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DEVELOPMENT OF GREEN PRODUCTION TECHNOLOGY AND RESEARCH OF HARPAGOPHYTUM PROCUMBENS ROOT DRY EXTRACT

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The aim *of this work is to study of standardization parameters and pharmacological properties of Harpagophytum procumbens (H. procumbens) root dry extract.*
Materials and methods *– obtaining of H. procumbens root dry extract was performed in accordance with developed scheme that is characterized by simplicity and cost-effectiveness of technology compared to analogous scheme. Study of standardization parameters of obtained extract was performed in accordance with the requirements of the monograph «Harpagophyti extractum siccum» of the State Pharmacopoeia of Ukraine 2.2., harmonized with the monograph «Harpagophyti extractum siccum» of the European Pharmacopoeia 9.5. Pharmacology study of analgesic and anti-inflammatory activities were conducted on the rat paw formalin-*induced *edema model.*
Results. *Obtained H. procumbens root dry extract is a free flowing, non-hygroscopic, light brown powder with taste and odor that is specific to raw material of H. procumbens. Determined loss on drying was 3.2±0.18 %. Content of heavy metals in dry extract was not more than 0.01 % (100 ppm). Harpagoside was identified by TLC method. Chromatographic zones on the obtained chromatograms of the reference solution and test solutions are the same in intensity of a color, cross-over and sharpness of development. Based on the results of the research performed by HPLC method, it has been established that content of harpagoside in investigated H. procumbens root dry extract is 2.50±0.02 %.*
According to the results of pharmacological research it was found that investigated dry extract (the content of harpagoside is not less than 37.0 mg/kg) showed a higher activity compared to reference drug («Phong Te Thap»).
Conclusions. *A new method of obtaining dry extract of H. procumbens (3.5:1) was proposed. The developed green production technology is simple; it does not require special equipment and expensive solvents. A correspondence of quality parameters with the requirements of State Pharmacopoeia of Ukraine and European Pharmacopoeia: appearance, identification of harpagoside and fructose, loss of drying, content of heavy metals, assay of harpagoside (2.50±0.02 %) was set. Analgesic and anti-inflammatory activities of H. procumbens dry extract has been established*
Keywords**:** *Harpagophytum procumbens, harpagoside, standardization parameters, analgesic* and *anti-inflammatory activities*

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1. Introduction

In the entire world, particularly in Ukraine, musculoskeletal system disorders occupy one of the leading places among the reasons of disability of population followed by accompanied premature loss of working efficiency. Every year losses of population-based labor work capacity are constantly increasing; this is a socially significant problem [1–3].

This group of diseases is characterized by inflammation of joints and chronic pain syndrome that requires long-term administration of synthetic medicines such as nonsteroidal anti-inflammatory drugs (NSAIDs). To avoid their negative effect on the patient's body during remission, herbal medicines can be treatment of choice. Due to the presence of various groups of biologically active substances (BAS) in plant raw material, herbal drugs have a wide spectrum of pharmacological activity and a more favorable toxicological safety profile, which is one of their advantages for a long-term use [4, 5].

Phytotherapy of musculoskeletal diseases is based on the selection of medicinal plant raw material, which have anti-inflammatory, diuretic, analgesic, sedative, health-promoting, desensitizing activity, in other words it has an effect on the etiological factors and clinical symptoms of diseases [6, 7]. The following plants are used in the international medical practice: *Zingiber officinale, Curcuma domestica, Harpagophytum procumbens, Scrophularia nodosa, Warburgia salutaris, Capsicum frutescens, Gaultheria procumbens* [8–10].

