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PHARMACOLOGICAL AND TECHNOLOGICAL STUDIES IN THE DEVELOPMENT OF TABLET COMPOSITION WITH ACORUS CALAMUS LEAF EXTRACT

Oleksiy Andryushayev, Yevhenii Samoilov, Valeriia Hnatiuk, Olena Ruban, Velia Mariia, Maryna Savokhina

The aim. *To determine the optimal qualitative and quantitative composition of auxiliary substances for tablets containing dry extract of Acorus calamus leaves and the solid dispersion of quercetin, their relatively therapeutic dose and antiexudative activity.*

Materials and methods. *This study determined a relatively therapeutic dose of dry extract of Acorus calamus leaves, investigated the impact of various auxiliary substances on the properties of tablets formulated with active ingredients – dry extract of Acorus calamus leaves and solid dispersion of quercetin, and assessed antiexudative activity these tablets. The comprehensive analysis entailed the utilization of standardized pharmacopoeial methods to evaluate the quality of the tablet samples. These methods encompassed a range of assessments designed to ensure that the tablets met the requisite pharmacological standards, focusing on key characteristics such as dissolution rate and stability. Determination of the relatively therapeutic dose and antiexudative activity made using standard pharmacological methods in laboratory rats.*

Results. *In-depth exploration during the study led to identifying Ac-Di-Sol and Lubripharm SSF as the most suitable auxiliary substances for the tablet composition. Detailed analysis revealed that Ac-Di-Sol, when utilized at a 10 % concentration, markedly improved the tablets' disintegration rate without adversely affecting their structural integrity. Concurrently, Lubripharm SSF was observed to significantly enhance the tablets' mechanical stability by reducing their friability.*

Conclusions. *As a result of the study, the relatively therapeutic dose of dry extract of Acorus calamus leaves, and the solid dispersion of quercetin, optimal auxiliary substances for the tablet formulation – Ac-Di-Sol and Lubripharm SSF – were established. The conducted research enabled the development of a tablet composition that aligns with the requisite pharmacotechnological specifications and conditions of the modern pharmaceutical industry and demonstrates high antiexudative activity relative to monocomponent substances and famous drugs*

Keywords: *Acorus calamus, quercetin, tablets, gastrointestinal tract, relatively therapeutic dose*

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1. Introduction

Inflammatory diseases of the gastrointestinal tract (GIT) are one of the most common diseases of internal organs. According to the data for 2019, 7.32 billion cases of gastrointestinal diseases were registered, 2.86 billion were dominant among other comorbidities, resulting in the death of 8 million people and disability of 277 million [1].

An important factor in the treatment of diseases of the GIT is a comprehensive approach to therapy at the first stages of identifying the symptoms of the disease. Such a line of therapy should be carried out taking into account the etiology, pathogenesis and clinical manifestations, and should consist of a combined approach to treatment [2–4].

Medicinal products developed using plant raw materials occupy an important place in the pharmacotherapeutic practice of treating GIT diseases. Their use is appropriate for reducing erosive-ulcerative changes and the inflammatory process in acute and chronic GIT diseases [5, 6].

Acorus calamus is a world-famous medicinal plant with a wide range of pharmacological activities: wound-healing, anti-inflammatory, antibacterial, antifungal, antidepressant, antioxidant, antihypertensive, and antihelmintic [6, 7]. From ancient times to the present, this plant has been the subject of close attention from scientists worldwide. Based on medicinal plant raw materials (MPRM) of the Acorus calamus, medicinal products have been developed to treat diseases of the hepatobiliary, nervous and cardiovascular systems [8]. It is important to note that in the production of these medicinal products, only the underground organs of Acorus calamus are used. However, the conducted studies indicate the almost complete identity of the qualitative composition of biologically active substances (BAS) of the rhizomes and leaves of this medicinal plant [8, 9]. Therefore, neglecting the above-ground parts of the plant leads to the irrational use of its natural resources and the reduction of the range of the species.

Previous studies have developed a technology for obtaining a liquid extract of the leaves of Acorus calamus:

the optimal method of extraction – remaceration with the use of ultrasound, as well as the temperature, duration and frequency of the process – has been experimentally determined. A positive effect of the surface-active substance – Polysorbate 80 on the process of extraction of the leaves of Acorus calamus was revealed [10].

A study was conducted on the development of a dry extract of the leaves of Acorus calamus to improve the technological properties of the obtained extract. A rational method of its preparation was chosen, which consists of introducing the carrier into the liquid extract followed by evaporation and drying under vacuum. This makes it possible to obtain a finished product with minimal losses and optimal technological properties, which allow the extract to be added to the composition of solid medicines without the use of additional auxiliary substances or sorbents.

During a literature review of plant components with anti-inflammatory properties, the flavonoid quercetin caught our attention. In modern scientific publications, many research works are devoted to this biologically active substance [11–13], and the number of drugs with this active pharmaceutical ingredient (API) is increasing on the pharmaceutical market. As of February 2024, 3 medical drugs containing quercetin are registered in Ukraine: lyophilisate for injections Corvityn®, which is prescribed for the treatment of cardiovascular diseases; chewable tablets Quertin® and granules/powder in packets of quercetin, used for treatment periodontitis, erosive and ulcerative diseases of the mucous membrane of the oral cavity and stomach; in complex treatment of climacteric, vertebral pain syndrome, neuro-reflex manifestations of osteochondrosis of the spine and others [14, 15].

Quercetin is a natural flavonoid that, like all flavonoids, stabilizes cell membranes, protects vessel walls and modulates immune function. Due to the presence of free radical scavenger properties and suppression of peroxidation processes, it protects body cells from damage [11, 16].

