UDC 615.451.1+582.991.1
DOI: 10.15587/2519-4852.2024.310837

EXTRACTS OF THE CANADIAN GOLDENROD (*SOLIDAGO CANADENSIS* L.) – PROMISING AGENTS WITH ANTIMICROBIAL, ANTI-INFLAMMATORY AND HEPATOPROTECTIVE ACTIVITY

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S. canadensis L. and S. virgaurea L. are the most common species of Solidago L. in Europe and with a long tradition of use in medicine for various therapeutic purposes: diseases of the urinary tract, gastrointestinal tract, diabetes, allergies and antiseptic purposes. Therefore, the research of species of the Solidago genus, which have a rich content of natural BACs, and the development of new effective medicinal products of plant origin are relevant.
The aim. *The method of work is the phytochemical and pharmacological study of extracts of the Canadian goldenrod herb, obtained by ethanol solutions of various concentrations, for the innovative perspective of creating new medicines based on them with antimicrobial, anti-inflammatory and hepatoprotective activity.*
Materials and methods. *The objects of the study were extracts of herb from Canadian goldenrod, obtained using purified water, 40 %, 70 % and 90 % ethanol. Phytochemical study of the extracts was performed by TLC and spectrophotometry. Study of acute toxicity, antimicrobial, anti-inflammatory and hepatoprotective activity of extracts was carried out according to standard methods.*
Research results. *A study of the phytochemical composition and pharmacological activity of extracts of herb from Canadian goldenrod obtained with different solvents. It was established that extracts of herb from Canadian goldenrod show anti-inflammatory activity. The most pronounced antiexudative activity is shown by the extract of the Canadian goldenrod herb (extractant – 40 % ethanol), reducing the degree of swelling by 24.77 %. Herb extracts from from Canadian goldenrod in acute toxic liver damage show moderate hepatoprotective activity, which is somewhat inferior to the comparative drug "Silibor". A more intense and effective effect on the hepatobiliary system was shown by the extract of the Canadian goldenrod herb (extractant – 40 % ethanol).*
Conclusions. *The perspective of creation based on herb of Canadian goldenrod new drugs with hepatoprotective activity. The most promising substance turned out to be an extract of herb from Canadian goldenrod obtained with a 40 % ethanol solution*
Keywords: *Canadian goldenrod (Solidago L.), extract, phenolic compounds, antimicrobial activity, anti-inflammatory activity, hepatoprotective activity*

How to cite:
Hrytsyk, Yu., Koshovyi, O., Hrytsyk, R., Raal, A. (2024). Extracts of the Canadian goldenrod (Solidago Canadensis L.) – promising agents with antimicrobial, anti-inflammatory and hepatoprotective activity. ScienceRise: Pharmaceutical Science, 4 (50), 78–87. http://doi.org/10.15587/2519-4852.2024.310837

1. Introduction

Species of the genus Solidago L. are perennial herbaceous plants of the Asteraceae family. More than 190 species are known in the world flora [1], 16 wild plants and 5–6 feral species grow on the territory of the CIS countries [2, 3]. The homeland of the plant is North America. In medical practice, it is prescribed as Canadian goldenrod (*S. canadensis L.*) or giant goldenrod (*S. gigantea L.*). Folk medicine uses common goldenrod (*S. virgaurea L.*). In Ukraine Canadian goldenrod is grown as an ornamental plant, which sometimes goes wild and grows as a weed in gardens or gardens [4, 5].

Species of the Solidago genus contain a large amount of BAC. Herb of Canadian goldenrod contains flavonoids, essential oil, hydroxycinnamic acids, coumarins, tannins, saponins, carotene, vitamins PP and C, macro- and microelements. Chemical composition of the usual has not been studied enough. It is known that the plant contains flavonoids, essential oil, saponins, organic acids, carotene, tannins, bitter and mucous compounds, resins [3, 6]. Therefore, considering the rich and diverse chemical composition of this raw material, it is important to research the species of the Solidago genus and develop new effective herbal medicines based on it.

In folk medicine, an infusion of goldenrod herb is used for diseases of the kidneys, urinary tract, liver, etc. Externally, goldenrod is used in the treatment of purulent wounds, furunculosis, gum abscesses in the form of washes and compresses [5, 7].

S. canadensis L. and *S. virgaurea L.* are the most common species in Europe with a long tradition of use in medicine for various therapeutic purposes: diseases of the urinary tract, gastrointestinal tract, diabetes, allergies and antiseptic purposes. Medicinal products of the goldenrod species have diuretic, choleretic, antibacterial, anti-inflammatory and astringent properties [8, 9]. To date, this

raw material is mostly used in the form of galenic agents, namely infusion and tincture, and there is no evidence that these solvents (water and 70 % ethanol) provide optimal extraction of biologically active components (BAC) from raw materials. Dry extract of Canadian goldenrod is part of complex medicines: Marelin, Phytolysin, Prostamed [10], while information about the solvents used to obtain these extracts is also not publicly available. To date, there is no monopreparation on the market of Ukraine based on biologically active compounds (BAC) of this raw material, therefore it is important to establish optimal concentrations of aqueous ethanol solutions that will ensure sufficient extraction of BAC from the *S. canadensis* herb and obtain products with the best pharmacological activity.