Harpagophytum procumbens (H. procumbens) (*Pedaliaceae* plant family, Devil's Claw) – is a traditional African plant which has been used in folk-medicine during a long time for the treatment of various diseases especially arthragra and rheumatism. It is a rare, highly valuable plant which is only found widely spread in the Kalahari

Desert of Southern Africa especially in South Africa, Namibia, Botswana, Zambia, Zimbabwe, and Mozambique. The fruit grows from the flower and is woody, radiating numerous long barbed spines. In fact, the name Devil's Claw refers to the barbs on the fruits and the plant owes its scientific name, *Harpagophytum*, to this unique characteristic; fruit with a grappling hook, *harpagos* in Greek. Roots of this plant are used as medicines that have anti-inflammatory, antirheumatic, analgesic, sedative and diuretic activity. Harpagoside and harpagide are main biologically active substances found in *H. procumbens* (Fig. 1), as well as chemical composition is represented with 8-p-coumaroylharpagide, 8-feruloylharpagide, 8-cinnamoylmyoporoside, pagoside, acteoside, isoacteoside, 6'-O-acetylacteoside, 2,6-diacetylacteoside, cinnamic acid, caffeic acid, procumbide, and procumboside [11, 12].

Recent clinical studies have shown presence of anti-inflammatory and analgesic action (inhibition of COX-2, iNOS), chondroprotective (decrease in quantity of mediator of cartilage destruction: TNFα, IL-1β, IL-6, MMPs, NO, elastase) and antioxidant (increase in superoxide dismutase, catalase, glutathione peroxidase activity, scavenging of superoxide and peroxy) activity of *H. procumbens* extract. However, it should be noted that *H. procumbens* extract therapy has more favorable safety profile than NSAID therapy [13–15].

Among the herbal medicines that are used for preventive treatment of musculoskeletal diseases are dominated by imported homeopathic medicines. One of the main plant components of which are raw materials or extracts of H. procumbens (Pedaliaceae plant family) [16, 17] (Table 1).

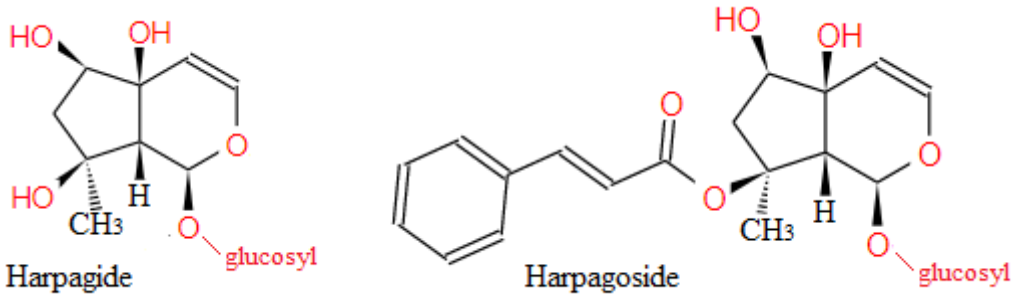

Fig. 1. Main BAS of *Harpagophytum procumbens*

Table 1

Results of analytical review of herbal medicinal products on the Ukrainian pharmaceutical market

No	Name of the medicine	Composition	Manufacturer	Indications
1	Sustamar	*Harpagophytum radice* dry extract (4.5-5:1)	Pharma Wernigerode GmbH, Germany	Acute and chronic musculoskeletal diseases
2	Rheumarcheb	*Extractum spissum* (2-4:1): *Harpagophyti radice*, *Echinaceae purpureae* herba, *Filipendulae ulmariae* flore	Poznan Herbal Company «Herbapol» S.A., Poland	Degenerative musculoskeletal diseases
3	Rheumafit	*Harpagophytum radice* dry aque extract (1.5-2.5:1)	Phytopharm Klenka SA, Poland	Pathologies of the musculoskeletal system
4	Carltillum	*Harpagophytum radice* dry extract, methylsulfonylmethane	LLC "Novalik-Pharm", Ukraine	Acute and chronic joint diseases

According to Table 1, all abovementioned medicines are single-component (include solid and dry extract of *H. procumbens* or multicomponent medicines that have other types of drug raw materials in their compositions. Absence of *H. procumbens* based medicinal products in Ukrainian market and their high therapeutic activity and safety proves the relevance of research concerning determination of standardization parameters and definition of pharmacological properties of *H. procumbens* dry extract for the development of phytotherapeutic agents on its base.

