acid (0.78 mg/ml) were predominant compounds. In the spectrophotometric determination of total content of phenolic compounds it was found that the amount for hydroxycinnamic acids calculated with reference to chlorogenic acid was 1.9 %, for flavonoids calculated with reference to rutin – 0.25 %, for polyphenolic compounds calculated with reference to gallic acid – 1.35 %. The dry residue was 24.63 %.

**Conclusions.** The significant content of extractive substances, flavonoids and hydroxycinnamic acids with predominance of rutin and chlorogenic acid in the alcohol-water extract from cleavers herb indicate the feasibility of its further pharmacological study.

**Key words:** Galium aparine L.; liquid extract; phenolic compounds
Cleavers (Galium aparine L.) herb of Rubiaceae Juss. family belongs to the cosmopolitan plants distributed in the temperate zone of Europe, Asia and North America. Cleavers herb is part of the homeopathic product Galium-Hel; it is also used in alternative medicine in many countries as a diuretic, choleretic drug in the treatment of skin diseases, epilepsy and gout [1].

As a result of numerous foreign studies it has been found that cleavers herb contains iridoids: asperulosidic acid and 10-deacetylasperulosidic acid [2], monotropein, asperuloside, acumine, aucubin; alkaloids: protopine, harmane, (±)-vasicinone, (-)-l-hydroxypeganine, (-)-8-hydroxy-2,3-dihydrodesoxypeganine [3]; n-hydroxybenzoic, chlorogenic, caffeic, n-coumaric acids; flavonoids, anthraquinones, cholesterol, campesterol, stigmasterol, sitosterol, Δ-[5]-avenasterol, Δ-[7]-stigmastanol, Δ-[7]-avenasterol [4]; polyphenolic compounds [5, 6]; phytosterols [7], saponins [8] and silicic acid. According to our previous studies sesquiterpenoids, squalene, aromatic compounds, higher alkanes and their derivatives, fatty acids, chlorophylls, and carotenoids were identified in cleavers herb [9].

The aim of our work was to study the content of phenolic compounds in the liquid extract obtained from cleavers herb. Materials and methods

Cleavers herb was collected at the flowering stage in the Botanical garden of the National University of Pharmacy (NUPh, Kharkiv, Ukraine) in June, 2017. The herbarium sample is stored in the Herbarum (No. 53/2017) at the Department of Pharmacognosy of the NUPh.

To obtain the liquid extract, 100.0 g of the air-dry crushed of cleavers herb was placed in a flask, 1000 ml of 60 % ethanol was added and heated with a reflux condenser on a boiling water bath for 30 min. After cooling the content of the flask was filtered, and the volume of the resulting solution was measured. The procedure was repeated twice under the same conditions. The filtrates were combined and concentrated under vacuum in a rotary vacuum evaporator to the ratio of the raw material – finished product of 1:1; thus, 100 ml of the liquid extract was obtained.

The content of extractives in the extract was determined by the gravimetric method [10]. Determination of the total content of different groups of phenolic compounds was carried out on an Evolution 60 S UV-Visible spectrophotometer (Thermo scientific): hydroxycinnamic acids – at \( \lambda = 327 \) nm calculated with reference to chlorogenic acid [11, 12], flavonoids – using the method of differential spectrophotometry with aluminum chloride at \( \lambda = 410 \) nm calculated with reference to rutin [13], the amount of polyphenolic compounds – at \( \lambda = 270 \) nm calculated with reference to gallic acid [14, 15].

The content of phenolic compounds was studied by the method of high-performance liquid chromatography (HPLC) on a Shimadzu LC20 Prominence liquid chromatograph in the modular system equipped with the 4-channel pump LC20AD, the column thermostat CTO20A, the automatic sampler SIL20A, the diode array detector SPD-M20A and ChemStation LC20. The chromatographic conditions were as follows: the Phenomenex Luna C18(2) chromatographic column with the size of 250 mm × 4.6 mm and the particle size of 5 µm; the column temperature – 35 °C; the detection wavelength – 330 nm (for hydroxycinnamic acids, glycosides of flavonoids), 370 nm (for aglycones of flavonoids), 280 nm (for tannins); the flow rate of the mobile phase \( \lambda = 1 \) ml/min; the injection volume – 5 µl.

The mobile phase was eluent A – 0.1 % solution of trifluoroacetic acid in water, eluent B – 0.1 % solution of trifluoroacetic acid in acetonitrile in a gradient mode (Tab. 1).