The aim of the work is the phytochemical and pharmacological study of Canadian goldenrod herb extracts obtained by ethanol solutions of different concentrations, to establish the prospect of creating new medicines based on them with antimicrobial, anti-inflammatory and hepatoprotective activity.

2. Planning (methodology) of research

The study protocol describing the different stages of the present research work is presented in the following flow chart (Fig. 1).

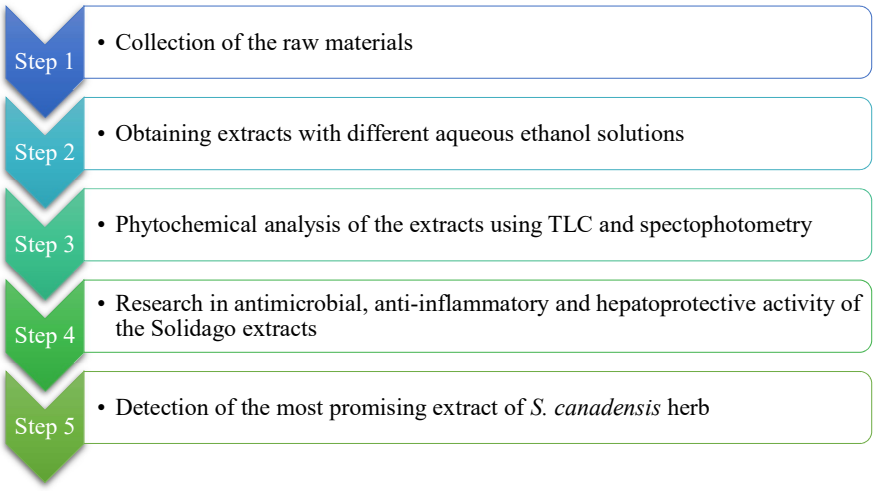

Fig. 1. Study protocol

3. Materials and methods

3. 1. Medicinal plant raw materials

The objects of the study were extracts of herb of Canadian goldenrod, which was collected at the plant collection site of the Druzhba State Dendrological Park named after Z. Pavlyk of the Carpathian National University named after Vasyl Stefanyk. The identity of the plant was established by the consulting assistance of professor A. R. Grytsyk from the Ivano-Frankivsk National Medical University (IFNMU) according to the botanical catalog [11]. Voucher specimens No. 455–457 were deposited at the Department of Pharmaceutical Management, Drug Technology and Pharmacognosy, Ivano-Frankivsk National Medical University. The raw material was dried for 14 days at room temperature in a well-ventilated area for ten days and stored in paper bags [12].

3. 2. Preparation of extracts from the Canadian goldenrod herb

Purified water, 40 %, 70 % and 90 % ethanol were used as extractants. The remaceration method was chosen for obtaining extracts from the Canadian goldenrod herb, as it is the most common, effective and available in household and laboratory conditions and is convenient for screening studies [13].

The raw material was poured three times with equal volumes of the extractant in the ratio herb: extractant – 1:10. When calculating the first portion of the extractant, the values of the absorption coefficient of the raw material were considered, which were established experimentally: for purified water – 3.65; 40 % ethanol – 4.05; 70 % ethanol – 3.39; 90 % ethanol – 2.93. Dried herb of Canadian goldenrod was ground on a mill "Miller-800" to a particle size of 3–5 mm. Crushed raw materials were loaded into the container for infusion, filled with the first portion of the extractant to a "mirror" thickness of 20–30 mm and left to swell for 4 hours. Then the rest of the extractant was added and the infusion was continued with occasional shaking for 20 hours. The infusion was drained, and the raw material was poured again with fresh extractant to the "mirror" and after insisting for 3 hours, a second drain was obtained. Similarly, the third downpour was received. After the end of the extraction, the raw materials were unloaded and squeezed, and the extracts obtained were combined. The resulting extracts are cloudy liquids containing a significant number of suspended particles. Cleaning was carried out by settling the extract at a temperature of 8–10 °C until a clear liquid was obtained. After settling for at least 2 days, the extracts were filtered through glass filters. The cleaned hoods were evaporated under vacuum at a temperature of 50–60 °C to a viscous consistency. The yield of extracts was calculated using the gravimetric method [12].

3.3. Phytochemical analysis

Identification of biologically active substances of phenolic nature was carried out by TLC method in comparison with solutions of standard substances of chlorogenic acid, quercitrin and rutin. The following mobile phase was used for the analysis: anhydrous formic acid P, water P, methyl ethyl ketone P, ethyl acetate P (6:6:18:30 V/V/V/V) and ethyl acetate P — water P — formic acid anhydrous P — acetic acid anhydrous P (72:14:7:7). Reference solution was dissolved 1.0 mg of chlorogenic acid P, 2.5 mg of quercitrin P and 2.5 mg of rutin in 10 ml of methanol R [14, 15]. The sample volume for the extract was 20 µl, and for the reference solution 10 µl. Detection of substances was carried out after spraying the plate with solutions of aminoethyl ether of diphenylboric

acid P, macrogol 400 P in methanol P while viewing the plate in UV light (365 nm) [14, 16].

Quantitative determination of tannins. Determination of the quantitative content of tannins was carried out according to the pharmacopoeial method [12, 17].