In extraction and separation processes, large quantities of organic solvents are used, in particular, methods of obtaining an extract [18]. Even though organic solvents have well-known advantages, their replacement with greener alternatives is necessary due to their toxic effects on the human health and the environment. Large part of these solvents is characterized as volatile organic compounds that contributes to increase the risks of fire and explosion. Moreover, these solvents are easily released in the atmosphere and can act as air pollutants, promoting the global warming [19].

A general definition of green chemistry is the invention, design and application of chemical products and processes to reduce or to eliminate the use and generation of hazardous substances. In relation to green extraction of natural products, this definition could be modified as follows: "Green Extraction is based on the discovery and design of extraction processes which will reduce energy consumption, allows use of alternative solvents and renewable natural products, and ensure a safe and high quality extract/product" [20].

Major solutions have been identified to design and demonstrate green extraction on laboratory and industrial scale to approach an optimal consumption of raw materials, solvents and energy: improving and optimization of existing processes and using non-dedicated equipment.

The aim of the research is the standardization of *H. procumbens* root dry extract according to the proposed scheme and investigation of its analgesic and anti-inflammatory properties.

2. Research planning (methodology)

Different scheme of obtaining of *H. Procumbens* based dry extracts were licensed by now [18], however, they have many disadvantages. Multistep, energy-consuming process and application of large quantity of organic solvents is one of their main disadvantages. Considering this, it is planned to develop green production technology of *H. Procumbens* dry extract, which decreases duration of manufacturing process, and does not require special equipment (Fig. 2).

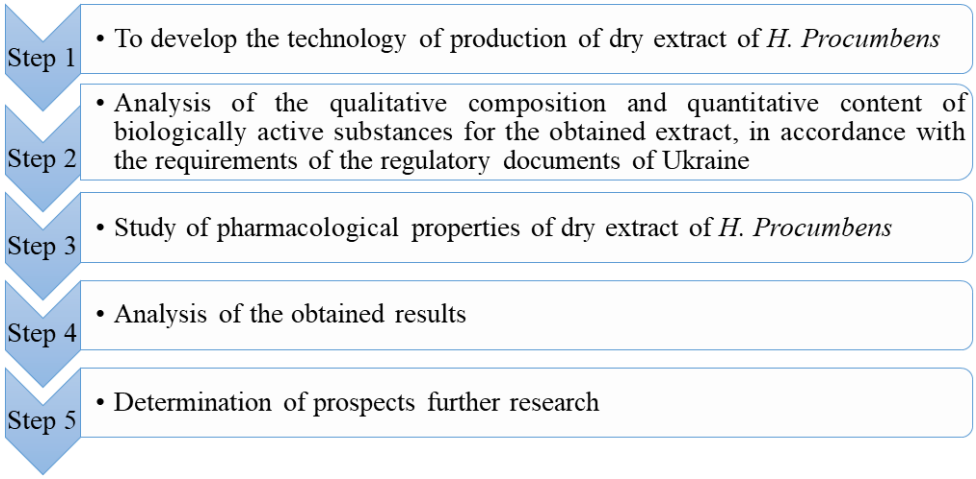

Fig. 2. Research planning scheme

3. Materials and methods

H. Procumbens root dry extract was obtained according the following scheme: crushed raw material (supplier is "Starwest Botanicals", USA) with a size of particles, which pass through a 3–5 mm sieve, was put into extractor with a magnetic stirrer. Ethanol was used as extracting solvent *(20 %, v/v)*. Extraction was performed using the following ratio of raw material – extracting solvent 1:10 during 2 hours, taking into consideration absorption coefficient of extracting solvent. After that, the resulting product was subjected to settling for a day and filtration. The concentrating of obtained liquid extract was performed using the rotary evaporator at 50–60 °C until stiff consistency (humidity was not more than 25 %). Obtained solid extract was dried to dryness in the vacuum oven at the 65–70 °C and pressure 800–870 mbar. Plant raw material/obtained extract ratio is 3.5:1.