Quercetin is able to inhibit inflammatory processes by suppressing neutrophil infiltration, promoting apoptosis of activated neutrophils, and reducing the level of inflammatory cytokines in plasma [17]. Regarding the protective effects of macrophage apoptosis, quercetin inhibited the expression of NLRP3 and lysate cysteine protease 1 in a concentration-dependent manner, as well as the expression of IL-1β and N-GSDMD, thereby preventing the apoptosis of THP-1 macrophages. In turn, quercetin can inhibit the activation of the NLRP3 inflammasome, the increase in the level of TLR2/MyD88 and p-AMPK induced by lipopolysaccharide/adenosine triphosphate, the inhibition of the TLR2/MyD88/NF-κB and ROS/AMPK pathways [18].

Studies indicate a significant effect of quercetin on the process of regeneration of damaged tissues, as well as on the stimulation of microcirculation processes in the mucous membrane of the stomach, which, in combination with anti-inflammatory activity, determines its gastroprotective properties [16].

Considering the above data, we decided to explore the possibility of combining Acorus calamus leaf extract and quercetin to develop a tablet-form medicinal product for the treatment of inflammatory diseases of the GIT.

However, during the study of the physicochemical characteristics of quercetin, it was found that this substance is poorly soluble in water, which results in its oral bioavailability being only 5.0±1.0 μmol/L. This amount does not allow for achieving the necessary concentration of the drug in the blood to exhibit the pharmaceutical activity of quercetin [19].

According to the literature, the use of solid dispersion technology in pharmaceutical practice is widely utilized as a promising direction for improving the biopharmaceutical characteristics of medicinal products by increasing solubility and release rate, creating prolonged-action drugs, with controlled release and targeted transport to the organ of interest, eliminating the side effects of APIs, etc. [20–22].

Taking into account the high technological nature of the solid dispersion of quercetin (SDQ) and the therapeutic potential of its combination with Acorus calamus leaves, the goal of our work was to establish the optimal excipients and their quantities for obtaining combined tablets containing dry extract of Acorus calamus leaves and SDQ.

2. Planning (methodology) of research

The development of the optimal and efficient tablet composition incorporating Acorus calamus leaf extract and quercetin involves a multifaceted approach that includes:

– determining a relatively therapeutic dose: to study the antiexudative activity of the dry extract of the leaves of Acorus calamus in different doses on models of acute aseptic carrageenan and zymosan inflammation;

– analysis of technological properties of active ingredients and their combination: this step encompasses an examination of the pharmacotechnological characteristics of Acorus calamus leaf extract with carrier and SDQ. The focus is on assessing their individual and combined properties, particularly evaluating the volumetric and flowability characteristics of the mixture for tableting;

– identification of suitable auxiliary substances: determining the most effective auxiliary substances for tablet formulation, focusing on their compatibility with the active ingredients and impact on the overall pharmacotechnological properties of the tablets;

– optimization of formulation ratios: establishing the ideal ratios of active ingredients to auxiliary substances, ensuring optimal therapeutic efficacy and stability of the final product;

– physicochemical characterization: conduct thorough physicochemical analyses, including assessment of tablet disintegration, dissolution rate, hardness, and friability, to ensure compliance with pharmacopoeial standards;

– determining the antiexudative activity of tablets with an extract of the leaves of Acorus calamus and quercetin: study of the antiexudative activity of tablets with extract of the leaves of Acorus calamus and quercetin with comparison drugs.

3. Materials and methods

The studies presented in this paper, conducted in 2022–2023, were carried out at the Educational and Scientific Institute of Applied Pharmacy of the National Pharmaceutical University (Kharkiv, Ukraine). All research on animals was carried out following the requirements of the Commission on bioethics of the National Academy of Sciences and the «General ethical principles of experiments on animals», which are consistent with the provisions of the «European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes» (Strasbourg, 1986) and the first National Congress on Bioethics (Kyiv, 2001). The experiment protocol was reviewed and approved by the bioethics commission of the National University of Pharmacy (NUPh), Kharkiv, Ukraine (Protocol No. 4 dated 2.10.2020).

The liquid extract of Acorus calamus leaves was obtained using 70 % ethyl alcohol by the method of remaceration with the use of ultrasound and a surfactant (0.1 % polysorbate 80). The extraction was carried out in a hermetically sealed ultrasonic extraction reactor PEX 1 (REUS, Contes, France); the ultrasound frequency was 35 kHz. The duration of the process was a 3-time remaceration for 45 minutes each; the process temperature was 65±5 °C. The extracts obtained were settled at a temperature of 8±2 °C for 2 days and then filtered through an ashless paper filter under vacuum [10, 23].

The standardization of the obtained extract of Acorus calamus leaves was conducted using a double-beam spectrophotometer, Specord 200 (Analytik Jena, Germany), with the method "Hawthorn leaves and flowers," according to the SPhU monograph. The amount of flavonoids was calculated in terms of hyperoside [24].

In the production of the dry extract of Acorus calamus leaves, carrier components (60 % Microcel-200 and 30 % Kollidon CL) were introduced into the composition of the liquid extract, after which the obtained suspension was evaporated on a rotary vacuum evaporator LabTech EV311 plus (Labtech S.R.L., Italy) under the following conditions: temperature – 45–60 °C, rotation speed – 100–150 rpm, vacuum depth – 900±10 mBar; residual moisture content – 25±2 %. Final drying to a residual moisture content of 3.0±0.5 % was carried out in a vacuum drying oven DZF-6050 (Zhengzhou Keda Machinery and Instrument Equipment Co., Ltd., China) at a temperature of 60±3 °C and a vacuum depth of 900±10 mBar. After drying, the dry extract was ground in a mortar and sieved through a mesh with a pore diameter of 1 mm.