Quantitative determination of the amount of flavonoids. Determination of the content of the amount of flavonoids was carried out according to the pharmacopoeial method in terms of hyperoside [12, 16].

Quantitative determination of organic acids. Determination of the quantitative content of free organic acids was carried out by the titrimetric method according to the pharmacopoeial method [12, 18]. The content of free organic acids in terms of malic acid in the absolutely dry extract in percent (X) was calculated using the formula:

$$X = \frac{V \times 0.0067 \times 250 \times 100 \times 100}{m \times 10 \times (100 - W)},$$

where 0.0067 is the amount of malic acid, corresponding to 1 ml of sodium hydroxide solution (0.1 mol/l), in grams; V – volume of sodium hydroxide solution (0.1 mol/l) used for titration, in ml; m – mass of raw material, in g; W – mass loss during drying of raw materials, in %.

Quantitative determination of ascorbic acid. Determination of the content of ascorbic acid was carried out according to the pharmacopoeial method [19]. The content of ascorbic acid in terms of the absolutely dry extract in percent was calculated according to the formula:

$$X = \frac{V \times 0.00088 \times 300 \times 100 \times 100}{m \times 1 \times (100 - W)},$$

where 0.000088 is the amount of ascorbic acid, corresponding to 1 ml of sodium 2,6-dichlorophenolindophenolate solution (0.001 mol/l), in grams; V – volume of sodium 2,6-dichlorophenolindophenolate solution (0.001 mol/l) used for titration, in ml; m – mass of raw material, in g; W – mass loss during drying of raw materials, in %.

Quantification of amino acids. The identification and determination of the content of amino acids in goldenrod herb extracts was carried out based on the SE "Ivano-Frankivsk Scientific and Production Center for Standardization, Metrology and Certification" (accreditation certificate No. 2H098 dated June 20, 2014) [20, 21].

Analysis was carried out using an amino acid analyzer AAA T-339 M. (Mikrotekhnika, Prague, CRSR) in comparison with the concentration of standard amino acid hydrolysates according to DSTU ISO 13903:2005.

To determine the amino acid composition, 5 ml of purified water and concentrated hydrochloric acid (1:1) were added to 0.1 g of the studied extracts and placed in a test tube for hydrolysis. Hydrolysis was carried out at a temperature of 120 °C for 15 minutes. The hydrolyzate was neutralized with dry sodium hydroxide to pH 11 and transferred to a porcelain cup for 1 hour to accelerate the evaporation of ammonia. Then a solution of hydrochloric acid was added until the pH was set to 2.2. The sample was filtered, 0.1–0.5 ml of liquid was taken, adjusted to a volume of 2 ml with a buffer solution with a pH of 2.2 and introduced into the amino acid analyzer (50 µl of solution). Detection was carried out using post-column staining with a solution of ninhydrin in DMSO at a wavelength of 570 nm. Quantification was performed using standard solutions of amino acids (TU 6-09-3147-83). The peak areas of the standard sample and the sample were measured for each amino acid. The content of amino acids in the analyzed sample, ω, g/kg, was calculated according to the formulas:

$$w = \frac{A_e \cdot c \cdot M \cdot V_e}{A_c \cdot m \cdot 1000},$$

$$w = \frac{A_e \cdot c \cdot M \cdot V_e}{A_c \cdot m \cdot 1000} \cdot \frac{A_{ic}}{A_{ie}},$$

where A_e is the area of the amino acid peak in the hydrolyzate and extract; c is the molar concentration of the amino acid in the standard solution, in mol/dm^3; M is the molecular weight of an amino acid; V_e is the total volume of hydrolyzate or calculated total volume of extract dilutions, ml; A_c is the peak area of the amino acid in the standard solution; m is the mass of the analyzed sample (adjusted for the initial mass for dry and/or defatted samples), in g; 1000 – conversion factor of volume units; A_{ic} is the peak area of the internal standard in the standard solution; A_{ie} is the peak area of the internal standard in the extract or hydrolyzate [22].

3. 4. Pharmacological research

Pharmacological studies were carried out taking into account ethical and moral and legal principles that guarantee humane treatment of experimental animals for scientific and educational purposes (protocol of the Ethics Committee of IFNMU No. 139/23 dated 11/16/2023) [22, 23].

Study of the acute toxicity of extracts of the Canadian goldenrod herb. The study of acute toxicity was carried out according to the methodology of preclinical studies of the harmlessness of medicinal products [25].

The animals were divided into 5 groups of 6 heads each. The studied extracts were administered intragastrically, in an amount not exceeding the maximum for this route of administration (0.6 ml). The first – fourth groups are animals that were injected with aqueous solutions of herb extracts of the Canadian goldenrod (extractants – purified water, 40 %, 70 % and 90 % ethanol); animals of the fifth group were intact, which were injected with purified water. Animals were observed for 14 days.

The degree of toxicity of substances was assessed by changes in the general condition of animals and mortality. The toxicity class was determined according to the generally accepted classification [25].

Study of antimicrobial and antifungal activity of extracts of the Canadian goldenrod herb. The study of the antimicrobial activity of the extracts was performed on clinical isolates of antibiotic-sensitive and antibiotic-resistant microorganisms.

Screening of the antimicrobial effect of plant extracts was carried out using the micro-diffusion in agar

method developed at the Department of Microbiology, Virology and Immunology of IFNMU [26, 27].

