

## STUDY OF ANTIBACTERIAL AND ANTIFUNGAL PROPERTIES OF THE LYOPHILIZED EXTRACT OF FIREWEED (*CHAMAENERION ANGUSTIFOLIUM* L.) HERB

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### Abstract

*Chamaenerion angustifolium* L. (*Epilobium angustifolium* L.) is commonly known also as fireweed, belongs to the family *Onagraceae*. The medicinal properties of this plant have been known many year. These properties are the result of the availability of many groups of biologically active. The herb of this plant is used in various diseases. The lyophilized extract of *Chamaenerion angustifolium* L. herb was studied to present antibacterial and antifungal properties. Phytochemical analysis of lyophilized extract indicated the presence of hydroxycinnamic acids. By thin-layer chromatography method in *Chamaenerion angustifolium* L. herb was identified the following hydroxycinnamic acids: chlorogenic, neochlorogenic, rosmarinic and caffeic acids.

Using the method of spectrophotometry, the quantitative content of hydroxycinnamic acids in the herb of the study plant was defined. The content of the total of hydroxycinnamic acids in *Chamaenerion angustifolium* L. herb, in recalculation at chlorogenic acid, which was (2.55 ± 0.03)%. Antibacterial and antifungal activities of the obtained extract were evaluated by two-fold serial dilutions in a liquid nutrient and agar diffusion ("wells" method). The bactericidal effect of a native lyophilized extract of *Chamaenerion angustifolium* L. herb on gram-positive microflora (*Staphylococcus aureus* – (20±2.3) mm; *Bacillus cereus* – (25±2.2) mm) was proved; bacteriostatic – up to fungi of the *Candida* genus (15±1.5) mm).

Therefore, the pharmacologically active substance in the form of the lyophilized extract of *Chamaenerion angustifolium* L. herb is highly effective and can find its application in the development of new drugs with antimicrobial action.

**Keywords:** *Chamaenerion angustifolium* L., herb, lyophilized extract, antibacterial activity, hydroxycinnamic acids

## Introduction

In broad years, the search for medicinal plants with a long history of use and small side effects is of interest to our society [1-3]. Nowadays, the world pharmaceutical industry extensively uses herbal raw materials as a basis for the creation of drugs [4, 5]. Conditioned the always growing needs of the industry in herbal raw materials for the manufacture of medicines, and the prominent task of pharmaceutical science is to increase existing and search for new sources [6-8]. The plants often use in the wrestle against many diseases [9-11].

There are many plants well known to contain numerous biologically active substances. Nowadays, plants rich in antioxidants and antibacterial properties are very important. Many drugs with high antioxidant potential are produced from these plants. On the other hand, new plants with such properties are constantly being sought. One of the plants that can help to meet health is the fireweed (*Chamaenerion angustifolium* L. or *Epilobium angustifolium* L.), which is one of the best known medicinal plants.

Fireweed (*Chamaenerion angustifolium* L.) (*Onagraceae*) is a well-known medicinal plant due to its anti-inflammatory, antioxidant, antibacterial, analgesic, and anti-cancer properties [12-15]. According to traditional medicine, its roots and herb were used as extracts and infusions in the treatment of gastrointestinal disorders, and skin diseases [16]. In classical folk medicine, the herbal parts of *Chamaenerion angustifolium* L. are used for the treatment of mainly benign prostatic hyperplasia (BPH), which is frequently associated with prostatitis and prostatic diseases [17].

The herb *Chamaenerion angustifolium* L. has been intensively studied concerning phenolic compounds which resulted in many flavonoids. Fireweed herb contains about 1–2% of flavonoids, such as the aglycones myricetin, kaempferol, 8-methoxykaempferol, and quercetin, and their glycosides [18, 19, 20]. The anti-inflammatory activity of *E. angustifolium* has been tightly related to its content of flavonoids evidenced by the finding of myricetin 3-O-glucuronide as a strongly active component in the rat paw edema test, a model of acute inflammation [21, 22].

