Biomathematical approach determination of a rational formula of complex phytocompositions

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Abstract. Despite the huge achievements of pharmaceutical chemistry, herbal medicines based on medicinal plant raw materials (MPRMs) are still in demand for the treatment of both humans and animals diseases. Their main advantage is the small number of contraindications and side effects. In this case, not one but several phytocompositions are often used, which makes it possible to enhance the effectiveness of the drugs and expand the spectrum of action. The key point in creating multicomponent (galenic) preparations is the development of an accurate phytocomposition formula that best meets the set goals. This process is quite complex and time-consuming. The purpose of this study was to determine the rational formula of complex phytocompositions based on a biomathematical approach. To reduce the number of performed experiments compared to blind enumeration and increase the reliability and efficiency of the quantitative formula of the phytocomposition, we tested a combination of the approach used in mathematics to search for the extremum of functions of many variables with a biological experiment on *Parametium caudatum*. In this case, the variables were the shares of each MPRM in the final recipe; function was understood as the pharmacological activity of a substance, determined through its membrane-stabilizing and antioxidant effect. This made it possible to determine the effective ratio of phytocomposition components faster and at lower costs. A comparison was made among more than 100 model samples. It has been established that all of them are non-toxic and have a good antioxidant and membrane-stabilizing effect. The final combination is by 10-20% more effective than other model samples and therefore it is advisable to use it in the future as the main ingredient for obtaining liposomal preparations for external use with a good pharmacological effect.

1 Introduction

The basis of the modern arsenal of medicines is the achievements of pharmaceutical chemistry. Preparations based on medicinal plant raw materials (MPRMs) are still in demand in the pharmacotherapy of chronic and indolent diseases, both in humans and

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animals. They account for up to 25% of all registered medicines [1]. Herbal medicines (HMs) have such advantages as a wide spectrum of action, low toxicity and allergenicity, availability of raw materials in conditions of sanctions in comparison with synthetic drugs, but at the same time, they have a number of disadvantages, such as difficulty in standardization, insufficient evidence of the level of clinical effectiveness, long course of use.

The preferred use of a wide range of herbal medicines is associated with an affordable price (especially for elderly patients), a small number of allergic reactions and side effects.

Among all HMs, extracted preparations (extracts, tinctures, oils) occupy second place (5.27%) after combined ones (8.54%) [2]. It should also be noted that in addition to this, extracts or biologically active substances (BAS) from MPRMs are widely used in cosmetics (primarily cosmeceuticals) and dietary supplements [3].

In this case, not one, but several plant (medicinal preparations), i.e. galenic preparations, most of which are obtained by extracting biologically active substances from plant and animal raw materials with water, alcohol, ether or mixtures thereof, are often used.

The use of not one, but several plants at once creates a synergistic effect, enhances the therapeutic effect and expands the scope of application due to the complex effect of the biologically active substances present in them. For example, violet and hypericum are added to the disinfectants. Violet contains phenols and has a disinfectant effect like carbolic acid, while hypericum contains substances that act as antibiotics. Different mechanisms of action mutually reinforce each other [4].

The key point in creating herbal preparations is to determine their optimal and rational formula that meets the goals set. However, determining the quantitative and qualitative recipe (phytocomposition) is a rather non-trivial and labor-intensive task [5].

This task is usually solved by an analysis of literature and folk recipes, as well as on the basis of researcher's experience. Unfortunately, this approach is not always possible. Recipes are often lost or contradict each other. In the literature, we talk more about the nomenclature of the plants used in the preparation and much less about their exact ratio. Meanwhile, the quantitative ratio is a necessary parameter for industrial production. As a rule, the ratio is selected empirically. To reduce the number of experiments performed compared to blind enumeration and to increase the reliability of the quantitative recipe of the phytocomposition, we proposed a combination of the approach used in mathematics to search for the extremum of functions of many variables with a biological experiment on *Parametium caudatum*. This allows us to determine, if not optimal, but at least a rational recipe more accurately and quickly [6].