The method is characterized by high sensitivity and discriminating ability, allowing to reliably differentiate active extracts from inactive ones [26]. 30 ml of agar was poured into Petri dishes located on a strictly horizontal and flat surface. After solidification of the medium, holes with a diameter of 4.0 mm were made with a special punch with even edges. The agar was evenly inoculated with a suspension of the test culture (concentration 1×10^7 CFU/ml). 20 µl of plant extracts were added to experimental wells, and 20 µl of extractants (40 %, 70 %, and 90 % aqueous ethanol) were added to the control wells. After cultivation for 24 hours, the diameters of the growth retardation zones of the bacterial test cultures were determined. Registration of fungistatic activity was carried out after 2 days, fungicidal activity – after 4 days of cultivation.

Study of anti-inflammatory activity of extracts of the Canadian goldenrod herb.

To determine the anti-inflammatory activity of extracts of the Canadian goldenrod, a model of formalin swelling of the paw of a rat caused by subplantar injection of a phlogogenic agent was used [25]. The model of aseptic formalin inflammation is based on the ability of a 2 % formalin solution to cause destruction of membrane proteins. Formalin-induced inflammation causes both local and systemic changes, with the release of inflammatory mediators, particularly prostaglandins, and is therefore suitable for screening studies. The maximum of the inflammatory reaction is observed 3 hours after the introduction of phlogogen.

For this purpose, 0.1 ml of a 2 % aqueous solution of formalin was injected under the aponeurosis of the sole of the hind paw, the ability of which to cause the destruction of membrane proteins has been repeatedly proven. Formalin-induced inflammation leads to both local and systemic changes, resulting in the release of mediators, particularly prostaglandins. The pathogenesis of formalin oedema is characterized by significant destruction of membrane proteins. The peak of the inflammatory reaction occurs in the third hour after the introduction of phlogogen.

The experiments were conducted on white non-linear male rats weighing 170–220 g. The animals were divided into six groups of 6 heads each. Animals of the first–fourth groups were injected intragastrically with solutions of extracts of Canadian goldenrod herb (extractants – purified water, 40 %, 70 % and 90 % ethanol), respectively; animals of the fifth group were injected with the comparative drug diclofenac sodium (Diclofenac-Darnytsia, solution for injections 25 mg/ml in ampoules of 3 ml, manufacturer – PrJSC "Darnytsia", Ukraine) [25]; animals of the sixth group are control.

2 hours before the introduction of the phlogogenic agent and immediately after its introduction, the animals of the first–fourth groups were injected with extracts of Canadian goldenrod herb, respectively, at a dose of 100 mg/kg of animal body weight, animals of the fifth group received the comparative drug diclofenac sodium at a dose of 8 mg/kg of animal weight, animals of the control group received water.

The assessment of the inflammatory reaction was performed oncometrically by the increase in the volume of the rat's paw, which was measured before the introduction of 2 % formalin solution, 1 h, 3 h, 5 h and 24 h after the introduction of the phlogogenic agent.

Study of the hepatoprotective activity of extracts of the Canadian goldenrod herb. The study of the hepatoprotective effect of extracts of the St. John's wort herb was conducted on the screening model of acute tetrachloromethane hepatitis [20, 25]. The pathology was modeled by a single subcutaneous injection of 50 % oil solution of tetrachloroethane.

Experiments were carried out on white male rats weighing 180-240 g, divided into 7 groups of 6 animals each: the first–fourth groups – animals that were injected intragastrically with solutions of Canadian goldenrod herb extracts (extractants – purified water, 40 %, 70 % and 90 % ethanol) at a dose of 25 mg/kg, respectively; the fifth group - animals that received the comparative drug "Silibor" (PE "Zdorovya") in a dose of 25 mg/kg; the sixth group – control, animals did not receive treatment; the seventh group - intact animals. Liver pathology was modelled by a single subcutaneous injection of a 50 % solution of oleic tetrachloromethane in a dose of 0.8 ml per 0.1 kg of the animal's weight for 2 days with an interval of 24 hours to the animals of the first to sixth groups. The investigated substances and the comparison drug were administered intragastrically to the animals of the first to fifth groups 1 hour before and 2 hours after the administration of the hepatotropic poison.

Rats were decapitated on the 3rd day after the first injection of tetrachloromethane. The conclusion about the pharmacotherapeutic effectiveness of the studied extracts was made based on biochemical and functional indicators of the state of the liver and blood serum, which were determined 24 hours after the last injection of tetrachloromethane. One of the criteria for the hepatoprotective effect of the studied substances is the percentage of animal survival [20, 25].

For the initial evaluation of the degree of hepatoprotective effect, the pharmacological screening determined the activity of alanine aminotransferase (AlAT) in blood serum and the rate of formation and content of LPO products in blood serum and liver tissue – thiobarbituric acid derivatives (TBA-reactants), which are hepatospecific markers of cytolysis.

AlAT activity was determined by the unified Reitman-Frenkel dinitrophenylhydrazine method using a standard set of reagents from the company "Filisit-Diagnostika", Ukraine.

The level of the LPO product – TBA-reactants was estimated by the reaction with 2-thiobarbituric acid spectrophotometrically according to the method of E. N. Korobeinikova [28, 29].