To prepare the solid dispersion of quercetin, 96 % ethanol was added to quercetin in a 1:1 ratio as a solvent and mixed for 20 minutes. The resulting solution was added to the melted PEO-6000 at 60 °C. It was thoroughly stirred for 10 minutes, during which the ethanol was evaporated. The obtained liquid mass was cooled to a temperature of 45–50 °C and mixed with Microcel 200 until the moisture was uniformly distributed throughout the mass [22].

Composition of the SDQ:

– Quercetin: 25 %

– PEO-6000: 50 %

– Microcel-200: 25 %

To improve the technological characteristics of the tablet mixture, the following excipients were used during the study: Tablettose 100 (Lactose, MEGGLE GmbH & Co, Germany), Parteck M 200 (Mannitol, Merck KGaA, Darmstadt, Germany), Parteck SI 400 (Sorbitol, Merck KGaA, Darmstadt, Germany), GalenIQ 721 (Isomalt, Pharma Excipients, Switzerland), Ac-Di-Sol (Croscarmellose Sodium, DuPont, USA), sodium starch glycolate (Sodium Starch Glycolate, DFE Pharma, Germany), Lubripharm SSF (Sodium Stearyl Fumarate, SPI Pharma, USA), LIGAMED MF BLS (Magnesium Stearate, Peter Greven GmbH & Co. KG, Germany), LIGAMED CPR-2-K (Calcium Stearate, Peter Greven GmbH & Co. KG, Germany), COMPRITOL 888 ATO (Glyceryl Behenate, Gattefossé, France), Aerosil A-380 (Fumed Silica, Evonik Degussa GmbH, Germany).

The technological properties of the dry extract samples and tablets obtained from them were assessed in accordance with the requirements of the State Pharmacopoeia of Ukraine (SPhU) [25].

The tablet-pressing process was carried out utilizing the single-punch TDP 5 Desktop Tablet Press (LFA Machines, Taiwan). For this process, 11 mm diameter dies were employed.

The conclusion about the flowability of dry extract samples was made according to the value of the Carr index:

$$K = 100 \times \frac{V_0 - V_f}{V_0},$$

where V_0 – bulk volume, ml; V_f – bulk volume after contraction, ml.

The tablet strength was determined by resistance to crushing using a Monsanto-type tablet hardness tester (Bexco, Belgium).

The disintegration time of the tablets was measured with a «PTZ AUTO» tablet and capsule disintegration device (Pharma Test, Germany).

To determine the «friability» index, a PJ-3 tablet four-usage tester rotating drum (China) was used.

Results were calculated using the formula:

$$F = 100\ \% \times \frac{P_{start} - P_{end}}{P_{start}},$$

where P_{start} – weight of the tablets before friability testing, g; P_{end} – weight of the tablets after friability testing, g.

Loss in weight after the test should not exceed 1 %.

The determination of the "flowability" index was conducted using the laboratory device model VP-12A (MZTO). The assessment of the powder's ability to flow in the vertical plane was carried out according to the given parameters, with the calculation of the ratio of the time required for outflow (in seconds and tenths of a second) to the volume of 100 g of the powder being tested.

In order to establish a relatively therapeutic dose of a dry extract of Acorus calamus leaves, the study was carried out on 72 white non-linear rats weighing 260±40 g, which were divided into 12 groups of 6 animals each, where groups 1 and 7 control pathology (CP). The sub-

stance under investigation – a dry extract of Acorus calamus leaves was administered in the form of a suspension intragastrically in doses of 10, 20, 30, 40 and 50 mg/kg 1 hour before the subplantar injection of phlogogens. Acute carrageenan inflammation was studied in groups 1–6 – 0.05 ml of 1 % carrageenan solution. Acute zymosan inflammation was studied in groups 7–12 – 0.1 ml of 2 % suspension. Foot volume was measured using a mechanical plethysmometer LE7500 (Spain). For carrageenan inflammation, measurements were made before and 1, 2, 3, and 4 hours after phlogogen administration; for zymosan inflammation – before and 0.5, 1, 2, and 3 hours after phlogogen administration. Conditionally relatively therapeutic dose was determined on the basis of the highest indicators of antiexudative activity in carrageenan and zymosan oedema [26].

The anti-exudative activity of the developed composition of tablets with dry extract of Acorus calamus and SDQ was determined on 42 white non-linear rats weighing 160–180 g in the model of acute aseptic carrageenan inflammation. The experimental animals were divided into 6 groups of 6 animals each: 1st group – control without treatment, 2nd group – animals that received a dry extract of Acorus calamus leaves (30 mg/kg), 3rd group – animals that received a tablet form of dry extract leaves of Acorus calamus (30 mg/kg)+quercetin (5 mg/kg), 4th group – animals that received the tablet form of dry extract of Acorus calamus (30 mg/kg)+SDQ (5 mg/kg in terms of quercetin in its pure form), 5th group – animals receiving the comparison drug «Vikair» tablets (number 0017780, manufactured by ARTERIUM) at a dose of 3 mg/kg with a calculation by the Acorus calamus, 6th group – animals receiving the comparison drug «Diclofenac-Euro» tablets (number PDU20002, manufactured by Unique Pharmaceutical Laboratories) in a dose of 8 mg/kg. All solid forms were administered as an intragastrical suspension. Acute aseptic carrageenan inflammation was reproduced by subplantar administration of 1 % carrageenan solution in a volume of 0.2 ml per animal 1 hour after intragastric administration of a suspension of the investigated medicinal product and comparative drugs [26]. Paw swelling in rats was measured after 1, 2, 3 hours using a mechanical plethysmometer LE7500 (Spain).