Identification of the components was carried out by the retention time and compliance of UV spectra with reference substances [16-18].

The content of substances in the liquid extract was calculated by the formula:

\[
X, \text{mg/ml} = \frac{A_{pr} \times C_p \times P}{A_{tr} \times 100},
\]

where: \( A_{pr} \) – is the peak area of the substance on the chromatogram of the test solution; \( A_{tr} \) – is the peak area of the substance on the chromatogram of the reference solution; \( P \) – is the activity of the reference standard, \%.
Results and discussion

The liquid extract obtained is a thick liquid of a brown-green color with a specific odor.

As a result of the studies conducted the content of the main groups of BAS of phenolic character in the liquid extract of cleavers herb was determined (Tab. 2).

Using HPLC 11 compounds of phenolic nature were reliably identified, including 5 hydroxycinnamic acids and 6 flavonoids, in the extract obtained (Fig., Tab. 3).

In addition to the phenolic compounds reliably identified the presence of derivatives of chlorogenic acid was also determined by the characteristic UV spectra.

### Table 1

| The time of chromatography (min) | Eluent A, % | Eluent B, % |
|----------------------------------|-------------|-------------|
| 0-5                              | 95          | 5           |
| 5-35                             | 95 → 75     | 5 → 25      |
| 35-40                            | 75          | 25          |
| 40-60                            | 75 → 50     | 25 → 50     |
| 60-65                            | 50 → 20     | 50 → 80     |
| 65-70                            | 20          | 80          |
| 70-85                            | 95          | 5           |

$C_u$ – is the concentration of substance in the reference solution, mg/ml.

### Table 2

| The BAS group                               | Content, % |
|---------------------------------------------|------------|
| The amount of hydroxycinnamic acids         | 1.90       |
| The amount of flavonoids                    | 0.25       |
| The amount of polyphenolic compounds        | 1.35       |
| The content of extractives                  | 24.63      |

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### Table 3

| Phenolic compounds of the liquid alcohol extract from Galium aparine herb |
|-----------------------------|-----------------|----------------|
| Compound                    | Retention time, min | The content in the extract, mg/ml | UV-spectrum |
| Neochlorogenic acid*        | 14.8-15.0        | 0.11           |             |
For the first time quercitrin, neochlorogenic acid, 3,5-dicaffeoylquinic and 4,5-dicaffeoylquinic acids were identified in the raw material of cleavers herb.

In the chromatographic study of the extract from *Galium aparine* herb apigenin and kaempferol were not detected. It confirms the data of previous researchers of the plant raw material of *Galium aparine* [6]. Therefore, it has been found by HPLC that the dominant phenolic compounds are rutin and chlorogenic acid (1.89 and 0.78 mg/ml, respectively). Flavonoids are pre-

| 1       | 2     | 3     | 4     |
|---------|-------|-------|-------|
| Catechin | 19.4  | 0.13  |       |
| Chlorogenic acid | 20.0-20.4 | 0.78 |       |
| Caffeic acid | 21.8-22.0 | 0.03 |       |
| Rutin    | 39.9-31.0 | 1.89 |       |
| Hyperoside | 31.8-32.4 | 0.31 |       |
| 3,5-Dicaffeoylquinic acid * | 35.0 | 0.48 |       |
| Quercitri | 35.5-35.9 | 0.02 |       |
| 4,5-Dicaffeoylquinic acid * | 37.0 | 0.13 |       |
| Quercetin | 46.6-47.2 | 0.03 |       |
| Isoquercitrin | 54.0 | 0.002 |       |

Note. * – determined and calculated with reference to chlorogenic acid due to the absence of reference standards.
dominantly presented by glycosides of quercetin and catechin.

CONCLUSIONS
1. The liquid extract from *Galium aparine* herb with the content of extractives of 24.63% has been obtained.  
2. Using HPLC in the liquid extract from *Galium aparine* herb 5 hydroxycinnamic acids – caffeic acid, chlorogenic acid, neochlorogenic acid, 3,5- and 4,5- dicaffeoylquinic acids; 6 flavonoids – catechin, quercetin, isoquercitrin, hyperoside and rutin have been identified, and their content has been determined.  
3. The content of hydroxycinnamic acids has been determined by the method of spectrophotometry, it is 1.90%, for flavonoids – 0.25%, and the amount of polyphenolic compounds – 1.35%.

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

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