3. 5. Statistical calculations

All practical material is processed by the method of variational statistics with the calculation of the arithmetic mean and its standard error. The reliability of the compared values was assessed according to the Student's test, the level of probability was accepted as $p \leq 0.05$ [12, 30]. Statistical processing of the obtained results was carried out using the package of Windows application programs – MS Excel 2007 and statistical programs "Statistica – 7.0" (Statsoft, USA) according to the methodology of the State Pharmacopoeia of Ukraine.

Table 1

Characterization and yield of extracts from the herb of the goldenrod

Raw material	Extractant	Conventional abbreviation	Organoleptic parameters of the extract			Extract yield, %
			Colour	Odor	Consistence	
Canadian goldenrod herb	Purified water	3K-B	Dark brown	Specific	Viscous	38.93
	40 % ethanol	3K-4	Dark brown	Specific	Viscous	34.86
	70 % ethanol	3K-7	Dark green	Specific	Viscous	28.72
	90 % ethanol	3K-9	Dark green	Specific	Viscous	22.24

4. Research results

4. 1. Characteristics of extracts

Thick extracts from the Canadian goldenrod herb were obtained using purified water, 40 %, 70 % and 90 % ethanol. The obtained extracts are viscous masses of dark brown and dark green colour with a specific smell. The yield of the obtained extracts was 22.24–38.93 %, depending on the extractant. The characteristics of the obtained thick extracts from the Canadian goldenrod herb are given in Table 1.

4. 2. Phytochemical analysis

The identification and quantitative determination of amino acids of extracts of the Canadian goldenrod herb was carried out based on the SE "Ivano-Frankivsk Scientific and Production Center for Standardization, Metrology and Certification" (accreditation certificate No. 2H098 dated 06/20/2014) [22, 31]. The results of the study are presented in Table 2.

Chlorogenic acid and 3 flavonoids: rutin, quercitrin and hyperoside were identified in Canadian goldenrod herb extracts by TLC method by comparison with standard substances (Fig. 2).

The quantitative content of tannins, flavonoids, ascorbic acid and organic acids in the studied extracts are shown in Table 3.

Table 2

Amino acid composition of extracts of the Canadian goldenrod herb

Amino acid	Amino acid content (%) in the obtained extract $\left(\bar{x} \pm \Delta\bar{x}, n = 3\right)$			
	Purified water	40 % ethanol	70 % ethanol	90 % ethanol
Non-essential				
Aspartic acid	1.01±0.03	1.21±0.03	1.07±0.04	0.96±0.03
Glutamic acid	0.93±0.02	1.14±0.04	1.32±0.05	0.92±0.02
Serine	1.91±0.04	2.09±0.05	2.11±0.07	1.43±0.06
Tyrosine	0.74±0.04	0.87±0.04	0.93±0.02	0.85±0.03
Glycine	0.76±0.03	0.58±0.01	0.43±0.01	0.35±0.02
Alanine	0.41±0.02	0.46±0.01	0.36±0.02	0.31±0.01
Essential				
Threonine	0.67±0.02	0.75±0.03	0.82±0.04	0.65±0.02
Valine	0.17±0.01	0.18±0.01	0.13±0.01	0.11±0.01
Leucine	0.54±0.02	0.62±0.03	0.71±0.01	0.57±0.02
Methionine	0.25±0.01	0.33±0.01	0.31±0.01	0.29±0.01
Isoleucine	0.81±0.03	0.86±0.04	0.93±0.03	0.87±0.02
Lysine	0.52±0.02	0.53±0.01	0.67±0.03	0.62±0.02
Phenylalanine	0.53±0.01	0.57±0.02	0.62±0.01	0.56±0.03
Conditionally essential				
Histidine	0.57±0.01	0.54±0.02	0.63±0.03	0.32±0.01

Table 3

BAC content in extracts from the Canadian goldenrod herb

BAC group	Contents of BAC in the obtained extracts $\left(\% \bar{x} \pm \Delta\bar{x}, n = 9\right)$			
	Purified water	40 % ethanol	70 % ethanol	90 % ethanol
Tannins	8.32±0.06	7.70±0.11	5.18±0.07	3.67±0.08
Flavonoids	2.98±0.03	3.96±0.05	4.93±0.04	3.09±0.06
Ascorbic acid	0.09±0.01	0.11±0.01	0.09 ±0.01	0.07 ±0.01
Organic acids	1.42±0.02	1.38±0.03	1.40±0.04	0.98±0.02

	Bluish-green, fluorescent zone
Quercitrin: yellow-brown fluorescing zone	Yellow-brown, fluorescent zone (quercitrin)
	Intense yellowish-brown zone (hyperoside)
Chlorogenic acid: blue, fluorescent zone	Blue, fluorescent zone (chlorogenic acid)
Rutin: yellow-brown, fluorescent zone	Yellow-brown, fluorescent zone (rutin)
Comparison solution	Extract solution

Fig. 2. Scheme of zones on the chromatogram in the analysis of extracts of the Canadian goldenrod herb by TLC method [12]

4. 2. Pharmacological research

Determination of toxicity of extracts.