Antiexudative activity was determined by the degree of reduction of oedema in experimental animals compared to controls and was calculated according to the formula [26]:

$$A = 100\,\% - \frac{(Mse - Mhe)\cdot 100}{Msc - Mhc},$$

where A – antiexudative activity, %; Mse – the volume of the swollen foot in the experiment; Mhe – the volume of the healthy foot in the experiment; Msc – the volume of the swollen foot in the control; Mhc – the volume of the healthy foot in the control.

Statistical analysis was conducted utilizing Microsoft Excel 2019. Each experimental measurement was performed three times, with the outcomes expressed as the average value±confidence interval (CI). A significance threshold was established at $p<0.05$.

4. Results

At the beginning of the study, a relatively therapeutic dose of the main component of the future tablets – the extract of the leaves of the Acorus calamus – was determined on models of acute aseptic carrageenan and zymosan inflammation. The study results are presented in Tables 1, 2.

As a result of the research, it was established that the anti-exudative activity of the extract increased up to a dose of 30 mg/kg. At higher doses, an increase in antiexudative activity was not observed, and even a decrease was recorded. Accordingly, a dose of 30 mg/kg (group 4) was accepted as relatively therapeutic and was used for further studies.

Table 1

Indicators of anti-exudative activity of a dry extract of the leaves of Acorus calamus on the model of acute aseptic carrageenan inflammation

Study group	Paw size (in units)/anti-exudative activity (%)					
	At the beginning	1 hour	2 hours	3 hours	4 hours	Average
Control (group 1)	1.82±0.20	2.44±0.36	2.11±0.19	2.08±0.14	2.03±0.10	–
10 mg/kg (group 2)	1.37±0.32	1.65±0.22 56.0	1.59±0.24 24.1	1.56±0.25 28.4	1.54±0.24 20.2	32.2
20 mg/kg (group 3)	1.65±0.27	1.89±0.32 61.1	1.78±0.35 55.2	1.83±0.30 29.0	1.72±0.32 64.5	52.5
30 mg/kg (group 4)	1.66±0.11	1.91±0.08 58.7	1.75±0.10 66.1	1.74±0.08 67.7	1.70±0.09 77.4	67.5
40 mg/kg (group 5)	1.63±0.12	1.82±0.08 69.2	1.76±0.08 54.6	1.70±0.08 70.3	1.69±0.09 69.4	65.9
50 mg/kg (group 6)	1.40±0.22	1.66±0.30 57.4	1.57±0.28 39.1	1.51±0.26 57.4	1.49±0.31 54.8	52.2

Table 2

Indicators of antiexudative activity of a dry extract of the leaves of Acorus calamus on the model of acute aseptic zymosan inflammation

Study group	Paw size (in units)/anti-exudative activity (%)					
	At the beginning	30 min	1 hour	2 hours	3 hours	Average
Control (group 1)	1.29±0.13	1.94±0.34	2.04±0.36	1.98±0.34	2.32±0.48	–
10 mg/kg (group 2)	1.19±0.12	1.64±0.17 32.1	1.72±0.17 29.0	1.88±0.14 1.4	2.04±0.08 17.7	19.8
20 mg/kg (group 3)	1.19±0.10	1.66±0.12 28.0	1.71±0.88 30.1	1.84±0.93 5.3	1.98±0.06 22.5	21.7
30 mg/kg (group 4)	1.30±0.10	1.76±0.18 29.5	1.77±0.13 37.4	1.89±0.12 15.4	2.04±0.14 27.9	27.7
40 mg/kg (group 5)	1.17±0.11	1.75±0.15 12.5	1.65±0.14 36.5	1.75±0.15 17.1	1.93±0.11 26.1	23.7
50 mg/kg (group 6)	1.33±0.15	1.79±0.20 29.8	1.86±0.22 28.5	1.95±0.19 10.6	2.12±0.12 22.6	22.9

To justify the choice of excipients in the tablet composition, an evaluation of the pharmacotechnological characteristics of three samples was conducted: Acorus calamus dry extract, SDQ, and their combination. The obtained data are presented in Table 3.

In the second phase of the study, an evaluation of the strength and disintegration rate of tablets containing a combination of dry Acorus calamus extract and SDQ was conducted. The results are presented in Table 4.

Table 3

Pharmacotechnological properties of API and their combination

Formulation	Bulk volume, ml	Bulk volume after compaction, ml	Carr's index, %	Hausner ratio	Flowability, g/s
SDQ	100.00± ±1.00	87.64± ±0.88	12.36± ±0.12	1.124± ±0.013	5.82± ±0.06
Dry extract of Acorus calamus	100.00± ±1.00	89.92± ±0.90	10.08± ±0.10	1.101± ±0.011	7.11± ±0.07
Dry extract of Acorus calamus+SDQ	100.00± ±1.00	89.21± ±0.89	10.79± ±0.11	1.108± ±0.012	6.78± ±0.07

Table 4

Pharmacotechnological characteristics of tablets

SPhU requirements	Crushing strength, N	Disintegration time, sec
	Not less than 40	Not more than 900
Dry Acorus calamus extract with carrier and SDQ	80.67±0.83	1500±14

The results indicated that the tablets under study had an insufficient disintegration time of 1500 sec, whereas according to the requirements of the SPhU, the disintegration time should not exceed 900 sec.

