



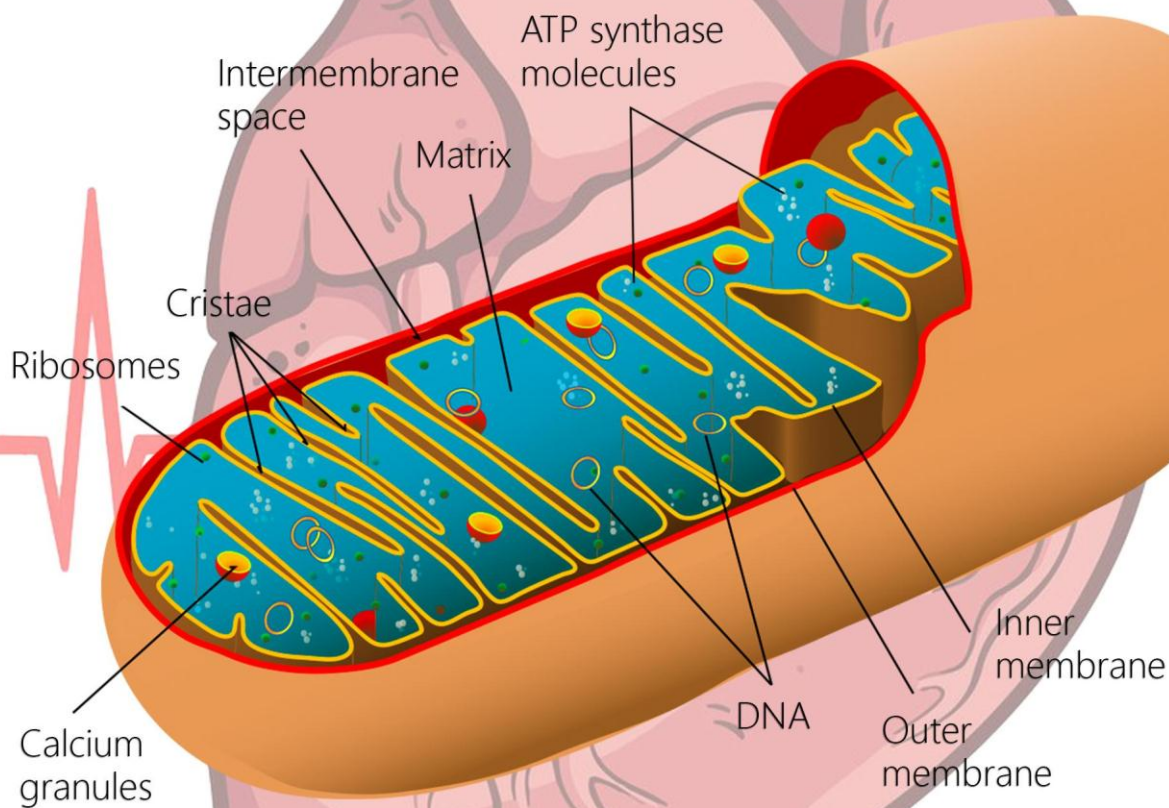
# Cardiometry

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Morphological examination of the heart to objectivize efficacy of  
mitochondrial therapy in myocardial infarction



