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Investigation of phenolic compounds content in *Chamerion angustifolium* L. herb freeze-dried extract

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Abstract

Phytochemical investigation of *Chamerion angustifolium* L. herb freeze-dried extract was performed. The presence of phenolic compounds and quantitative content of their main groups was established. The extract contains 9.76% of hydroxycinnamic acids in terms of chlorogenic acid, 11.92% of flavonoids in terms of rutin, 24.23% of tannins in terms of pyrogallol and 20.3% of phenolic compounds in terms of gallic acid were determined.

Keywords: Freeze-dried extract, *Chamerion angustifolium* L., hydroxycinnamic acids, flavonoids, phenolic compounds, tannins

1. Introduction

Phytotherapeutic treatment and prevention of diseases is recognized by traditional medicine and it has quite reasonable basis – biologically active chemical components of medicinal plants. The herbal medicines realized their pharmacological effects through certain biologically active substances in its composition, which can affect physiological processes in the body [1].

Herbal medicines commit softer effect, have a wide range of pharmacological activity, practically don't cause addiction and have more potent safety indexes in compare with synthetic drugs. They have relatively low toxicity and can be administered for a long time, especially for patients' rehabilitation [2-4]. Due to the presence of numerous groups of biologically active substances with variety pharmacological effects in plants they can be used for treatment a lot of diseases.

Therefore, one of the most perspective fields of pharmacy and pharmacology is the study of new medicinal plants species and creation of new medicines on their base [5, 6].

The search of medicinal plant materials containing phenolic compounds is a leader among current issues of pharmacy and herbal medicine.

C. angustifolium L. belongs to *Onagraceae* family *Chamerion* genus. This genus counts more than 400 species in the world's flora. They are the most common in large parts of Western Europe, Siberia, China, Japan, and North America and also in our country.

Infusion of *C. angustifolium* L. flowers and herb or decoctions of rhizomes are widely used in folk medicine. It is known at herbal medicine as astringent, enveloping, anti-inflammatory, analgesic, hemostatic, restorative and anticancer agent. *C. angustifolium* L. extracts are effective in the treatment of gastric and duodenal ulcer, gastritis, colitis, various gastrointestinal disorders such as diarrhea and dysentery. Also it is can be used for the treatment of prostate diseases or inflammation of the urethra [7]. It was experimentally proved that *C. angustifolium* L. extract has analgesic [8], anti-inflammatory [9], antibacterial and antifungal effects [10].

Medicines based on *C. angustifolium* L. are practically absent at Ukrainian pharmaceutical market. That is why the investigation is relevant in order for creation a new drugs.

The aim of this study was to investigate the content of phenolic compounds at *C. angustifolium* L. herb freeze-dried extract.

2. Materials and methods

The air-dried herb of *C. angustifolium* L. was collected in Ternopil region (Ukraine) in the period of flowering in July 2015.

The extraction was performed by modified repercolation method. Extract was lyophilized by SP Scientific VirTis Freeze-Drier/Lyophilizer.

The quantitative and quantitative content investigations of the phenolic compounds,

flavonoids, hydroxycinnamic acids and tannins amounts were performed according to the Ukrainian Pharmacopeia. The measurements were carried out using spectrophotometer Lambda 25.

Using identification reactions it was determined that *C. angustifolium* L. herb freeze-dried extract contains phenolic compounds. For this purpose were used cyanidin test and reaction with 10% ethanol-water solution of potassium hydroxide (identification of flavonoids), 1% solution of iron (III) chloride (hydroxycinnamic acids, simple phenols), solution of iron (III) ammonium sulfate, 1% solution of gelatin (tannins) [11].

Determination of qualitative composition of the flavonoids in the extract was performed by thin layer chromatography. Extract solution (50 ml) was applied to the start line of the chromatographic plate. In parallel, at a distance of 10 mm were applied 10 ml of alcohol solutions of hyperoside, rutin, quercetin, kaempferol, apigenin and luteolin standard samples.