To determine the influence of substances from the fillers group on the strength and disintegration indicators of the tablets, they were incorporated into the tablet mixture at a concentration of 30 %. The results are presented in Table 5.

The obtained data indicate that the incorporation of excipients from the fillers group does not have a sufficiently positive effect on the disintegration time of the tablets.

In the next stage, the influence of disintegrants on the disintegration time and strength indicators of the tablets based on a combination of dry extract of Acorus calamus leaves with carriers, and SDQ was investigated. The results are presented in Fig. 1, 2.

Table 5

Indicators of crushing strength and disintegration time for tablets with auxiliary substances of the fillers group

Auxiliary substance	Crushing strength, N	Disintegration time, s
Tabletose 100	65.12±0.65	1500±12
Partec M 200	52.53±0.51	1380±14
Partec Si 400	35.78±0.80	1200±11
Galen IQ 721	49.11±0.49	1680±17

According to the study results, the most optimal approach was to introduce the disintegrant Ac-Di-Sol at a concentration of 10 %. This allows achieving the best balance between accelerating disintegration and maintaining the tablets' strength.

The next step was to determine the influence of excipients from the lubricants group on the indicators of friability, disintegration time, and tablet strength. The quantitative content of the listed substances – 1 % – was chosen according to the manufacturer's recommendations and literature data [12, 13]. The results are presented in Table 6.

Based on the study results, Lubripharm SSF was chosen as the most effective excipient for improving friability and maintaining an acceptable disintegration time and tablet strength.

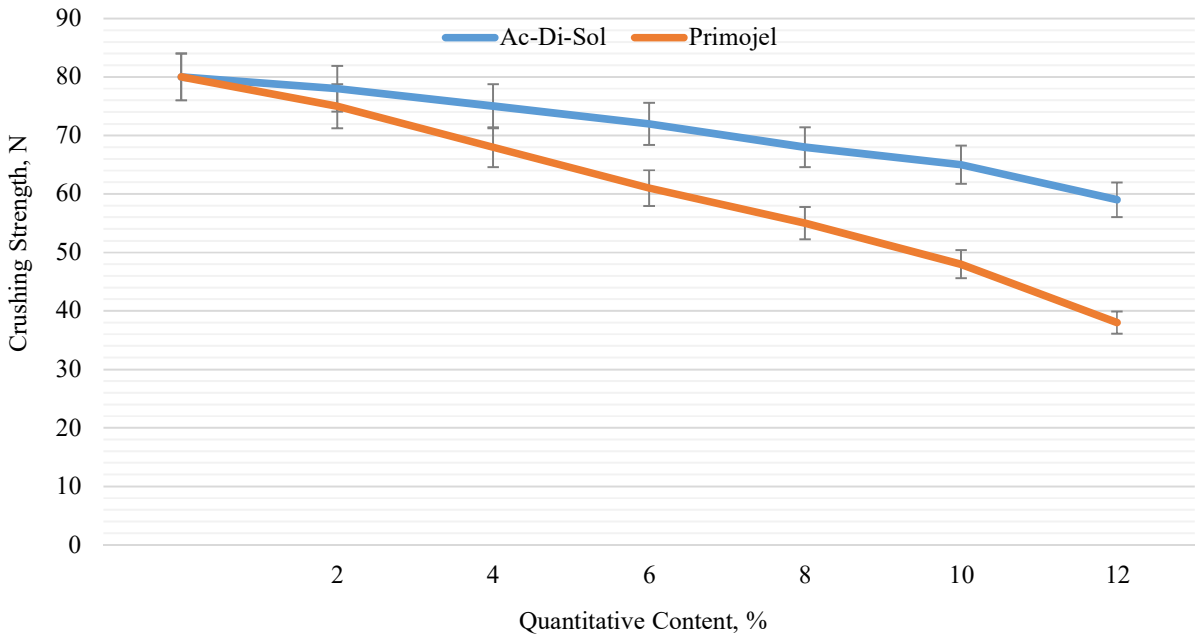

Fig. 1. Study of the influence of disintegrants on the crushing strength indicator