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- 62 **cAMP concentrations in cardiac mitochondria and serum in the C57BL/6 mice under independent melanoma B16/F10 growth versus melanoma B16/F10 growth linked to chronic neurogenic pain**  
*Oleg I. Kit, Elena M. Frantsiyants, Valeria A. Bandovkina, Irina V. Neskubina, Alla I. Shikhlyarova, Irina V. Kaplieva, Ekaterina I. Surikova, Yulia A. Pogorelova, Natalia D. Cheryarina, Lidia K. Trepitaki, Irina A. Goroshinskaya, Larisa N. Vaschenko, Yulianna S. Shatova, Lyudmila P. Kuchkina, Vera A. Kovalenko, Lyudmila A. Nemashkalova*  
 The aim of this research work is to study the cAMP level in the cardiac mitochondria and serum in the C57BL/6 strain mice of both genders under the independent melanoma B16/F10 growth versus the melanoma B16/F10 growth linked to chronic neurogenic pain (CNP).
- 69 **Development of adaptive stereotactic radiotherapy method in treatment of primary malignant glial tumors in the brain**  
*Stanislav G. Vlasov, Marina A. Yengibaryan, Alla I. Shikhlyarova, Pavel G. Sakun, Vitaly I. Voshedsky, Olga G. Rodionova, Elena A. Karnaukhova, Anna A. Solntseva, Vladislav E. Khatyushin, Olga V. Pandova, Natalia S. Kuznetsova, Sergey N. Kabanov, Maria A. Teplyakova*  
 At present, there are some scattered evidence data pertaining to the use of an adaptive technique of radiotherapy in treatment of malignant glial tumors of the brain. Our evidence data obtained in MRI in the course of treatment show that the initial treatment plan may become irrelevant due to some changes observed in the tumor configuration. .
- 77 **Intelligent digitalization of cardiovascular risks**  
*Yuri Y. Gromov, Aleksei V. Gorbunov, Viacheslav Mikhailovich Tyutyunnik*  
 The aim of the study was to develop a mathematical model of the risks of the cardiovascular system based on the selected factors affecting cardiovascular diseases (CVD) and to test the developed mathematical model on a sample of clinical examples. CVD risk factors was grouped by types: biological indicators (anthropometric, biochemical, morphological, physiological), disease indicators, social indicators.
- 95 **The use of cardiometry in development of self-control skills by means of game sand modeling**  
*Vladimir A. Zernov, Elena V. Lobanova, Elvira V. Likhacheva, Lyubov' P. Nikolaeva, Aleksandr S. Ognev, Nadezhda A. Ogneva, Mikhail Yu. Rudenko*  
 The article presents experimental evidence of the validity of our hypothesis that progress in the formation and development of self-control can be fixed by assessing heart rate variability using the calculation of the Baevsky stress index (SI).
- 100 **Multiple factor analysis applied to calculation of blood pressure based on pulsometry data**  
*Igor S. Yavelov, Georgy L. Danielyan, Andrey V. Rochagov, Anatoly V. Zholobov*  
 Development of algorithms capable of determining arterial pressure (AP) according to the contour of a pulse wave (PW) remains a topical issue to be solved with long-time efforts by researchers who work for many years in the field of hemodynamics. There is no doubt that the PW contour contains the data on AP, however due to a great number of factors influencing AP to reveal the relationship between them is extremely complicated.
- 106 **Adrenaline heart**  
*Mikhail Y. Rudenko, Irina A. Berseneva, Marat P. Deberdeev, Mariam V. Pasikova, Alexander V. Tychinsky, Amalia I. Mitilineo-Obukhova, Botir A. Yuldashev, Malika D. Murodova*  
 The role of adrenaline in the body, especially in athletes, is considered. Special attention is paid to the cardiovascular system performance in conditions of the continuous release of adrenaline. The ECG shape and the possible therapy in case of adrenaline heart are discussed.
- 108 **Using the method of mathematical planning of the experiment in the development of a quality control method of aerva lanata l. herb**  
*Viloyatkhon N. Abdullabekova, Vosil R. Khaydarov, Saodat T. Sharipova, Oygul Rakhim qizi Rakhimova, Aipashsha J. Tajieva*  
 Today, among the urgent tasks facing the pharmaceutical science of the Republic of Uzbekistan is the study of introduced medicinal plants. Of the promising plants Erva woolly - Aerva lanata Juss of the amaranth family (Amaranthaceae) deserves special attention.

# Using the method of mathematical planning of the experiment in the development of a quality control method of *Aerva lanata* L. herb

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## Abstract

Today, among the urgent tasks facing the pharmaceutical science of the Republic of Uzbekistan is the study of introduced medicinal plants. Of the promising plants *Erva woolly* - *Aerva lanata* Juss of the amaranth family (Amaranthaceae) deserves special attention. A perennial or biennial herbaceous plant grows in many countries of Africa, Saudi Arabia, India, Indonesia, etc. For the first time, *Aerva lanata* Juss. was introduced on the territory of the former USSR by the Transcaucasian zonal experimental station VILR (Kobuletti) in the mid-70s, then in Ukraine, Uzbekistan and Kazakhstan.

*Aerva lanata* is a dioecious plant. The flowers of *A. lanata* are nectariferous. Pollination of *Erva woolly* occurs under the influence of gravity inside the flower, wind, insects and rainwater. *Erva woolly* with more than one pollination and seed dispersal mode is able to invade a variety of habitats with different environmental conditions and grow as widespread weeds.

Among other *Aerva* species, *A. lanata* cultivar has a wide range of pharmacological activities such as sedative, antiulcer, antiasthma, antidiarrheal, antioxidant, antihyperglycemic, antimalarial, hypolipidemic and other activities. It is used as a diuretic, for acute kidney damage, as well as for ulcerative wounds, rheumatism.

Given the availability of the raw material base necessary to meet the possible growth in consumer demand and its widespread use in traditional and scientific medicine to create effective, low-toxic drugs intended for the prevention and treatment of diseases of the urinary system, *Aerva lanata* Juss L. was chosen as the object of research.

An important stage in the standardization of the final product is the quality control of the technological process and the raw

materials entering the production to obtain the finished product. Particular attention is paid to the method of quantitative determination of one of the active substances or groups of biologically active substances that affect the therapeutic effect of finished pharmaceutical products.