Herbal and plant-based sources reported to have antibacterial properties may provide a cost-effective and viable source for the development of new antibacterial drugs such as the herb of *Chamaenerion angustifolium* L. Respectively, a systematic review of the literature related to fireweed herb were undertaken to identify potential new plant-based extracts and compounds with potential antibacterial properties. The aim of this work was to evaluate the antimicrobial activity of the extract of the herb of *Chamaenerion angustifolium* L.

## Methods

### Plant materials

The object of the research was the herb of *Chamaenerion angustifolium* L. This raw material was collected in the Temopil region (Western Ukraine) in the period of flowering in July 2017. The study raw material was authenticated by prof. Svitlana Marchyshyn (I. Horbachevsky Temopil National Medical University, Temopil, Ukraine) [23, 24]. A voucher specimen of *Chamaenerion angustifolium* L. is kept at the Department of Pharmacognosy and Medical Botany, TNMU [25]. The herb was dried using the conventional method and then stored in paper bags in a dry, protected from direct sunlight place [26, 27].

### Obtain of extract

The herb of *Chamaenerion angustifolium* L. was powdered with the help of a crusher. The extraction was performed by a modified repercolation method. Extract was lyophilized by SP Scientific VirTis Freeze-Drier/Lyophilizer.

### Chemicals and reagents

Iron (III) chloride, *n*-butanol, acetic acid were of the highest purity available and purchased from the Ltd. Sfera Sim (Lviv, Ukraine).

### Microorganisms

In research used a standardized daily suspension of testing strains of microorganisms, namely: *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* NCTC 74, *Escherichia coli* ATCC 25922, and *Candida spp.* ATCC 885-653. Cell concentration was 0.5 McFarland (used to compare the standard turbidity).

#### Detection and identification of hydroxycinnamic acids

Hydroalcoholic extract of the herb of *Chamaenerion angustifolium* L. was used for the detection of hydroxycinnamic acids.

Reaction with Iron (III) chloride. 2 drops of 1 % solution of Iron (III) chloride were added to 1 ml of the extract. The appearance of a green-gray color appeared will be indicating the presence of phenolic compounds, including hydroxycinnamic acids, in the object under study.

Hydroxycinnamic acids were detected by thin-layer chromatography in a solvent system of n-butanol – acetic acid – water (4:1:2) [28, 29]. Chromatographic plates Silufol were used for chromatography (Silufol UV 254; 15x15, Kavalier; Czech Republic). The chromatogram was dried in a fume hood and examined in daylight and UV light before and after treatment with ammonia vapor or Iron (III) chloride solution.

#### Total hydroxycinnamic acids content

The content of the total hydroxycinnamic acids was determined out by the spectrophotometric method on a spectrophotometer Lambda 25 UV (Perkin Elmer, USA) [30].

2 g (exact sample) of the cut raw materials was placed in a 200 ml flask and poured into 70 ml of 20% ethanol R. The flask was connected to a reflux condenser and heated in a water bath for 15 min. The extraction was performed three times. The extract was cooled and filtered through a paper filter using a Buchner funnel. The extract was quantitatively transferred to a 250 ml volumetric flask and brought the volume of the solution with 20 % ethanol P to the mark (solution A).

1 ml of solution A was added to a 50 ml volumetric flask and brought to the mark with 20% ethanol R. The optical density of the solution was measured on a spectrophotometer at a wavelength of 327 nm in a cell with a layer thickness of 10 mm.

For comparison, 20% ethanol R was used [31].

The determination of the content of hydroxycinnamic acids was carried out in a five-fold repetition [32].

#### Antibacterial and antifungal test

The antimicrobial and antifungal activity of the obtained lyophilized extract of *Chamaenerion angustifolium* L. herb was studied *in vitro* according to the State Pharmacopoeia of Ukraine [33].