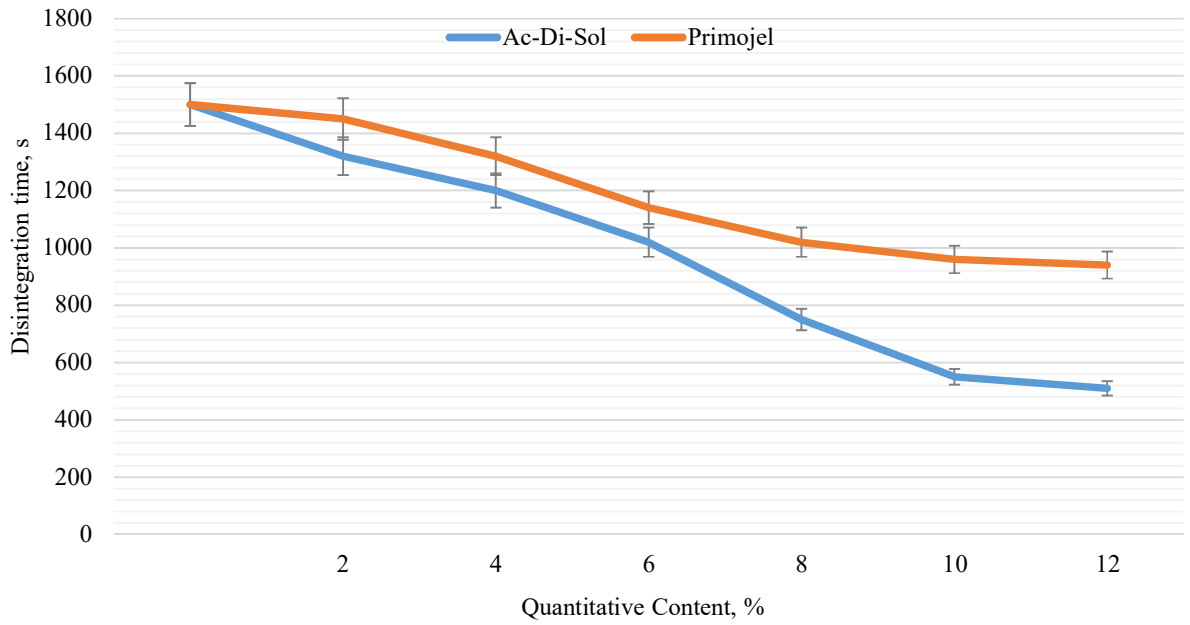

Fig. 2. Study of the influence of disintegrants on the disintegration time indicator

In order to confirm the expediency of combining Acorus calamus extract with SDQ, a study of the effectiveness of tablet samples for their anti-exudative activity on the model of acute aseptic carrageenan inflammation was conducted (Table 7).

As a result of the research, it was found that the composition of dry extract of Acorus calamus+SDQ in the form of tablets had the highest anti-exudative activity among combinations of Acorus calamus with quercetin – 71.3 %.

Table 6

Quality indicators of tablets with lubricants

Quality indicator	Without lubricant	Lubripharm SSF	LIGAMED MF BLS	LIGAMED CPR-2-K	COMPRITOL 888 ATO	Aerosil A-380
Friability, %	0.871±0.008	0.156±0.001	0.322±0.003	0.305±0.003	0.273±0.003	0.224±0.002
Disintegration time, sec	546±5	570±6	792±8	750±7	684±7	588±6
Strength, N	64.12±0.64	59.34±0.61	56.67±0.57	55.43±0.54	60.02±0.59	53.93±0.54

Table 7

Anti-exudative activity of tablets of dry extract of Acorus calamus leaves and SDQ on the model of acute aseptic carrageenan inflammation

Study group	Paw size (in units)/anti-exudative activity (%)				
	At the beginning	1 hour	2 hours	3 hours	Average
Control	1.27±0.05	1.85±0.04	1.70±0.05	1.60±0.05	–
Dry extract of Acorus calamus, 30 mg/kg	1.11±0.02*	1.36±0.04* 58.2	1.26±0.03* 65.8	1.21±0.03* 69.8	63.5
Dry extract of Acorus calamus 30 mg/kg+quercetin 5 mg/kg	1.18±0.06	1.38±0.05* 65.0	1.31±0.05* 68.8	1.26±0.05* 75.4	68.8
Dry extract of buck Acorus calamus wheat +SDQ, 30 mg/kg+5 mg/kg	1.04±0.04*	1.23±0.03*/**/# 68.5	1.17±0.03*/# 69.6	1.11±0.04*/# 78.4	71.3
Vikair, 1.26 mg/kg	1.09±0.03*	1.39±0.03*/## 47.3	1.32±0.03*/## 45.8	1.26±0.03*/## 47.2	46.8
Diclofenac, 8 mg/kg	1.09±0.03*	1.27±0.03*/# 70.2	1.19±0.04*/# 78.1	1.13±0.03*/# 87.9	77.1

*Note: * – significance relative to control, p<0.05; ** – significance relative to dry extract of Acorus calamus, p<0.05; # – significance relative to vicar, p<0.05; ## – significance relative to diclofenac, p<0.05.*

5. Discussion

Determining the average effective dose of API is the basis of any pharmacological study because it is an indicator of the future effectiveness of drugs [26]. When researching plant extracts, as a rule, due to their low toxicity and the inability to calculate an average effective dose, a relatively therapeutic dose is determined based on screening studies of certain types of activity depending on the pharmacological properties of the active substance [27, 28]. Acorus calamus leaves contain flavonoidin and essential oils that have anti-inflammatory, reparative, antispasmodic and other effects [29]. Therefore, on the basis of the pharmacognostic characteristics of the extract of the leaves of the Acorus calamus, we conducted studies to determine the relatively therapeutic dose of the extract based on the

calculation of antiexudative activity on models of acute carrageenan and zymosan inflammation. According to the results of the study indicated in Tables 1, 2, it was established that the average indicators of the antiexudative activity of the dry extract of Acorus calamus leaves on the model of acute carrageenan inflammation were the following: group 2 – 32.2 %, group 3 – 52.5 %, group 4 – 67.5 %, group 5 – 65.9 %. A further increase in the dose of dry extract to 50 mg/kg (group 6) did not lead to an increase in antiexudative activity and amounted to 52.2 %. Determination of the antiexudative activity of the dry extract of Acorus calamus on the model of acute zymosan inflammation also showed the presence of anti-inflammatory activity of the substance under study. At the same time, the level of antiexudative activity was lower compared to carrageenan oedema, but the trend of increasing activity depending on the dose remained: group 2 – 19.8 %; group 3 – 21.7 %, group 4 – 27.7 %, group 5 – 23.7 %, group 6 – 22.9 %. Conducted screening studies showed that the highest indicators of anti-exudative activity are shown by the extract of the leaves of the Acorus calamus at a dose of 30 mg/kg (group 2), which was taken as a relatively therapeutic dose.

According to the results presented in Table 3, it was found that the combination of dry Acorus calamus leaf extract with a carrier and SDQ has excellent bulk characteristics and flowability (6.78 g/s). This study justified the need to analyze the pharmacotechnological characteristics of tablets made from this mixture.