The plate was dried and placed in a chromatographic chamber with solvent system n-butanol-acetate acid-purified water (4: 1: 2). Then plate was removed, dried and kept over the pairs of potassium hydroxide or ammonia. Chromatogram was analyzed in ultraviolet light at a wavelength of 365 nm.

Determination of qualitative composition of hydroxycinnamic acids in the extract also was performed by thin layer chromatography.

The standard samples of rosemary, p-coumaric, neochlorogenic, chlorogenic, caffeic and ferulic acids and the solvent system n-butanol-acetate acid-purified water (4: 1: 2) were used. Chromatograms were analyzed in a daylight and UV-light before and after processing by pairs of ammonia and 3% solution of iron (III) chloride.

Determination of phenolic compounds quantitative content was performed and calculated on gallic acid. The optical density of the solution was measured at a wavelength 270 nm. Determination of flavonoids quantitative content was performed using a spectrophotometric method and calculated on rutin at a wavelength 410 nm. The method is based on the property of flavonoid aglycones to form complex with $AlCl_3$.

Determination of hydroxycinnamic acids content in *C. angustifolium* L. herb freeze-dried extract was performed and calculated on chlorogenic acid. The optical density of the solution was measured at a wavelength 327 nm [12].

The determination of total polyphenols, polyphenols unabsorbed on hide powder (nontannin polyphenols) and tannins was performed by the spectrophotometric method with phosphorous-volframic acid and hide powder at a wavelength 760 nm [11] and calculated on pyrogallol.

2.1 Statistical analysis

The content of all investigated phenolic compounds was evaluated in five independent analyses and data were expressed as means \pm SD. Values were determined using Statistica v 10.0 (StatSoft I nc.) program.

3. Results and discussion

Identification reactions showed, that *C. angustifolium* L. herb freeze-dried extract contains flavonoids, mainly flavonol derivatives (in conducting of cyanidin test the color of test solution changed to reddish-pink, in reaction with alkali appeared yellow color), condensed tannins (in the reaction solution of iron (III) ammonium sulfate appeared dark green color, in reaction with 1% gelatin solution appeared turbidity

which disappeared with an excess of gelatin); hydroxycinnamic acids (observed the appearance of green-gray color, which indicates the presence of phenolic compounds) [13].

In *C. angustifolium* L. herb freeze-dried extract were identified rutin, luteolin, apigenin, isoquercetin and hyperoside (Fig. 1).

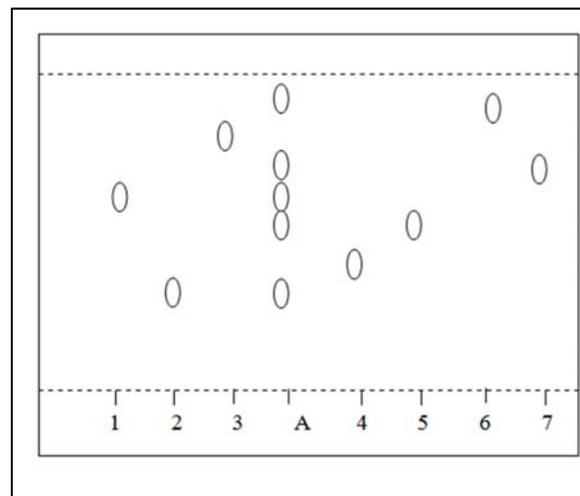


Fig 1: The scheme of *C. angustifolium* L. herb freeze-dried extract chromatogram: 1 – hyperoside; 2 – rutin; 3 – kaempferol; 4 – quercetin; 5 – apigenin; 6 – luteolin; 7 – isoquercetin; A – *C. angustifolium* L. herb freeze-dried extract. Solvent system - n-butanol- acid acetate -purified water (4: 1: 2).