It is known that the quantitative determination of the active substance is carried out by its extraction from plant materials. In this regard, the problem arises - finding the optimal conditions for the process of extracting the active substance. In view of this, it becomes necessary to conduct research to determine the optimal conditions for the extraction process.

**The objective of the research:** the use of the mathematical planning method of the experiment for the complete extraction of flavonoids from the herb *Aerva lanata*.

**To achieve this objective,** the following tasks were defined:

- selection of the mathematical planning method of the experiment;
- selection of method for quantitative determination of flavonoids amount;
- statistical analysis of the obtained results.

Optimization of the process of extracting the amount of flavonoids from the herb of *Aerva lanata*, which is predominant in its raw materials, was carried out using the method of mathematical planning of experiments by Box and Wilson. The optimization problem was reduced to determining the values of technological parameters that ensure the maximum yield of total flavonoids from the plant. As an optimization parameter, *Y* was taken - the content of the sum of flavonoids in the extract, in % of its content in the raw material.

## Conclusion.

This article presents the results of modeling and optimization of the process of obtaining the sum of flavonoids from the herb *Aerva lanata* Juss. L. A comprehensive quantitative assessment of the influence of three experimental factors (alcohol concentration, hydromodulus and extraction time) on the yield of the total flavonoids was carried out using the method of mathematical planning of the Box-Wilson experiment.

The objective was achieved by building a mathematical model based on the first order regression equation:

$$Y=1.20+0.185X_1+0.091X_2+0.111X_3$$

The adequacy of this process is shown, a mathematical model and parametric identification of this model are presented.

As a result of the statistical analysis of the obtained data, the following optimal conditions for the process of extracting the amount of flavonoids from the herb of *Aerva lanata* were revealed in the quantitative analysis of raw materials: alcohol concentration - 50%; hydromodule - 1:60; extraction time - 30 min.

The maximum yield of the sum of flavonoids was 1.52% by weight of dry raw materials.

The resulting mathematical model of the extraction process will allow further optimization of the extraction of compounds of natural origin, taking into account the quantitative influence of each factor.

### Keywords

Aerva lanata herb, extraction, sum of flavonoids, experiment design, statistical analysis, planning matrix

### Imprint

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### Introduction

Erva woolly - *Aerva lanata* L Juss. belongs to the amaranth family - *Amaranthaceae*. In 1977, seeds of domestic reproduction *Aerva lanata* were obtained from plants of Ceylon origin under greenhouse conditions. The plant grows rapidly, blooms profusely and bears fruit in the open ground, and also develops well, does not die at the first frost. In the zone of humid subtropics of Georgia, it is possible to carry out within 2-3 cuttings of the above-ground mass during the growing season [1,2,3].

According to the literature data [4], *Aerva lanata* has traditionally been used in the treatment of cough, hemiplegic migraine and kidney-related diseases.

Numerous pharmacological studies report a wide range of therapeutic properties of *Aerva lanata*. Because of its medicinal properties, it has traditionally been used by the common people.

*Aerva lanata* is known for its antioxidant, antibacterial, anti-inflammatory, hepatoprotective, anti-diabetic, anticancer, antihyperlipidemic and nephroprotective activities. This plant is promising not only from a medical point of view, but also for a number of other scientific purposes. The review also discusses the synthetic significance and toxicity of these plants. The study aims to comprehensively summarize what has already been done and what still needs to be done in future research related to *Aerva* species [4,5,6].

Based on experimental studies by Indian scientists, the protective effect of the ethanol extract of *Aerva lanata* (EEAL) in preventing the toxic effects of acetaminophen (ACN) on the liver was evaluated. EEAL was prepared and its hepatoprotective effect was studied. The study was carried out both on isolated primary hepatocytes in vitro and on Sprague Dawley rats in vivo. Studies have shown that mean levels of GOT (glutaminoxaloacetic transaminase) and GPT (glutamicpyruvic transaminase) decreased only after EEAL treatment. Moreover, only ALP (alkaline phosphatase) and LDH (Lactate dehydrogenase) in serum were at normal levels after EEAL treatment, while GOT and GPT showed lower levels in controls. Treatment with ACN increased the expression of the pro-inflammatory genes COX 1 (cyclooxygenases) and COX 2 (cyclooxygenases), and the levels of these genes decreased with EEAL treatment. EEAL pretreated rats exposed to ACN were found to retain normal liver structure compared to rats treated with ACN alone. In conclusion, EEAL was noted to have significant anti-inflammatory and hepatoprotective effects against ACN-induced liver injury. The results obtained are confirmed by molecular and histopathological analyses. The content of polyphenols, flavonoids and alkaloids in *A. lanata* contributes to the manifestation of its activity [7].