Study of standardization parameters of obtained extract was performed in accordance with the requirements of the monograph "Harpagophyti extractum siccum" of the State Pharmacopoeia of Ukraine 2.2 (SPhU) [21], harmonized with the monograph "Harpagophyti extractum siccum" of the European Pharmacopoeia 9.5 (Ph. Eur.) [22].

Method of thin layer chromatography (TLC) was carried out for identification test in the solvent system: *water R* : *methanol R* : *ethyl acetate R* (*8:15:77 V/V/V*). Analytical standard *Harpagoside R* (Sigma-Aldrich, No 68527) and *fructose R* (Sigma-Aldrich, No PHR1002) were used as active markers. Determination of characteristic chromatographic zones was performed after spraying with 10 g/L *phloroglucinol R* solution in *ethanol R (90 %, v/v)* and then with *hydrochloric acid R* followed by heating at 80 °C for 5–10 min. Result of determination was estimated in the daylight.

The assay part of standardization involved determination of the content of harpagoside using high performance liquid chromatography (HPLC). Chromatographic determination was performed on the liquid chromatograph Varian Prostar model. As active marker were used analytical standard *Harpagoside R* (Sigma-Aldrich, No 68527). Chromatographic conditions:

– Column Phenomenex Luna C18(2) size: 150×4.60 mm, with size particle 3 µm.

– Column temperature: 40 °C;

– Mobile phase: *methanol R – water R* (*50:50 V/V*);

– Flow rate: 0.7 mL/min;

– Detection: spectrophotometer at 278 nm;

– Injection volume: 10 µL;

– Run time: 15 min.

Study of analgesic and anti-inflammatory activity of obtained extract was performed using the model of formalin-induced rat hind paw oedema. Formalin test has a high prognostic value at the estimation of analgesic properties that helps to assess the impact of medicinal products on the primary pain afferents and on the pain syndrome, caused by inflammatory process [23]. Preclinical studies comply with modern scientific standards and ethical principles (Minutes of the 5th meeting of the Commission on Bioethics of the National University of Pharmacy, dated March 25, 2021).

Medicinal product «Phong Te Thap» (manufacturer is «LTD FITO FARMA Co.», Vietnam) was selected as a reference drug. This medicinal product is registered as liquid extract for internal use that belongs to the

medicines used for the treatment of musculoskeletal disorders (M09 AX10) according to the ATC classification. Besides this medication is a plant extract with anti-inflammatory and analgesic properties for symptomatic treatment of rheumatic arthralgias, mixed arthritis of different etiology and osteochondrosis [17].

The experiments were performed on 50 white outbred male rats weighing 150–180 g. The animals had free access to food and water during their stay under the standard conditions of vivarium. The experiments were conducted in compliance with the General Ethical Principles for Conducting Experiments on Animals adopted at the First National Congress on Bioethics, which agree with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [23–25]. The animals were divided into 5 groups: control and four experimental groups. 1 hour before the formalin injection, animals were administered intragastrically necessary doses of medicinal products via a gastric tube. Control group of animals received equivalent quantity of purified water and dry extract of *H. Procumbens* that was dissolved in 1–2 mL of purified water. Experimental model of formalin-induced edema was caused by sub-plantar injection of 0.1 mL of 2 % formalin solution in the rat hind paw of [23].

Analgesic activity was estimated by the total time of episodes of painful reactions of first (1–10 min) and second (30–50 min) phase including paw clamping, licking, gnawing or shaking. Anti-inflammatory activity in the formalin test model was determined 3 hours after the phlogogen injection using automatic oncometer [22, 23].