By TLC-method in *C. angustifolium* L. herb freeze-dried extract were determined 5 hydroxycinnamic acids such as chlorogenic, neochlorogenic, caffeic, rosemary, and p-coumaric acids.

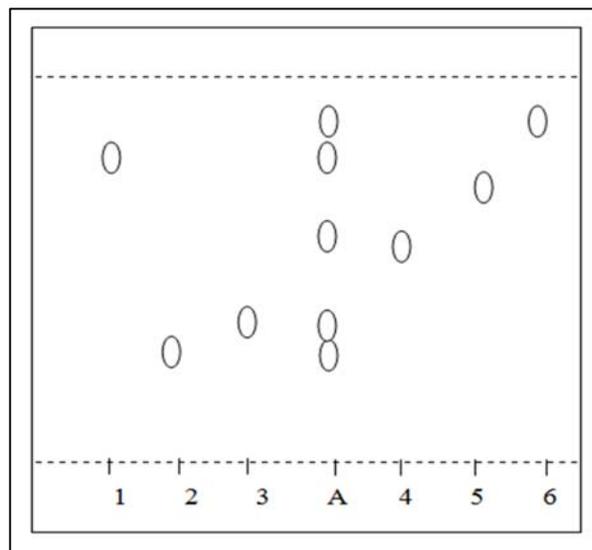


Fig 2: The scheme of *C. angustifolium* L. herb freeze-dried extract hydroxycinnamic acids chromatogram: 1 – rosemary acid; 2 – neochlorogenic acid; 3 – chlorogenic acid; 4 – caffeic acid; 5 – ferulic acid; 6 – p-coumaric acid; A – *C. angustifolium* L. herb freeze-dried extract. Solvent system - n-butanol-acetate acid-purified water (4: 1: 2).

Table 1 shows results of the quantitative analysis of phenolic compounds.

Table 1: The content of main biologically active substances (% = Mean \pm SD, n = 5)

Compound	Content,%
Phenolic compounds	20,3 \pm 0,003
Hydroxycinnamic acids	9,76 \pm 0,01
Flavonoids	11,92 \pm 0,04
Tannins	24,23 \pm 0,01

Considering the above information, it can be argued that the *C. angustifolium* L. herb freeze-dried extract contains higher quantities of tannins in comparison with all others. The content of natural phenolic compounds was also significant. Phenolic compounds have different pronounced pharmacological activity. Hydroxycinnamic acids exhibit anti-inflammatory, anti-tumor activity, affect lipid metabolism, prevent the development of coronary artery disease [14-16]; flavonoids and condensed tannins have a strong antioxidant activity, which causes their hepatoprotective, hypocholesterolemic and anti-sclerotic effects, membrane stabilizing properties, antimicrobial, anticancer and antiviral action [17-19]. Therefore, we can anticipate such kinds of pharmacological activity for future investigation of *C. angustifolium* L. herb freeze-dried extract as therapeutic agent.

4. Conclusions

1. The phytochemical analysis of *C. angustifolium* L. herb freeze-dried extract and established the presence of natural phenolic compounds: flavonoids, hydroxycinnamic acids, tannins was performed.
2. Main flavonoids of *C. angustifolium* L. herb freeze-dried extract such as rutin, isoquercetin, hyperoside, apigenin, luteolin was identified by TLC method. The main hydroxycinnamic acids are chlorogenic, neochlorogenic, caffeic, rosemary and p-coumaric acids.
3. Quantitative content of phenolic compounds in *C. angustifolium* L. herb freeze-dried extract was determined using spectrophotometric methods. The extract content hydroxycinnamic acids (9,76 \pm 0,01)%, flavonoids (11,92 \pm 0,04)%, tannins (24,23 \pm 0,01)%, phenolic compounds (20,3 \pm 0,003)%.
4. The results of our investigation indicate the advisability of further pharmacological study of *C. angustifolium* L. herb freeze-dried extract as anti-inflammatory, antioxidant and antimicrobial medicine.

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