The study of acute toxicity of plant extracts of goldenrod was carried out on white non-linear sexually mature male mice weighing 19–21 g, grown in the nursery of the vivarium of the IFNMU, which were standardized according to physiological and biochemical indicators and were in accordance with the requirements of sanitary and hygienic standards on a standard diet.

According to the LD_{50} for the studied extracts, the maximum administered doses were conditionally accepted, since they did not cause the death of animals. The conducted studies showed that after the intragastric administration of extracts of the Canadian goldenrod herb at a dose of 6000 mg/kg, no deaths were observed: the animals were tidy, had a satisfactory appetite, responded normally to sound and light stimuli, the processes of urination and defecation were normal, breathing disorders and seizures were not observed.

Study of antimicrobial and antifungal activity.

To study the antimicrobial and antifungal activity, extracts of the Canadian goldenrod herb were used (extractants – water, 40 %, 70 % and 90 % ethanol, respectively).

Evaluation of the activity of the studied extracts of the Canadian goldenrod herb was conducted on clinical strains of microorganisms that were characterized by various degrees of resistance to modern antimicrobial drugs. To study the antimicrobial activity of Canadian goldenrod herb extracts were chosen *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Escherichia coli*, *Staphylococcus epidermidis*, *Candida albican*, *Penicillium sp.*, *Staphylococcus aureus*, which are causative agents of skin pustular infections, eye infections, pneumonia, infections of the genitourinary system [32], etc. (Table 4). Since the aqueous extract did not show significant activity, it is not listed in Table 4.

Table 4

Results of the study of antimicrobial and antifungal activity of extracts of the Canadian goldenrod herb

Test cultures of microorganisms	Zone of inhibition of the microorganism's growth, mm					
	3K-4	3K-7	3K-9	40 % ethanol	70 % ethanol	90 % ethanol
Staphylococcus aureus MSSA	0	0	5.61±0.5	0	4.98±0.48	6.12±0.64
Staphylococcus aureus MRSA	[6.27±0.45]	[5.13±0.76]	8.95±0.80	[6.14±0.45]	5.89±0.17	6.00±0.41
S. haemolyticus MRSH	0	5.50±0.28	7.00±0.23	0	0	5.31±0.16
S. epidermidis MLS-S (Bc)	0	5.46±0.32	6.45±0.39	0	0	5.37±0.18
S. epidermidis MLS-S (Bc)	[5.60±0.43]	[6.20±0.20]	[10.95±1.25]	[5.43±0.43]	0	[5.82±0.54]
S. epidermidis MLS-R (Bc)	0	0	6.62±0.43	0	0	5.71±0.58
S. epidermidis MLS-R (Bc)	0	[8.57±0.40]	[22.14±0.56]	0	0	[7.32±0.42]
Enterococcus faecalis	0	5.27±0.27	5.65±0.19	0	5.06±0.40	5.90±0.29
Klebsiella pneumoniae	0	0	0	0	0	4.77±0.23
Escherichia coli	0	0	0	0	0	0
Providencia stuartii	0	0	0	0	0	0
Pseudomonas aeruginosa	0	0	0	0	0	0
Candida albicans	0	5.65±0.52	[5.44±0.67]	0	[5.69±0.46]	5.85±0.79
Candida tropicalis	0	5.21±0.51	0	0		5.15±0.43
Candida lipolytica	0	[4.60±0.33]	0	0	4.89±0.46	5.37±0.48

Note: Data are relative to the respective solvent.

Study of anti-inflammatory activity.

The effect of the studied extracts was assessed by their ability to suppress the development of formalin-induced edema of the paw of rats in comparison with animals of the control group (Table 5).

Table 5

Anti-inflammatory activity of extracts of the Canadian goldenrod

Group of animals	Dose mg/100g	Increase in the volume of a rat's paw, c.u.: $\bar{x} \pm \Delta\bar{x}$, n=6		
		In 1 hour	In 3 hours	In 5 hours
Control	–	123.10±2.73	135.30±2.12	152.30±4.40
3K-B	10	113.07±2.11*/**	116.80±3.35*/**	122.97±1.34*/**
3K-4	10	106.82±1.97*	109.52±0.76*/**	114.57±2.77*
3K-7	10	107.60±2.96*	109.83±2.02*/**	116.37±2.81*/**
3K-9	10	114.23±2.76*/**	123.35±2.50*/**	135.62±3.98*/**
Sodium diclofenac	0.8	105.60±1.59*	107.15±1.45*	112.53±1.43*

Antiexudative activity				
Group No.	Name of the drug	Indicator of suppression of the inflammatory reaction, %		
		In 1 hour	In 3 hours	In 5 hours
1	3K-B	8.15	13.67	19.26
	3K-4	13.22	19.05	24.77
2	3K-7	12.59	18.82	23.59
	3K-9	7.21	8.83	10.95
3	Sodium diclofenac	14.22	20.81	26.11

*Note: * – reliability of deviations in relation to the data of the control group (p≤0.05); ** – the reliability of the deviations in relation to the data of the group of animals that received diclofenac sodium (p≤0.05).*

Study of hepatoprotective activity.

Treatment of chronic diseases of the liver and biliary tract requires long-term use of drugs. The relative limitation of the arsenal of domestic hepatoprotectors determines the urgency of the search for new hepatoprotective agents, in particular of plant origin.