Two isolated compounds (quercetin and betulin) from *A. lanata* were tested for anti-urolithic potential in male Wistar albino rats induced by stones (ethylene glycol 0.75% v/v). By oral administration of 2 mg/kg body weight per day as a test dose for 28 days, urine volume was found to increase significantly from  $12.76 \pm 0.10$  ml to  $21.35 \pm 0.20$  ml in rats treated with quercetin and  $21.50 \pm 0.21$  ml in rats treated with betulin. Urinary microscopy showed a significant decrease ( $p < 0.001$ ) in stone size and a significant increase ( $p < 0.001$ ) in calcium, oxalate, and phosphate excretion, while magnesium levels were elevated [8].

The phytochemical composition and antioxidant properties of the aqueous extract of *Aerva lanata* (*A. lanata*) were studied in vitro. During a preliminary phytochemical analysis, the aqueous extract of *A. lanata* was tested for the presence of carbohydrates, proteins, phenolic compounds, oils and fats, saponins, flavonoids, alkaloids, tannins and phytosterols. The antioxidant activity of the extract was determined by the activity of scavenging 2-diphenyl-1-picrylhydrazyl radicals, the chelating activity of metals, the decrease in energy activity, and the activity of inhibition of

DNA damage. The analysis of phenolic compounds was performed by the method of Folin-Ciocalto reagents and by the method of gradient high-performance liquid chromatography. Preliminary phytochemical analysis showed the presence of phenolic compounds, saponins, flavonoids, tannins and phytosterols as the main phytochemical groups. The extract exhibited high 2-diphenyl-1-picrylhydrazyl radical scavenging activity ( $IC(50) = 110.74\mu\text{g/mL}$ ), metal chelating activity ( $IC(50) = 758.17\mu\text{g/mL}$ ), decreased energy activity, and DNA damage inhibition efficiency. The extract has been reported to have a high total phenol content and some have been identified as gallic acid (3,4,5-OH), apigenin-7-O-glucoside (apigenin), quercetin-3-O-rutinoside (rutin) and myricetin (3,5,7,3,4,5-OH) by high performance liquid chromatography. Hemolytic analysis showed that the extract is non-toxic to human erythrocytes ( $IC(50) = 24.89\text{ mg/ml}$ ). These results led to the conclusion that *A. lanata* has a high antioxidant activity and can be used to create natural and safe antioxidant compounds [9].

Extracts of *A. lanata* have been tested for antioxidant, antimicrobial, and antilytic potential in order to scientifically support its traditional use. The research results showed that the methanol extract has the highest antioxidant activity, and the aqueous extracts have the lowest in the following order: methanol extract > ethyl acetate > chloroform > water. The results of this antimicrobial study indicate that the methanolic extract of *A. lanata* can be used as antimicrobial agents. In the future, this study can be recognized as a major new report, which focuses on the use of *A. lanata* (Linn) as an antioxidant, antimicrobial, and antiurolytic agent [10]. A group of researchers [11] studied the phytochemical composition of various extracts of *Erva woolly* obtained in solvents such as ethanol, ethyl acetate, chloroform, acetone, water, and methanol. The analysis confirmed the presence of flavonoids, glycosides, terpenoids and alkaloids and other components in them. Simultaneously, the extracts were tested for antibacterial activity against microbial pathogens and antioxidant properties. The results showed that the solvent extracts showed marked activity against the tested strains. The plant extract was highly effective against *E. coli* and *E. aerogenes* and the MIC was 5 mg/mL. In addition, the extracts had promising effective antioxidant activity.

*Aerva lanata* was subjected to successive extractions using solvents (petroleum ether, ethyl ace-

tate and ethanol) in ascending order of polarity. The extracts thus prepared were subjected to a preliminary phytochemical analysis. The extracts were then tested for analgesic activity and anti-inflammatory activity in Wistar rats using diclofenac sodium and indomethacin as standard drug, respectively. The results showed that all extracts had significant analgesic activity. Ethanol extract at a dose of 800 mg/kg body weight was more significant than other extracts [12].

The aim of this study was to study the chemical composition of phenolic acid (PA) enriched fractions isolated from methanolic extracts of *A. lanata* (L.) Juss. herbs using a liquid/liquid extraction method and their potential antioxidant, anti-inflammatory, and anti-diabetic properties. Free PA (FA) fraction, PA (FB) fraction released after acid hydrolysis, and PA (FC) fraction obtained after alkaline hydrolysis were analyzed using liquid chromatography/electrospray ionization triple quadrupole mass spectrometry (LC-ESI-MS). /MS.). Thirteen compounds were detected and quantified in all samples, including some substances not previously found in this plant species. The phenolic profile of each sample showed high antiradical and antidiabetic activity [13].