Anti-inflammatory antiexudative properties of investigated drugs were estimated on the model of carrageenan-induced paw edema in the rat over time. Medicinal product «Phong Te Thap» was selected as a reference drug. Acute aseptic inflammation of hind paw was simulated on the outbred female rats weighing 180 – 200 g at the sub-plantar injection of 0.1 mL of 1 % carrageenan solution. Animals were divided on 5 groups: control and 4 experimental groups (10 animals per every group). This standard model allows to investigate ability of test-samples to influence inhibition of cyclooxygenase pathway of arachidonic acid transformation. A hour before the phlogogen injection rats were intragastrically injected solutions of tested *H. Procumbens* dry extract (the content of harpagoside is not less than 37.0 mg/kg) and reference drug (340 mg/kg of *Homalomena* roots – main component of medicinal product), while animals of control group were injected with purified water. Edema was measured 3 hours after the injection of carrageenan using automatic oncometer.

Antiexudative activity was calculated according to the following formula:

$$A = 100\ \% - [(P_{study} / P_{contr}) * 100],$$

where A – antiexudative activity, %;

$P_{contr.}$ – mean difference in volume of swollen and healthy paw in control group;

P_{study} – mean difference in volume of swollen and healthy paw in study group;

Loss on drying and content of heavy metals in dry extract were Determined according to the methods of articles SPhU 2.0 *"Weight loss on drying" (2.2.32)* and *"Heavy metals" (2.4.8)* respectively.

Experimental data were processed with the method of variation statistics using "Statistica 8.0" standard program package. Data comparison was carried out using analysis of variance statistical models (ANOVA) [26].

4. Research results

Obtained *H. procumbens* root dry extract is a free flowing, non-hygroscopic, light brown powder with taste and odor that is specific to raw material of *H. procumbens*. Determined loss on drying was 3.2±0.18 %. Content of heavy metals in dry extract was not more than 0.01 % (100 ppm).

The obtained chromatogram by TLC meets the requirements described in the monograph "Harpagophyti extractum siccum" SPhU 2.2 and Ph. Eur. 9.5 [19, 20].

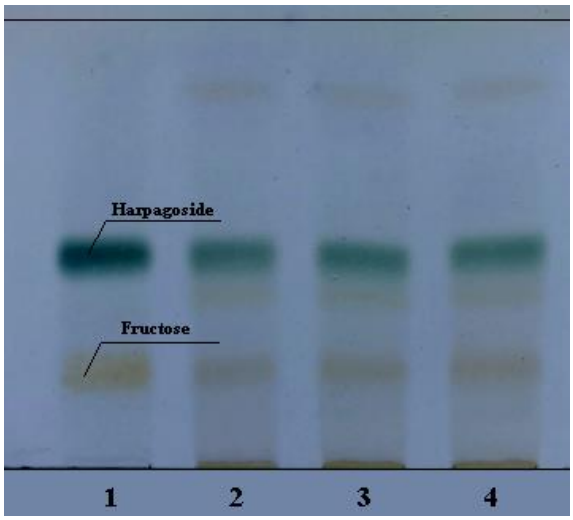

Fig. 2. Chromatogram in daylight, obtained under the conditions described in the SPhU 2.2 and Ph. Eur. 9.5 (1 – reference solution, 2-4 – test solutions)

As shown on the Fig. 2 chromatographic zones of *harpagoside P*, *fructose P*, reference and test solutions coincide with color intensity, cross-over and sharpness of development.

Assay of harpagoside in the investigated dry extract was performed by HPLC and it was next stage of our research. Results of system suitability test are given in Table 2.

Table 2

Results of system suitability test

Criteria	Requirements	Results
Column efficiency	>2000	2500
Symmetry factor	From 0.8 to 1.5	0.89
Relative standard deviation, %	<2.0 %	0.87 %

Retention time of main peak of *harpagoside R* is about 9 minutes under the chromatographic conditions used for its determination. Chromatograms obtained test solution and reference solution are presented on the Fig. 3 and Fig. 4, respectively.