The determination of the tablets' pharmacotechnological characteristics (Table 4) showed that the experimental samples had good strength (80.67 N). However, the disintegration time (1500 sec) did not meet the requirements of the State Pharmacopoeia of Ukraine (900 sec) and required adjustment with the appropriate excipients.

According to the data presented in scientific sources, excipients of carbohydrate nature from the fillers group are often chosen to improve the disintegration time of tablets. Carbohydrates are known for their solubility and ability to interact with other components, which can significantly affect the disintegration time and crushing strength of tablets. These modifying excipients demonstrate various effects on the pharmacotechnological characteristics of tablets. They can significantly enhance the structure and strength of the tablets while also affecting their disintegration time, which is especially important for ensuring the effectiveness and speed of action of tablet formulations [30–32].

Based on the above, to optimize the pharmacotechnological characteristics of the tablets, a decision was made to explore the possibility of using carbohydrate excipients from the fillers group to improve their disintegration time. The substances were added to the composition of the dry Acorus calamus extract with carrier and SDQ in an amount of 30 % relative to the mass of the mixture.

The results presented in Table 5 indicate the low effectiveness of carbohydrate nature substances regarding the disintegration time of tablets within the studied mixture. The best disintegration time was observed in the sample with Partec Si 400 – 1200 sec. However, this disintegration time was significantly longer than that stipulated by the SPhU standards. Additionally, a significant decrease in tablet crushing strength (35.78 N) was observed.

Since the introduction of carbohydrate excipients from the fillers group did not yield positive results, the next stage of the study decided to examine the impact of disintegrants (expanding agents) directly on the strength and disintegration indicators of the tablets. Substances from this group are added to tablet formulations to improve their breakdown into small fragments in an aqueous environment, facilitating the quicker release of API.

An analysis of modern literature sources on the mechanism of action, effectiveness, and technological properties of expanding agents was conducted to establish the qualitative and quantitative composition of disintegrants. It was found that there are several groups of disintegrants relative to the mechanism of action. The main ones include substances with swelling (starch, sodium starch glycolate, croscarmellose sodium (Ac-Di-Sol)) and capillary (various forms of polyvinylpyrrolidone (PVP)) mechanisms of action.

It is important that Kollidon CL, a cross-linked PVP present in the carrier for the dry extract of Acorus Calamus leaves, exhibits disintegrating activity through a capillary mechanism in addition to carrier properties. As some researchers point out the rationality of combining several expanding agents with different mechanisms of action, substances with a swelling mechanism were used in further studies: sodium starch glycolate (Primojel) and croscarmellose sodium (Ac-Di-Sol). The concentrations of these disintegrants (1–12 %) were determined according to the literature and the manufacturer's recommendations stated in the brochure [33].

The results, illustrated in Fig. 1, 2, confirm the positive effect of Ac-Di-Sol and Primojel on the speed of tablet disintegration. It is worth noting that both components actively accelerate the disintegration process, but Ac-Di-Sol proved to be the most effective, providing a significantly faster result compared to Primojel.

In addition, another important aspect should be considered: compared to Primojel, Ac-Di-Sol has less impact on reducing the tablet's crushing strength (Fig. 1). This is an important aspect since the physical integrity of the tablets is a key factor in ensuring their effectiveness and maintaining quality. Thus, analyzing the obtained data, the most optimal is the use of Ac-Di-Sol at a concentration of 10 %.

Friability is an important pharmacotechnological quality indicator of the finished dosage form. To ensure the friability indicator complies with the regulatory documentation requirements (SPhU), substances-lubricants are added to the composition of the developed product. The introduction of such excipients helps prevent the formation of conglomerates and friction between individual particles of the medicinal product, overall leading to increased accuracy of the API mixture dosing.

Considering the data mentioned above, the following substances were selected to improve friability:

1. Sodium Stearyl Fumarate (Lubripharm SSF): this substance has good lubricating properties and can help reduce friction between particles during tabletting, which can improve the quality of tablets and prevent sticking to the tablet press. The substance is inert and hydrophilic. The low electrical charge of sodium stearyl fumarate improves its distribution in the tablet mixture during mixing, providing more uniformity in the composition and better lubrication.

2. Magnesium Stearate (Ligamed MF BLS): used to prevent powder from sticking to equipment during tabletting, it also improves the flowability of the powder mixture. Thanks to its hydrophobic properties, magnesium stearate reduces mutual friction between particles and equipment, improving the quality of the finished product and stabilizing the production process.

3. Calcium Stearate (Ligamed CPR-2-K): helps reduce the sticking of powder mixtures to tablet presses and improves mixture flowability. This substance has hydrophobic properties, helping ensure tablet uniformity and protect the finished product from moisture.

4. Glycerol Behenate (Compritol 888 ATO): this substance is a solid lipid used as a lubricant and matrix agent in the production of tablets and capsules. It provides efficient lubrication, improving the quality of finished tablets and facilitating the manufacturing process. COMPRITOL 888 ATO can also be used to control the release rate of active substances in tablet forms.

5. Colloidal Silicon Dioxide (Aerosil A-380): used as a glidant in tabletting to improve the flowability of powder mixtures. This substance promotes the even distribution of ingredients in the tablet and reduces the likelihood of lump formation. Thanks to its unique surface properties, Aerosil A-380 provides better mixture flowability and improves the quality of finished tablets.

According to the results of the study (Table 6), the following correlation of the main pharmacotechnological parameters was observed:

– friability: a significant reduction in friability was observed when using all types of lubricants compared to the variant without a lubricant. The lowest friability was with Lubripharm SSF (0.156 %), indicating its effectiveness in improving the mechanical stability of tablets. Aerosil A-380 also showed good results (0.224 %).