In this paper [14], the authors presented the results of studies on the synthesis of silver nanoparticles (AgNP) with an aqueous extract of the herb *Aerva lanata* (AL) and an assessment of the effect of the resulting substance on wound-associated bacteria. Phytochemical analysis of the AL extract confirmed the presence of flavonoids (rutin, quercetin and kaempferol), polyphenols (gallic and ellagic acids) and saponins. The production of AL-AgNPs nanoparticles was proven by the appearance of brown color and absorption of light at a wavelength of 430 nm. The reduced levels of phytochemicals and the Ag<sup>+</sup> ion in the reaction mixture further confirmed the bioresonating efficiency of AL. Microscopic and spectral analysis showed that AL-AgNP particles have a face-centered cubic crystalline nature and are a 50 nm spherical shape with a rough surface (10.3157 nm), zeta potential (-27.1 mV). The results of this study showed that the resulting AL-AgNPs have good in vitro biocompatibility and antibacterial activity against wound bacterial infections. The authors suggest that AL-AgNP may be pharmaceutically useful in the treatment of infectious diseases.

The authors conducted studies on the acute toxicity of an aqueous extract of *Aerva lanata* growing in Ni-

geria. Acute toxicity tests of the extract, administered orally at a dose of 1-30 g/kg and intraperitoneally at a dose of 0.1-2 g/kg, were carried out on albino mice; while the test for subchronic toxicity was carried out by daily oral administration of the extract at a dose of 40-1000 mg/kg to albino rats for 90 days. During the study of subchronic toxicity, anthropometric, biochemical and hematological parameters were carried out, as well as histological studies of vital organs. The results of this study indicate that an aqueous extract of *A. lanata* is relatively safe for acute oral exposure, moderately toxic for acute intraperitoneal administration, and relatively safe with antioxidant activity for long-term exposure [15].

The pharmacological actions listed above are associated with the presence of valuable nutrients and biochemical compounds such as sterols/terpenes, flavonoids, alkaloids, phenols and sugars. The expedient use of substances obtained from this plant can increase the possibility of its use in medical practice [4,5,8].

Phytochemical components present in the plant include alkaloids, flavonoids (kaempferol, quercetin, isorhamnetin), benzoic acid acetate,  $\beta$ -sitosterol acetate and tannic acid [4].

Flavonoid glycosides, such as kaempferol, isorhamnetin, quercetin, flavanone, are the main components of this plant, and apigenin, ferulic, syringic and vanillic acids are secondary. The authors believe [5] that it is necessary to quickly use analytical methods to determine various concentrations of substances present in the alcoholic extract of the *Aerva lanata* plant.

An important stage in the standardization of the final product is the quality control of the technological process and raw materials to obtain the finished product. Particular attention in assessing the quality of manufactured products is given to the quality and method of quantitative determination of one of the active substances or groups of biologically active substances (BAS) of raw materials.

As can be seen from the above data and our own research, flavonoid compounds, which are of great value for medical practice, are of significant importance among the biologically active substances of the *Aerva lanata* herb.

Classical scientific research is associated with experiments that require large expenditures, effort and time. They are based on alternately varying individual independent variables while others tend to remain constant. Experiments, as a rule, are considered multi-

factorial and are associated with optimizing the quality of raw materials, finding the best conditions for conducting technological processes, developing more optimal equipment systems, etc. The objects of such studies are systems that are often considered complex and cannot be theoretically studied in a short time.

Despite the significant amount of research work carried out, as well as the lack of a real opportunity to sufficiently fully study a large number of objects of the research, almost all decisions are made on the basis of random information, and therefore are far from optimal. And so there is a need to find a solution that allows to conduct research work at an accelerated pace and ensure decision-making that is close to the best results. Statistical methods of experiment planning appeared in this way [16].

The plan of the experiment depends on the type of mathematical model, and not infrequently, the success of further research depends on the correct choice of the plan for conducting a purposeful experiment. At the same time, a correctly chosen plan makes it possible not only to reduce the amount of research, but also to minimize the influence of unaccounted for, uncontrolled factors on the result of the study.

In this regard, the issues of organizing the experiment, reducing the cost of conducting it and processing the results are relevant. Modern methods of planning an experiment and processing its results, developed on the basis of probability theory and mathematical statistics, can significantly reduce the number of experiments.

The widespread use of statistical methods of planning experiments in applied research makes the work of researchers the most focused and organized, significantly increases both labor productivity and the reliability of the results obtained [17-20].

Currently, in analytical practice, for optimizing processes, such methods of experiment planning as the Box-Wilson method or the steep ascent method [21] and the successive simplex optimization method (SSM) [17, 21] are widely used.

The steep ascent method, or the Box-Wilson method, combines the positive aspects of other existing methods, in particular the Gauss-Seidel method [22], the gradient method [22] and the method of full (or fractional) factorial experiment [22] as a means of obtaining a mathematical models. The solution of the problem by the method of steep ascent is carried out so that the stepping movement is carried out in the

direction of the fastest increase (or decrease). The use of the Box-Wilson method makes it possible to find the equation of the mathematical model for the area with the maximum value of the degree of absorption.