2 Materials and Methods

Based on the analysis of literature and recipes of traditional medicine, the recipe of a certain phytocomposition was determined. It is necessary to determine the proportions (shares) of a particular plant in the final formula, ensuring its maximum specified pharmacological effect in the finished product. From a mathematical point of view, this is a typical problem of finding the extremum of a function of many variables, which is usually represented as follows.

Given values: a set X and a function f (x) defined on X. It is required to find the minimum or maximum points.

$$f(x) \rightarrow \min$$
$$(\max) x \in X, \quad (1)$$

where $f(x)$ is objective function;

X is admissible set;

$\forall x \in X$ ia admissible point of the problem.

In our study, we are dealing with a finite dimensional optimization problem; in this case, the admissible set X lies in the Euclidean space R^n ($x \in R^n$).

The point $x^* \in X$, which is a solution to the problem, can be a global or local minimum point.

There are many ways to determine the extremum of functions of many variables. However, what they all have in common is that we calculate the values of the function itself or its derivatives and move in the direction of the extremum, given different values of the variables. In our case, the difficulty is that the type of the objective function is unknown. Therefore, we decided to use a biological test instead of a computational experiment. In this case, the points (variants of recipe) at which experiments are carried out are determined using an algorithm similar to the method of coordinate descent [7]. This approach reduces research time and increases the reliability of the results compared to the random search method.

The variables in our case are the shares of each MPRM in the final recipe. In other words, knowing the qualitative recipe of the phytocomposition, it is necessary to determine the quantitative recipe. We obviously need to understand the required pharmacological effect as a function.

Currently, in vitro tests on materials from cell cultures, subcellular fractions or individual enzymes are widely used to assess the initial pharmacological activity of drugs and active substances [8]. This approach allows us to minimize the number of experiments on animals and reduce the time and cost of preclinical studies.

In many cases the initial pharmacological activity of drugs or substances with a wide range of action is assessed by their membrane stabilizing and (or) antioxidant effect. The presence of a membrane-stabilizing and antioxidant effect can, for example, indirectly characterize the anti-inflammatory and anti-arthritic effects of the drug. As noted above, to determine recipe variants, the algorithm applied in the coordinate descent method is used.

The method of coordinate descent (also called the Gauss-Seidel method) is one of the simplest and corresponds, in our opinion, to the essence of the problem under study at the same time.

The essence of the method is an alternative search for the extremum of the function along one of the coordinates (in our case, this is the amount of a given type of MPRM plant in the phytocomposition). In this case, all other coordinates (ingredients) are fixed at some initial values. Thus, instead of a multidimensional problem, we solve the problem of finding the minimum (maximum) of a one-dimensional function at each stage. In our case, since the total share of all components is equal to 1 (100%), then, by decreasing or increasing one of the variables, the rest are uniformly increased or decreased in proportion to the step length, accordingly (change in the share of the component in the recipe) so that the sum of all shares remains equal to 1.

Since the type of function is unknown to us, we cannot calculate the value of the function and its derivatives, but instead, we used a biological experiment on paramecia to determine the values of the function. This is the main difference of the proposed approach. In other words, the points at which we conduct the experiment are determined using a modified algorithm of the coordinate descent method, while the values of the criteria by which we select the recipe of the phytocomposition are based on a biological experiment.

The algorithm of the method can be presented in the following form.

Input: function $f(x)$

Output: found point of optimum x^*

1. Initialization with some value $x_0 \in R^n$
2. Repeat: for $i = 1 \cdots n$

3. Fix the values of all variables except x_i to obtain one-dimensional function $f(x_i)$.

4. Carry out one-dimensional optimization for the variable x_i using the dichotomy method, so that the sum of all variables remains equal to one.