Four tubes were prepared with two consecutive dilutions of the herbal medicine according to the method of serial dilutions in a liquid nutrient medium.

To each tube with dilutions of the herbal medicine, as well as to the control, add 0.2 ml of the prepared suspension of the test culture of bacteria at the rate of  $10^5$ - $10^6$  microbial bodies in 1 ml depending on the type of microorganism. The tubes were incubated in a thermostat for 24 h at 37 °C. After this period, the results were evaluated visually.

Determination of the activity of antibacterial drugs was performed on two layers of dense nutrient medium poured into Petri dishes. "Starving" unsown media were used in the lower layer. The lower layer was a substrate 10 mm high, on which thin-walled stainless steel cylinders with a diameter of 8 mm and a height of 10 mm were horizontally mounted. Around the cylinders the upper layer was poured, consisting of nutrient agar medium, melted and cooled to 40°C, in which the appropriate standard daily culture of the test microbe was introduced. Preliminarily, the upper layer was mixed well to form a homogeneous mass. After solidification, the cylinders were removed with sterile tweezers and a solution of a lyophilized extract of *Chamaenerion angustifolium* L. (0.06 ml) was placed in the formed wells. The dishes were dried for 30-40 min at room temperature and incubated in a thermostat at 37° C for 24 h [34, 35].

The antimicrobial activity of the obtained lyophilized extract of *Chamaenerion angustifolium* L. herb was estimated by consistent serial dilutions, which permit investigating the minimum bactericidal and minimum inhibitory concentrations. To define minimum inhibitory concentrations was prepared serial twofold dilutions of the extract in the liquid nutrient environment; after on, it was identified by

the lowest concentration of a substance not giving increase to the culture's growth. The bactericidal concentration of the lyophilized extract of *Chamaenerion angustifolium* L. herb was determined by seeding dilutions into a dense nutrient medium [36].

#### Statistical analysis

Results were determined using Statistica v 10.0 (StatSoft Inc.) program [37]. Statistical significance of differences between mean values was assessed by the Student test [38]. The difference between the values was considered reliable if the likelihood was  $*p < 0.05$ .

### Results and Discussion

As a result of the reaction with Iron (III) chloride appeared green-gray color, indicating the presence of phenolic compounds, including hydroxycinnamic acids, in the object under study.

The method of thin-layer chromatography in the solvent system *n*-butanol-acid-acetate-water (4:1:2) in a hydro-alcohol extract from the *Chamaenerion angustifolium* L. herb chlorogenic (3-(3,4-dihydroxycinnamoyl)quinic acid), neochlorogenic (5-O-(*trans*-3,4-Dihydroxycinnamoyl)-D-quinic acid), rosmarinic (3,4-dihydroxycinnamic acid (R)-1-carboxy-2-(3,4-dihydroxyphenyl) ethyl ester) and caffeic (3,4-dihydroxycinnamic acid) acids were identified.

The content of the total of hydroxycinnamic acids in *Chamaenerion angustifolium* L. herb, in terms of chlorogenic acid, was  $(2.55 \pm 0.03)\%$ .

Many substituted hydroxycinnamic acid is a precursor to better complex compounds, while some of them play metabolic and regulator roles. Only a minor fraction exist acids as free.

Studies show that chlorogenic acid is active against *Staphylococcus aureus* and *Escherichia coli* strains. It is enzymatically oxidized forms show antiviral activity against herpes pathogens. Extracts rich in this hydroxycinnamic acid inhibit the expression of HIV reverse transcriptase. Hypocholesterolemic, hepatoprotective, hypoglycemic and antitumor action of chlorogenic acid has also been noted [39, 40].