Studies have been carried out to optimize the process of extracting a complex of flavonoid substances to ensure their maximum yield from plant raw materials. Optimization of the process of extracting the amount of flavonoids from the *Aerva lanata* herb was carried out by the method of mathematical planning of experiments by Box and Wilson (steep ascent). The optimization problem was reduced to determining the values of technological parameters that provide the maximum yield of total flavonoids. As an optimization parameter,  $Y$  was taken - the content of the sum of flavonoids in the extract, in % of its content in the raw material.

It is known [23,24,25] that the yield of biologically active substances from plant materials is influenced by various factors, such as the methods and conditions of the extraction process, the hydromodule, and the equipment used.

**The objective of the research:** the use of the mathematical planning method of the experiment for the complete extraction of flavonoids from the herb *Aerva lanata*.

**Materials and research methods.** The object of the study was the local raw material *Aerva lanata*, which is pieces of leafy stems with inflorescences, as well as with roots, individual whole short-petiolate, ovate or elliptical, pointed or blunt at the top, entire or partially crushed leaves, spike-shaped, felt-pubescent inflorescences and roots, individual whole small inconspicuous flowers, fruits, bean-shaped, black, shiny, very small seeds. The smell is weak. The taste of the aqueous extract is bitter with a slimy feeling.

Methods are of classical extraction of plant material with ethyl alcohol and mathematical planning of experiments by Box and Wilson. Authenticity and quantification studies were carried out by the spectrophotometric method on a UV-1800 double-beam scanning spectrophotometer equipped with a liquid crystal display and a cuvette with a thickness of 1 cm (Shimadzu, Tokyo, Japan). All reagents and solvents for research were purchased from Merck (Germany).

Prior to the extraction process, samples of dried raw materials were weighed on a Gibertini Europe 1700 analytical balance (Novate Milanese, Milan, Italy). The raw material samples had a moisture content of 18%.

Based on preliminary experiments, the following factors were chosen as independent variables: extractant concentration, %, liquid modulus, and extraction time (min).

**Research results.** The experiments were carried out as follows: about 2 g (accurately weighed) of crushed raw materials are placed in a flask with a 150 ml thin section, 30 ml of 50% alcohol are added, the flask is attached to a reflux condenser and heated in a boiling water bath for 30 minutes, periodically shaking to rinse particles of raw materials from the walls. The hot extract is filtered through cotton wool into a volumetric flask with a capacity of 100 ml so that the particles of the raw material do not fall on the filter. Cotton wool is placed in an extraction flask and 30 ml of 50% alcohol is added. After cooling, the extraction volume was adjusted to the mark with 50% alcohol and mixed (solution A).

5 ml of solution A is placed in a 100 ml flask with a thin section, 10 ml of a 10% sulfuric acid solution is added, added to a reflux condenser and heated in a boiling water bath for 1 hour. Then the solution is evaporated to half of the original volume, cooled for 15 minutes in a vessel with ice and filtered through a paper filter (TU 6-09-1706-82) with a diameter of about 5 cm. The flask in which the hydrolysis is carried out and the residue on the filter is washed with water 4 times by 10 ml. Then, 40 ml of 96% alcohol are poured into the same flask in portions, heated to 50°C, and the residue is dissolved on the filter. The solution is collected in a volumetric flask with a capacity of 50 ml, the solution is brought to the mark with 95% alcohol and mixed. 7.5 ml of the resulting solution is transferred into a volumetric flask with a capacity of 25 ml, the volume of the solution is adjusted to the mark with 50% alcohol and mixed. The optical density of the resulting solution is measured on a spectrophotometer at a wavelength of 370 nm in a cuvette with a layer thickness of 10 mm. 50% alcohol is used as a reference solution.

To identify the optimal modes of the process of extracting the amount of flavonoids in the quantitative determination, taking into account the simultaneous influence of the most suitable various factors, the method of mathematical planning of the experiment was used.

The design of the experiment allows to vary all the factors simultaneously and obtain a quantitative assessment of the main effects and the effects of in-

teraction. Effects of interest are determined with less error than with traditional research methods. Ultimately, the design of experiments greatly increases the efficiency of the experiment while significantly reducing their number. You can reduce the number of experiments if you use the so-called sequential plans proposed by Box and Wilson. This method is a statistical mathematical method that uses a minimum of resources and quantitative data from an appropriate experimental design to determine and simultaneously solve multivariate equations for process optimization.

The core of such designs is a full factorial experiment 23 for n=3 or a half-replica of it for n>3. The possibility of using half-replicas as the core of the plan for n>3 is due to the fact that even a semi-replica provides obtaining unmixed estimates for linear effects of pair interaction.