5. If the criterion for stopping the process of approximate finding of the maximum is met (variants are described below), then return the current value $x = (x_1, \cdots, x_n)$.

Criterion for stopping the process of approximate finding of the maximum.

The criteria for stopping the process of approximate finding of the minimum can be based on various considerations. We used two:

1. $\left\|x^{[k+1]} - x^{[k]}\right\| \leq \varepsilon_1$
2. $\left\|f(x^{[k+1]} - f(x^{[k]})\right\| \leq \varepsilon_2$

Here, $-x^{[k]}$ и $f(x^{[k]},$ are the values of the variable and the objective function, respectively, obtained after the k^{th} optimization step, ε_1 and ε_2 are predetermined positive numbers.

Taking into account the experimental errors and the approximate nature of the calculations, we assume ε_1=0,005, ε_2=0.025, which is sufficient for practical calculations.

The biological activity of model phytocompositions was determined using the culture of *Parametium caudatum* (slipper animalcule). This method has proven itself well in the study of various plant objects. *Paramecium caudatum* is easy to cultivate, and a large amount of digital information can be quickly obtained by studying its growth and reproduction. Paramecia are very convenient objects of research, combining the functions of a cell and a living organism at the same time. The motor activity of paramecium is largely formed due to work of ion channels in the membrane of the cilia, and is a characteristic that reflects the functional state of the cells.

The protective effect (antioxidant membrane stabilizing) of the studied compositions was assessed by the effect on the duration of the active period of paramecium in an environment with the addition of toxic substances. As a toxicant, which breaks down into peroxide radicals and damages mainly the lipid part of the membrane *in vivo*, we used a 1% solution of hydrogen peroxide, and as a toxicant that mainly damages the protein structures of the biomembrane we used a solution of 14% ethyl alcohol.

During the experiment, growth, reproduction, movement pattern and stopping time of ciliates were analyzed. The method of testing on paramecia is described in sufficient detail in various works, for example.

3 Results and Discussion

The experimental results showed that the introduction of 5% of any phytocomposition variant into the original environment is beneficial for paramecia. Although their size under the influence of the compositions (60-80 μm) was slightly smaller than in the control group (70-90 μm), by the 3rd day the number of paramecia exceeds the control by 3 or more times.

However, the main criteria when choosing phytocomposition formula are all their antioxidant and membrane-stabilizing properties, which are determined by the time the paramecium stops in the corresponding toxicant solutions. Let us introduce the following criteria name:

w1 – activity time of paramecium in a solution of 14% ethyl alcohol,

w2 – activity time of paramecium in a solution 1% hydrogen peroxide.

We need to choose the appropriate phytocomposition in order to

$w_{1=}f_1(x_k) \rightarrow max, w_{2=}f_2(x_k) \rightarrow max$

Thus, we have two criteria. If there are several criteria, Pareto optimization is usually used, when the optimal values are the best values, i.e. points that cannot be better selected based on all criteria. Table 1 presents several variants from the entire range of phytocompositions considered.

Table 1. Variants for the composition of phytocompositions

Component name	The ratio of components in the phytocomposition				
	Variant 1 (initial)	Variant 2 (2nd algorithm step)	Variant 3 (3rd algorithm step)	Variant K (k-th algorithm step)	Variant X* (finite)
Chamomile (flowers)	0.075	0.08	0.07	0.02	0.02
Calendula (flowers)	0.075	0.08	0.07	0.025	0.025
Cumin (fruits)	0.075	0.08	0.07	0.015	0.015
Pine (buds)	0.10	0.04	0.16	0.25	0.25
Yarrow (herb)	0.075	0.08	0.07	0.025	0.025
Mint (leaf)	0.075	0.08	0.07	0.08	0.08
Rosehip (fruits)	0.075	0.08	0.07	0.025	0.025
Fennel (fruits)	0.075	0.08	0.07	0.13	0.15
Licorice (root)	0.075	0.08	0.07	0.16	0.12
Wormwood (herb)	0.075	0.08	0.07	0.16	0.18
Thyme (herb)	0.075	0.08	0.07	0.075	0.075
Hypericum (herb)	0.075	0.08	0.07	0.025	0.025
Celandine (herb)	0.075	0.08	0.07	0.01	0.01
13 components	1.00	1.00	1.00	1.0	1.0

The results of determining the protective effect of various variants of phytocompositions in relation to cellular poisons are presented in Table 2.