Gram-positive flora (*Staphylococcus aureus*, *Bacillus cereus*) as evidenced by the bactericidal effect was the most sensitive to the native lyophilized extract of *Chamaenerion angustifolium* L. according to the results of the study by serial dilutions. Regarding fungi of the genus *Candida* spp. the studied substance showed bacteriostatic activity. Gram-negative bacteria (*Escherichia coli*) were not sensitive to lyophilized extract of *Chamaenerion angustifolium* L. herb. Multiple dilutions of native solutions did not lead to bactericidal or bacteriostatic effects (Tables 1, 2.).

The study of antimicrobial activity of native lyophilized extract of *Chamaenerion angustifolium* L. herb by agar diffusion confirmed the results obtained by serial dilutions (Table 3). The growth retardation of test cultures was most pronounced when using a 50 % solution of a lyophilized extract of *Chamaenerion angustifolium* L. herb against *Staphylococcus aureus* ( $d = 20 \pm 2.3$  mm) and *Bacillus cereus* ( $d = 25 \pm 2.2$  mm), less pronounced against fungi of the genus *Candida* spp. ( $d = 15 \pm 1.5$  mm).

### Conclusions

Research results showed that the lyophilized extract of *Chamaenerion angustifolium* L. herb has antibacterial and antifungal properties. The bactericidal effect of a native lyophilized extract of *Chamaenerion angustifolium* L. herb on gram-positive microflora (*Staphylococcus aureus* –  $(20 \pm 2.3)$  mm; *Bacillus cereus* –  $(25 \pm 2.2)$  mm) was proved; bacteriostatic – up to fungi of the *Candida* genus ( $15 \pm 1.5$  mm). These properties of the lyophilized extract of *Chamaenerion angustifolium* L. herb are the result of the presence of phenolic compounds, including hydroxycinnamic acids. The content of the total of hydroxycinnamic acids in *Chamaenerion angustifolium* L. herb, in recalculation at chlorogenic acid, which was  $(2.55 \pm 0.03)\%$ .

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**Table 1.** Analysis of antibacterial activity of 30% solution of lyophilized extract of *Chamaenerion angustifolium* L. herb by serial dilutions method

Testing culture of microorganisms	Native solution	Dilutions			
		1:2	1:4	1:8	Control
<i>Staphylococcus aureus</i> ATCC 6538	++	—	—	—	—
<i>Bacillus cereus</i> NCTC 74	++	—	—	—	—
<i>Candida spp.</i> ATCC 885-653	+	—	—	—	—
<i>Escherichia coli</i> ATCC 25922	-	—	—	—	—

Notes: ++ – bactericidal properties;

+ – bacteriostatic properties;

— no bactericidal and bacteriostatic properties.

**Table 2.** Analysis of antibacterial activity of 50% solution of lyophilized extract of *Chamaenerion angustifolium* L. herb by serial dilutions method

Testing culture of microorganisms	Native solution	Dilutions			
		1:2	1:4	1:8	Control
<i>Staphylococcus aureus</i> ATCC 6538	++	—	—	—	—
<i>Bacillus cereus</i> NCTC 74	++	—	—	—	—
<i>Candida spp.</i> ATCC 885-653	+	—	—	—	—
<i>Escherichia coli</i> ATCC 25922	—	—	—	—	—

Notes: ++ – bactericidal properties;

+ – bacteriostatic properties;

— no bactericidal and bacteriostatic properties.



**Table 3.** Quantitative analysis of antibacterial activity of lyophilized extract of *Chamaenerion angustifolium* L. herb by "wells" method

Testing culture of microorganisms	Diameter of delayed microbial growth exposed to various dilutions of lyophilized extract of <i>Chamaenerion angustifolium</i> L. herb, mm	
	30% solution of lyophilized extract	50% solution of lyophilized extract
<i>Staphylococcus aureus</i> ATCC 6538	17.5±1.5	20±2.3
<i>Bacillus cereus</i> NCTC 74	17.0±1.2	25±2.2
<i>Candida</i> spp. ATCC 885-653	≤ 10±1.2	15±1.5
<i>Escherichia coli</i> ATCC 25922	≤ 10±1.1	≤ 10±1.1