One of the criteria for the hepatoprotective effect of the studied extracts is the percentage of animal survival. During the experiment, the death of animals was noted in the control group and among the animals that received the extract obtained with 90 % ethanol. Mortality in these groups was 16.7 %, in other groups all animals remained alive until the end of the experiment.

The study of biochemical indicators was carried out on the basis of the Center of Bioelementology of the Ivano-Frankivsk National Medical University (certificate of technical competence No. 037/19 from June 13, 2019 to June 12, 2024). The results of biochemical studies are presented in the Table 6.

5. Discussion

Thick extracts, which are viscous masses of dark brown and dark green colour with a specific smell, were obtained from the herb of the Canadian goldenrod using purified water, 40 %, 70 % and 90 % ethanol. The yield of the obtained extracts was 22.24 – 38.93 %, depending on the extractant.

15 amino acids were identified in extracts of the Canadian goldenrod herb, 10 of which are monoaminomonocarboxylic: phenylalanine, threonine, tyrosine, serine, methionine, leucine, isoleucine, glycine, valine and alanine; monoaminodicarboxylic 2: glutamic and aspartic acids; diaminomonocarbonic 2: lysine and arginine; heterocyclic 1 – proline. Among the detected amino acids, 7 essential amino acids (phenylalanine, isoleucine, methionine, lysine, threonine, leucine and valine) and 1 conditionally essential amino acid (histidine) were identified.

In addition, chlorogenic acid and 3 flavonoids were identified in the extracts: rutin, quercitrin, and hyperoside (Fig. 2), which made it possible to choose appropriate methods for quantitative determination of phenolic compounds.

According to the results of the research (Table 3), it was established that the content of ascorbic acid in extracts from the Canadian goldenrod herb is 0.07–0.11 %. The obtained results indicate that the content of organic acids is in the range of 0.98–1.42 %, depending on the type of extractant. The obtained results of the study indicate that the content of tannins in the extracts from the Canadian goldenrod herb ranges from 3.67 % to 8.32 %, respectively. The highest content of tannins was found in the extract of the Canadian goldenrod herb, obtained with purified water. Based on the research results, it was established that the greatest extraction of flavonoids is provided by a 70 % ethanol solution.

Table 6

Effect of plant extracts on biochemical parameters of blood serum and liver condition in acute hepatitis

Biochemical and hematological indicators	Research objects						
	3K-B	3K-4	3K-7	3K-9	«Silibor»	50 % oily solution CCl_4	Intact animals
Dose, mg/0.1 kg	2.5	2.5	2.5	2.5	2.5	0.8 ml	–
Blood serum							
AlAT, μmol/h.ml	0.80±0.05*/**	0.56±0.04*/**	0.87±0.04*/**	1.03±0.08*/**	0.28±0.03*/**	1.37±0.04*	0.25±0.02
TBA-reactants nmol/ml	4.21±0.20*/**	4.12±0.18*/**	4.73±0.16*/**	5.03±0.19*/**	3.85±0.20**	5.86±0.45*	3.54±0.10
Liver homogenate							
TBA-reactants μmol/g	39.7±1.78*/**	35.62±1.61*/**	47.8±1.90*/**	46.3±1.38*/**	33.9±1.64*/**	62.4±1.86*	26.6±1.63

*Note: * – the reliability of the deviation in relation to the data of the group of intact animals (p≤0.05); ** – the reliability of the deviation in relation to the data of the control group of animals (p≤0.05).*

Since the extracts turned out to be rich in phenolic compounds that have pronounced antioxidant properties, it is advisable to investigate their anti-inflammatory and hepatoprotective activity.

It was established that the intragastric administration of extracts of the Canadian goldenrod herb at a dose of 6000 mg/kg did not lead to the lethality of animals, which indicates the absence of toxic effects of the extracts at this dose, and characterizes them as practically non-toxic (toxicity class V, LD_{50}>5000 mg/kg).

The results of the research are given in the Table 4, show that antimicrobial activity is detected by extracts of the Canadian goldenrod herb obtained using 70 % and 90 % ethanol. Extracts obtained with the use of water show no activity, and with the use of 40 % ethanol, show weak antimicrobial activity. Extracts obtained using 70 % ethanol show better antimicrobial and antifungal activity. No extract shows activity against *Pseudomonas aeruginosa*.

The obtained data (Table 5) indicate the effect of the experimental extracts on the exudative phase of inflammation. The antiexudative effect of sodium diclofenac for 5 hours of the experiment was 26.11 %. 5 hours after the start of the experiment, the investigated extracts of the Canadian goldenrod herb reduced the degree of swelling by 19.26 % (extractant – purified water), 24.77 % (extractant – 40 % ethanol), 23.59 % (extractant – 70 % ethanol), 10.95 % (extractant – 90 % ethanol). Extracts obtained with 40 % and 70 % ethanol exhibited an effect close to the reference drug. The least pronounced anti-exudative effect was noted when using an extract of the Canadian goldenrod obtained with 90 % ethanol, and the most pronounced – with 40 % ethanol.

The pharmacological effect of the Canadian goldenrod is explained by the presence of a rich content of BAC, in particular flavonoids and tannins, which are part of the extract, because of which the permeability of blood vessels decreases, which leads to the involution of edema [33, 34].