Consider the construction of orthogonal plans based on three factors that significantly affect the amount of total flavonoids in the extraction process. On the basis of experimental data and known extraction laws in the "solid-liquid" system, the main factors influencing the extraction of raw materials were chosen. These are: alcohol concentration - 1 (%), water content - 2 (ml) and extraction time - 3 (min).

When optimizing processes, ¼ replicas from a full factorial experiment were used, using planning. Based on the staged experiments, the levels of factors and the intervals of their variation were selected, which are shown in Table 1.

Table 1  
Experiment planning conditions

Factors	Main level	Variation interval	Lower level	Top level	Unit of measurement
x <sub>1</sub>	35	15	20	50	%C
x <sub>2</sub>	1:40	1:20	1:20	1:60	ml
x <sub>3</sub>	20	10	10	30	min

The studies were carried out according to the matrix of three-factor planning of experiments. The planning matrix and the experimental results are presented in Table 2.

The process under study for given intervals of variable variation can be described by the following linear relationship in the form of a regression equation:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 \quad (1)$$

where b<sub>0</sub>, b<sub>1</sub>, b<sub>2</sub>, b<sub>3</sub>, – regression coefficients of an incomplete quadratic equation.

Table 2  
Planning Matrix and Experimental Results

Experiment №	x <sub>1</sub>	x <sub>2</sub>	x <sub>3</sub>	x <sub>0</sub>	y <sub>1</sub> %	y <sub>2</sub> %	y <sub>aver</sub> %
1	+	+	+	+	1.54	1.50	1.520
2	+	-	+	+	1.45	1.42	1.435
3	-	+	+	+	1.28	1.21	1.245
4	-	-	+	+	1.12	0.98	1.050
5	+	+	-	+	1.34	1.48	1.410
6	+	-	-	+	1.10	1.26	1.18
7	-	+	-	+	1.05	0.94	0.995
8	-	-	-	+	0.70	0.85	0.775

Using the least squares method using the formula (2), the values of the regression coefficients were calculated.

$$b_j = \frac{\sum_{i=1}^N X_{ij} y_i}{N}, \quad (2)$$

where: i – experiment number (1, 2...8);

j – factor number (1, 2...3);

X<sub>ij</sub> – coded value of factors;

N – number of experiments in the matrix.

We substitute the numerical values of the coefficients into the equation:

$$Y = 1.20125 + 0.185X_1 + 0.09125X_2 + 0.11125X_3 \quad (3)$$

Thus, a mathematical model of the process was obtained, which is a first-order regression equation.

Further, in order to verify the correctness of the experiments, the adequacy of the obtained model, statistical processing of the obtained data was carried out.

To determine the variation in the values of repeated experiments, we used the S<sub>i</sub><sup>2</sup> dispersion calculated by formula (4):

$$S_i^2 = \sum_1^n (Y_q - \hat{Y})^2 \quad (4)$$

where: Y<sub>q</sub> – the result of individual experiment;

Ŷ – arithmetic value;

f = (n – 1) – the number of freedom degrees equal to the number of repeated experiments minus one.

For two repeated experiments, the formula took the following form:

$$S_i^2 = \frac{2\Delta Y^2}{1}, \quad (5)$$

The homogeneity of the dispersion was carried out according to the Cochran criterion:



$$G = \frac{S_{\max}^2}{\sum_i^N S_i^2} \leq G_{cr}, \quad (6)$$

Here, the Cochran tabular test is equal to  $G_{cr} = 0.6798$ .

$$G_{\exp} = 0.2397 < G_{cr} = 0.6798.$$

It can be seen that the resulting dispersion satisfies the Cochran condition and the dispersion is homogeneous.

To check the adequacy of the resulting model, the adequacy variance was determined  $S_{ad}^2$ .

$$S_{ad}^2 = \frac{\sum_{i=1}^N (\Delta Y')^2}{f}, \quad (7)$$

for calculations we first found the value  $S_{ad}^2$ ,  $\Delta Y'_q$  was determined by the following formula (8):

$$\Delta Y'_q = Y'_{cp} - Y_q \quad (8)$$

The results are shown in table 3.