– disintegration time: the fastest disintegration was observed in tablets with Lubripharm SSF (570±6 sec) and Aerosil A-380 (588±6 sec). The use of Ligamed MF BLS and Ligamed CPR-2-K significantly increased the disintegration time, indicating low technological suitability regarding this parameter.

– strength: all lubricants reduced the strength of the tablets compared to the variant without a lubricant, but this reduction was within the norms of the State Pharmacopoeia of Ukraine. The highest strength was shown by tablets with Compritol 888 ATO (60.02 N) and Lubripharm SSF (59.34 N), indicating their ability to maintain the structural integrity of the tablets.

Based on the study results, it was decided to choose Lubripharm SSF as the optimal excipient effectively improving friability while maintaining an acceptable disintegration time and tablet strength.

In the process of searching for medical products that show anti-inflammatory activity, it is mandatory to determine the anti-exudative activity [26]. According to the results shown in Table 7, the highest anti-exudative activity among the investigated products based on the dry extract of Acorus calamus leaves was found by the composition of dry Acorus calamus extract+SDQ in the form of tablets – 71.3 %, which was higher than the indicators of dry Acorus calamus extract and the combination of dry Acorus calamus extract with quercetin in its pure form. The addition of quercetin to the dry extract of the leaves of the Acorus calamus increases the antiexudative activity from 63.5 % to 68.8 %, which is explained by the combination of effects on various inflammatory mediators: cyclooxygenase (the effect of the Acorus calamus extract) [7] and leukotrienes (quercetin) [11]. The use in combination with SDQ further increases the anti-exudative activity of tablets due to the higher bioavailability of the studied substances.

Indicators of anti-exudative activity of the composition of Acorus calamus extract+SDQ determined at different time intervals were significantly better compared to «Vikair», and the average indicator was 24.5 % higher. At the same time, the anti-exudative activity of the composition was lower compared to the synthetic drug «Diclofenac» and lower by 6 %, with a significant difference only in the first hour after the start of the experiment. The conducted studies indicate the high potential of the new combination and the expediency of its further studies in order to determine more specific pharmacological properties and the possibilities of treatment of inflammatory diseases of the GIT.

Practical relevance. The study identifies optimal auxiliary substances and formulation parameters, paving the way for new therapeutic options in treating inflammatory gastrointestinal diseases and enhancing patient outcomes with a more effective and stable pharmaceutical product.

Study limitations. Research aimed at identifying the best excipients for the dry extract of Acorus calamus leaves and quercetin's solid dispersion does not cover all potential risks related to applying this technology in a production setting. Further investigations using the industrial equipment at the manufacturer's disposal are necessary.

Prospects for further research. Future research endeavours could focus on finalizing the composition of an innovative pharmaceutical product based on Acorus calamus leaves and quercetin. This would encompass extensive pharmacological studies to ascertain the drug's efficacy and safety profile. Additionally, the development and standardization of technological, regulatory documentation for its production will be crucial. Further studies should also include verifying the alignment of the developed drug with established quality parameters and substantiating the technological processes involved in its manufacture. This comprehensive approach will pave the way for the introduction of a novel and effective treatment option in the pharmaceutical market.

6. Conclusions

1. A relatively therapeutic dose of dry Acorus calamus extract has been determined, which is 30 mg/kg, and can serve as a basis for creating a new tablet formulation based on this plant.

2. The optimal qualitative and quantitative composition of excipients for producing tablets containing dry sweet flag leaf extract and SDQ has been established. The obtained combination ensures high efficiency and stability of the final pharmaceutical product.

3. Studies on the effect of different disintegrants have shown that Ac-Di-Sol most effectively improves the disintegration time of tablets. At the same time, this disintegrant has a lesser impact on reducing the strength of tablets compared to other excipients studied.

4. It has been determined that a 10 % concentration of Ac-Di-Sol is optimal for achieving the desired results regarding tablet disintegration time. This provides an effective balance between disintegration time and tablet strength.

5. The study of various lubricants showed that Lubripharm SSF effectively reduces tablet friability, increasing their mechanical stability. It is important to note that the introduction of Lubripharm SSF did not lead to a significant deterioration of other pharmacotechnological characteristics, such as strength and disintegration time.

6. The highest anti-exudative activity of 71.3 % was demonstrated by a sample of tablets with a dry extract of Acorus calamus and SDQ.

Conflict of interests

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Data availability

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Use of artificial intelligence

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

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Oleksiy Andryushayev*, PhD Student, Department of Industrial Technology of Drugs, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002

Yevhenii Samoilov, PhD Student, Department of Pharmacology and Pharmacotherapy, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002

Valeriia Hnatiuk, Doctor of Medicine Sciences, Associate Professor, Department of Pharmacology, Bogomolets National Medical University, Tarasa Shevchenka blvd., 13, Kyiv, Ukraine, 01601

Olena Ruban, Doctor of Pharmaceutical Sciences, Professor, Head of Department, Department of Industrial Technology of Drugs, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002

Mariia Velia, PhD, Assistant, Department of Pharmacy, Bukovinian State Medical University, Teatralna sq., 2, Chernivtsi, Ukraine, 58002

Maryna Savokhina, PhD, Associate Professor, Department of Pharmacology and Pharmacotherapy, National University of Pharmacy, Pushkinska str., 53, Kharkiv, Ukraine, 61002

***Corresponding author:** Oleksiy Andryushayev, e-mail: linuks454@gmail.com*