Thus, the obtained results indicate that the extracts of the Canadian goldenrod herb exhibit anti-inflammatory activity. The most pronounced anti-exudative activity is shown by the extract of the Canadian goldenrod herb (extractant – 40 % ethanol).

The results of the research are given in the Table 6, show that damage to the liver by tetrachloromethane was accompanied by significant impairment of its functional state. A 5.48-fold increase in AlAT activity in blood serum indicates the development of cytolysis of hepatocytes. An increase in the content of TBA-reactants in blood serum and liver homogenate of animals of the control group by 1.65 and 2.34 times, respectively, compared to the indicators of intact animals, indicates the presence of oxidative stress.

Application of herb extracts of the Canadian goldenrod and the comparative drug "Silibor" against the background of experimental hepatitis had a positive effect on the condition of the liver of animals. Under the influence of the studied drugs, a decrease in inflammatory processes in the liver of animals of the first to fifth groups was observed, as evidenced by a decrease in the activity of the AlAT enzyme and a decrease in the level of TBA-reactants in blood serum and liver homogenate in comparison with the control group of animals.

When 3K-B, 3K-4, 3K-7, and 3K-9 extracts were administered, the activity of AlAT decreased by 1.71 times, 2.45 times, 1.57 times, and 1.33 times, respectively. Simultaneous administration of the hepatotropic poison and the studied extracts led to a decrease in the level of TBA-reactants in blood serum by 1.39 times (3K-B extract), 1.42 times (3K-4 extract), 1.24 times (3K-7 extract) and 1.16 times (3K-9 extract), and in the liver homogenate – 1.57 times, 1.75 times, 1.31 times, and 1.34 times, respectively. The use of the drug "Silibor" led to a decrease in AlAT activity by 4.89 times and a decrease in the level of TBA-reactants in blood serum and liver homogenate by 1.52 and 1.84 times, respectively, compared to animals of the control group.

Thus, the obtained results indicate that herb extracts of the Canadian goldenrod in acute toxic liver damage show moderate hepatoprotective activity, which is somewhat inferior to the comparison drug "Silibor" [20, 35]. The use of 3K-4 extract at a dose of 25 mg/kg of the animal's body weight showed a more intense and effective effect on the hepatobiliary system compared to 3K-B, 3K-7 and 3K-9 extracts.

Practical relevance. It has been proven that the extract of the Canadian goldenrod herb, the obtained 40 % ethanol solution is the most promising substance from this raw material for the creation of new drugs with antimicrobial, anti-inflammatory and hepatoprotective activity.

Study limitations. Only one sample of raw materials was used in the study, so it is advisable to investigate other series of similar extracts in the future. Also, at the time of conducting the study, it was not possible to perform HPLC analysis of the obtained extracts.

The prospects for further research. In the future, it is advisable to conduct HPLC analysis of the obtained extracts to determine the content of individual phenolic substances, develop quality control methods for the most promising extract and create dosage forms based on it.

6. Conclusions

The study of the phytochemical composition and pharmacological activity of extracts of the Canadian goldenrod herb obtained with various solvents was carried out, and the prospect of creating new drugs with hepatoprotective activity based on them was shown. The most promising substance turned out to be an extract of Canadian goldenrod herb obtained with a 40 % ethanol solution, which showed the greatest anti-inflammatory and hepatoprotective activity.

In extracts of the Canadian goldenrod herb chlorogenic acid and 3 flavonoids were identified: rutin, quercitrin and hyperoside; and 15 amino acids, and the quantitative content of tannins, flavonoids, ascorbic acid and organic acids was determined. The highest content of tannins was found in the extract of Canadian goldenrod herb, obtained with purified water, and the greatest extraction of flavonoids is provided by a 70 % ethanol solution.

Antimicrobial activity is revealed by extracts of the Canadian goldenrod herb obtained using 70 % and

90 % ethanol. The most pronounced antiexudative activity is shown by the extract of the Canadian goldenrod herb (extractant – 40 % ethanol), reducing the degree of swelling by 24.77 %. Extracts of the Canadian goldenrod herb in acute toxic liver damage show moderate hepatoprotective activity, which is somewhat inferior to the comparative drug "Silibor". A more intense and effective effect on the hepatobiliary system was shown by the extract of the Canadian goldenrod herb (extractant - 40 % ethanol), reducing the activity of AlAT by 2.45 times and TBA-reactants in blood serum by 1.42 times.

Thus, extracts of *Solidago canadensis L.* are promising agents with antimicrobial, anti-inflammatory and hepatoprotective activity, and require further thorough and in-depth study.

Conflicts of interest

The authors declare that they have no conflict of interest concerning this research, whether financial, personal, authorship or otherwise, that could affect the research, and its results presented in this paper.

Funding

This work was supported by the European Union in the MSCA4Ukraine project "Design and development of 3D-printed medicines for bioactive materials of Ukrainian and Estonian medicinal plants origin" [ID number 1232466].

Data availability

The datasets used and/or analyzed during the current study are available from the author and/or corresponding author on reasonable request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

Acknowledgement

The authors sincerely thank all the defenders of Ukraine who made the performance of this study possible. The authors sincerely appreciate the support of the partners who stand with Ukraine.

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Received date 28.05.2024
Accepted date 20.08.2024
Published date 30.08.2024

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