Table 3  
Statistical analysis of the obtained results

Experiment No	$\Delta Y$	$\Delta Y^2$	$S_i^2$	$Y_q$	$\Delta Y_q = Y'_{cp} - Y_q$	$(\Delta Y')^2$
1	0.02	0.000400	0.00080	1.589	0.07	0.0050
2	0.01	0.000225	0.00045	1.406	0.03	0.0010
3	0.03	0.001225	0.00245	1.219	0.03	0.0010
4	0.07	0.0049	0.00980	1.036	0.01	0.0000
5	0.07	0.004900	0.00980	1.366	0.04	0.0020
6	0.08	0.006400	0.01280	1.154	0.00	0.0000
7	0.06	0.003025	0.00605	0.996	0.00	0.0000
8	0.08	0.005625	0.01125	0.814	0.04	0.0020
$\Sigma$	0.03	0.026700	0.05000	9.610	0.00	0.0099

After that, the reproducibility variance was determined  $S_Y^2$  according to the formula (9):

$$S_Y^2 = \frac{\sum_1^N \sum_1^n (Y_{iq} - \hat{Y})^2}{N * (n-1)}. \quad (9)$$

For two repeated experiments, formula (10) took the form:

$$S_Y^2 = \frac{2 * \sum_1^N (Y_{iq} - \hat{Y})^2}{N} = \sum_1^N S_i^2. \quad (10)$$

According to the obtained formula, was calculated the value  $S_Y^2 = 0.006675$ .

Next, the adequacy variance was found by the formula (7):

where:  $q = \kappa + 1$ ,  $\kappa$  – number of regression coefficients

$$S_{ad}^2 = 0.004931$$

The adequacy of the model was checked by the Fisher criterion:

$$F_{\exp} = \frac{S_{ad}^2}{S_y^2}, \quad (11)$$

The model is adequate if its absolute value is less than the table value.

$$F_{calc} = 0.739 < F_{tabular} = 3.3$$

It can be seen that the model is adequate.

To check the significance of the coefficients (regression), first of all, it is necessary to find the variance of the regression coefficients  $S_{bi}^2$  using the formula:

$$S_b^2 = \frac{S_y^2}{N}, \quad (12)$$

$S_{bi}$  – squared error of the regression coefficient.  
 $S_{bi} = \pm \sqrt{S_{bi}}, (11)$

$$S_{bi} = 0.0289$$

Then a confidence interval was built  $\Delta b_i = -t S_{bi}$ .

Here  $t$  is the tabular value of the Student's test at  $P = 95\%$  and the number of freedom degrees (7) used to determine  $S_y^2$  at the selected significance level (usually 0.05)  $\Delta t_{cr} = 2.31$ ;

$$\Delta b_i = t * S_{bi} = 0.00667$$

The coefficient is significant if its absolute value is greater than the confidence interval (Table 4).

Table 4  
Significance of coefficients

$b_i$ – values	Icons	$\Delta b_i$ – values	Results
1.20125	>	0.06673	The coefficient is significant
0.18500	>	0.06673	The coefficient is significant
0.09125	>	0.06673	The coefficient is significant
0.11125	>	0.06673	The coefficient is significant

In our case, all coefficients are significant, then the equation takes the following form:

$$Y = 1.20 + 0.185X_1 + 0.091X_2 + 0.111X_3$$

One of the tasks of extraction optimization by the method of mathematical planning of an experiment is a quantitative assessment of the contribution of each of the selected factors to the extraction result. By quantitative contribution, the factors are arranged in the following order:  $X_1 > X_3 > X_2$

A steep ascent was not carried out, as a yield of 1.54% was obtained, which is quite acceptable for this extraction process. Thus, the equation adequately describes the quantitative determination of flavonoids amount in the feedstock.

Thus, as a result of a number of studies on optimizing the process of extracting the amount of flavonoids from *Aerva lanata* herb, the region of the supposed optimum was reached.

By statistical analysis of the data obtained, the following optimal conditions for the process of extracting the amount of flavonoids from raw materials were revealed during its quantitative analysis: alcohol concentration – 50%; hydromodule – 1:60; extraction time – 30 min. The following linear regression equation for the extraction process was obtained:

$$Y = 1.20 + 0.185X_1 + 0.091X_2 + 0.111X_3$$

## Conclusion

As a result of the research, in order to highlight the maximum extraction of the amount of flavonoids from *Aerva lanata* herb, the method of planning the Box-Wilson experiment was chosen. This method made it possible to reveal the influence of several factors, in particular, the concentration of ethyl alcohol, the ratio of raw materials to the extractant, and the extraction time on the process under study.

By statistical analysis of the data obtained, the following optimal conditions for the process of extracting the amount of flavonoids from raw materials were revealed during its quantitative analysis: alcohol concentration – 50%; hydromodule – 1:60; extraction time – 30 min. A linear regression equation for the extraction process is obtained in the form:

$$Y = 1.20 + 0.185X_1 + 0.091X_2 + 0.111X_3$$

The adequacy of the obtained mathematical model is proved.

It should be noted that the proposed optimization method made it possible to maximize the amount of flavonoids in the amount of 1.52% by weight of the raw material.

Experimental and theoretical data from studies on the extraction of plant compounds will subsequently provide theoretical models that will make it possible to optimize the extraction of compounds of natural origin, taking into account the quantitative influence of each factor.

## Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

## Conflict of interest

None declared.

## Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

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