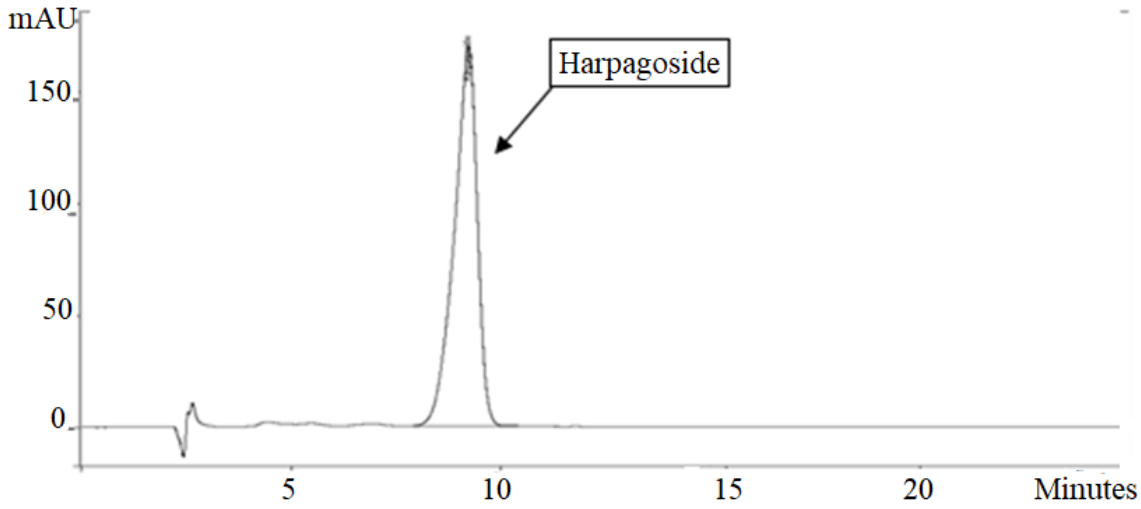

Fig. 3. Chromatogram of reference solution

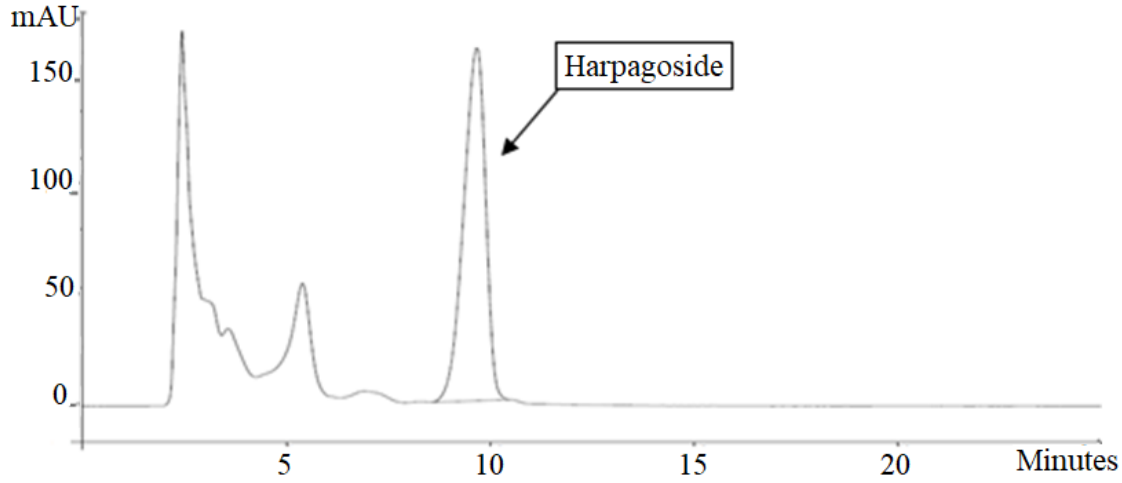

Fig. 4. Chromatogram of test solution

Based on the research findings it has been established that content of harpagoside in investigated *H. procumbens* root dry extract is 2.50±0.02 %.

Analgesic activity of standardized extract from roots of *H. procumbens* was studied during I phase of pain reaction caused by sub-plantar introduction of 2 % formalin solution in the hind paw of rat.

This reaction lasted 10 minutes after injection. Any activity was not observed indicating the absence of effect on main mechanisms of pain transmission.