Table 2. Assessment of the degree of protection of paramecium from the action of toxicants by the time of stopping the movement of ciliates (acute experiment)

Number of the variant	**Paramecium stopping time in 14% ethanol solution, min (w1)**	**Paramecium stopping time in 1% hydrogen peroxide solution, min (w2)**
Control	0.2 ± 0.01	0.09 ± 0.01
Variant 1 (initial)	9.3 ± 0.30	4.9 ± 0.02
Variant 2 (2nd algorithm step)	9.1± 0.30	4.7 ± 0.02
Variant 3 (3rd algorithm step)	9.2± 0.30	5.0± 0.02
Variant K (4rd algorith step)	10.0 ± 0.30	5.3± 0.02
Variant X* (finite)	11.0 ± 0.30	5.9 ± 0.02

From the analysis of the data, it should be concluded that all variant of the phytocomposition significantly lengthen the time of paramecia stopping under the influence of cellular poisons - ethyl alcohol and hydrogen peroxide. The extension of the time of stopping the movement of paramecia under the influence of ethyl alcohol characterizes the membrane-stabilizing activity of the developed phytocomposition, where the selected components, in a qualitative and quantitative ratio, prevent damage to the protein part of the biomembrane. Antioxidant activity was tested by extension the time of paramecium movement under the influence of a solution of hydrogen peroxide, which is associated with the ability of the components of the developed combination to inhibit lipid peroxidation of the membrane.

As an initial point (variant 1), a composition with the same percentage of all components and a slight excess in the number of pine buds was chosen. At the second step of the algorithm, we reduced their content by 6% while simultaneously proportionally increasing the content of the remaining components. The result was worse than the previous one. Therefore, at step 3 we moved in the opposite direction - we increased the number of pine buds by 6% with a uniform proportional decrease in the remaining components. The result was positive. Then, we moved in this direction, if necessary reducing the step length by two times (change in the percentage of the component) until the further change remains insignificant ($0{,}025 \leq \varepsilon 2$) or the step becomes less than the specified value ($0{,}005 \leq \varepsilon 1$). Next, we fix the resulting value and do not change it. We repeat this for all other components.

From the analysis of Table 2, it follows that each subsequent variant is better or worse than the previous one according to both criteria at once, which makes it possible to avoid the procedures of searching for Pareto-optimal solutions or collapsing criteria. This makes it easy to choose a rational option. The final composition extended the time of paramecium stopping by 10-20% compared to other model options, which means that a drug based on it will be more effective.

4 Conclusion

When creating soft forms of medicinal preparations for various purposes based on MPRMs, the correct quantitative and qualitative composition of a complex of active plant components is extremely important. This task is quite complex and time-consuming. To solve this problem, we proposed a technique that combines elements of a modified coordinate descent method for selecting recipe variants (experiment points) and used Paramecium caudatum as biomodels to determine the biopharmaceutical activity of substances based on their membrane-stabilizing and antioxidant effects.

This biomathematical approach makes it possible to increase the reliability and reduce the research time, as well as to obtain phytocompositions with high pharmacological effects. All proposed phytocompositions extend the stopping time of paramecia under the influence of cellular poisons - ethyl alcohol and hydrogen peroxide - by more than 50 times, which indicates the presence of good membrane stabilizing and antioxidant effects.

The final composition extended the stopping time of paramecium by 10-20% compared to other model options, which make it a promising agent for creating various herbal ointments for external use.

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