The latent period that lasted on the average for 20 minutes came at the end of I phase. Then II phase of pain reaction came, which was caused by the algogens of irritation. Signs of pain sensitivity in rats were registered for 20 minutes (at 30–50 minutes after injection).

Administration of investigated *H. procumbens* root dry extract significantly affected the reduction of time duration of pain reaction at II phase this decrease was 1.54 times less than control, but in comparison to reference drug, this reduction was less by 1.15 times (Table 3).

Table 3

Analgesic activity indexes of *H. procumbens* root dry extract in formalin test ($\overline{X}$ ±S_X)

Experimental group (n=10)	Total time of pain reaction of I phase, min	Total time of pain reaction of II phase, min
Control pathology	7.42±0.61	14.18±1.07
Dry extract of roots of *Harpagophytum procumbens*	7.35±0.41	9.21±0.84*/**
Reference drug	7.06±0.52	12.38±1.24

*Note: * – possible differences with respect to animals of control pathology group (p≤0.05); ** – possible differences with respect to animals of reference drug group (p≤0.05)*

Evaluation of anti-inflammatory activity was carried out 3 hours after phlogogen administration during prostaglandin phase of irritation. *H. procumbens* root dry extract demonstrated significant antiexudative action compared to the reference drug not only on the model of formalin-induced rat paw edema but also on the model of carrageenan-induced rat paw edema. Investigated extract decreased formalin-induced edema by a mean of 17.8 %, but in the case of carrageenan-induced edema this decrease was by 25.1 % (Table 4).

Table 4

Antiexudative activity indexes of *H. procumbens* root dry extract in formalin test ($\overline{X} \pm S_X$), %

Experimental group (n=10)	Formalin-induced edema	Carrageenan-induced edema
Harpagophytum procumbens root dry extract	17.8 %	25.1 %
Reference drug	12.6 %	19.3 %

Results of analgesic and antiexudative activity determination shows that *H. procumbens* root dry extract (the content of harpagoside is not less than 37.0 mg/kg) showed a higher activity compared to reference drug.

5. Discussion of research results

New method of obtaining of *H. procumbens* root dry extract (3.5:1) was proposed. The developed green production technology is simple; it does not require special equipment and expensive solvents. A correspondence of quality parameters (description, identification of harpagoside and fructose, loss of drying, heavy metals, and assay of harpagoside (2.50±0.02 %) of the obtained extract with the requirements of SPhU 2.2 and Ph. Eur. 9.5 was established.

Today published the results of pharmacological studies on the anti-inflammatory and analgesic activity of the roots of *H. procumbens*, as well as extracts based on it [13–15]. These extracts are obtained from raw materials of various territorial origin, as well as according to various production technology schemes.

We carried out similar studies in order to bring the pharmacological activity of the dry extract obtained according to the technology described in the article. Analgesic and anti-inflammatory activity of *H. procumbens* root dry extract was found. After single intragastric administration of dry extract to rats, this medicinal product showed analgesic and anti-inflammatory effect that was statistically significant in comparison with the reference drug. It was found that after this single administration of dry extract formalin edema decreased by an average of 17.8 % while the carrageenan edema decreased by an average of 25.1 %.

Study limitations. The proposed methods for the determination of harpagoside can be used for medicinal plant raw materials, as well as preparations based on it.

Prospects for further research. Obtained and standardized *H. procumbens* dry extract is proposed for introduction into the formulation of domestically made dietary supplements soft gelatin capsules “Osteovert”.

6. Conclusions

Obtained *H. procumbens* root dry extract complies with the requirements of monograph *“Harpagophyti extractum siccum”* SPhU 2.2 and monograph *“Harpagophyti extractum siccum”* Ph. Eur. 9.5. Investigated dry extract after single intragastric administration to rats demonstrated analgesic and anti-inflammatory action that exceeded activity of reference drug by all indicators.

The proven pharmacological effectiveness of *H. procumbens* root dry extract determined the feasibility of creating on its basis dietary supplement of soft gelatin capsules.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Financing

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