

Optimization of a Method for Obtaining and Determination of Carotenoids in Tambukan Mud

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Abstract—Therapeutic sulphide silt mud from Tambukan lake has been formed for a long period of time. Balneological value of silt mud from that specific lake is high because of high level of ferrous sulphides and water-soluble salts in it's content, including recognized as therapeutically active bromine and boric acid; high concentration of lipids and carotenoids, which have anti-inflammatory, anti-bacteria, immune stimulating and bio stimulating effects. **Goal:** development of the method for stimulation of the peloid extract obtaining process from the Tambukan mud by an ultrasound-assisted extraction with further determination of biologically active substance using a spectrophotometric method.

The research is based on formerly known and patented methods, offered by Kh.G.Karagulov. The method is reasonable, allows to extract carotenoid in full. The disadvantage of it is the energy-demanding and the usage of an organic solvent.

As the object of the research a deep seated mud sample from the Tambukan lake was taken. After an ultrasound-assisted extraction there was obtained an oily extract from the mud sample. Quantitative content estimation was held out using a spectrophotometric method in visible region of absorption.

The ability to optimize the process of peloid extraction from the Tambukan mud by the ultrasound-assisted extraction has been proven. Offered method for extraction and determination of carotenoid amount in Tambukan mud is less resource- and energy-demanding. Alongside with that it provides best possible carotenoid amount in the outcome.

Effective and less expensive method to obtain peloid from Tambukan mud has been developed. The research results point out that the level of carotenoids in Tambukan silt mud has been reduced resulting in serious reduction of therapeutic effect.

Keywords—*Tambukan lake, silt mud, ultrasound-assisted extraction, carotenoids, spectrophotometry.*

I. INTRODUCTION

Lake Tambukan, located in the South of Russia on the border of Predgorny district of the Stavropol region and

Zolskyi district of the Kabardino-Balkar Republic, is a source of therapeutic sulphide silt mud. A large amount of wastewater saturated with chemical fertilizers flowing from adjacent fields, as well as the proximity of the federal highway, have a negative effect on the animal and plant life of the lake. In order to avoid "depletion" of the flora and fauna of the lake, it is necessary to take measures to preserve the ecological purity of Tambukan lake. It is necessary to create a multilevel automatic information system of environmental control in the region of Caucasian Mineral Waters. The use of such a system will allow for round-the-clock monitoring of the ecological environment with the transfer of data to relevant authorities, to predict the development of certain events to prepare rational management decisions in the resort region, ways to control and improve it.

The experience of using the silt mud refers to the beginning of XX century. Professor A.L. Shinkarenko's work was the most valuable, where she studied in details hydrophobic mud fraction. She offered a product based on the lipophilic fraction of Tambukan therapeutic mud with anti-bacteria, recovering, anti-inflammatory actions named Shinkarenko's extract [1]. However, the production of the drug was not established due to the complex technological scheme for the production and use of flammable solvents (alcoetherial mixture).

Many years of experience of using therapeutic mud to treat mucosal diseases show that microbes contained in mud do not infect an organism and have an inhibiting action on pathogenic flora [2,3]. Black ductile mud from the Tambukan lake is high-mineralized high-sulphide, has great amount of organic substances along with ions of chloride, sodium, potassium, magnesium. The crystalline skeleton consists of fine clay and sand. Tambukan mud is on the first place in Russia according to the ferrous sulfide content [4]. Also mud solution contains biologically active substances, lysozyme, penicillin-like compounds that provide bactericidal activity of mud. It was used successfully while the World War II in hospitals of Caucasian Mineral Waters as mud dressings on wounds.

Therapeutic mud from the bottom of the lake is not homogeneous but consists of several layers. On the top there are silts bedding at width at 10-30 cm. Under that layer there are heavy-mixed dead algae, also called "felt" - from 2-13

cm. Under the felt there is a layer of black mud at width at 30-80 cm, then there is dark-grey mud (30-85 cm). Mud is undelays by steel-grey clay at width of 3.8-4.3 cm. Bottom layer- brown and dark-grey clay of a Maikop age. Silt, black and dark-grey mud are obtained and used for therapeutic procedures [5].

However, the problem of desalination of the Tambukan lake, which for more than 100 years has been a source of therapeutic mud, is an actual theme in the field of medicine and ecology.

Desalination leads to reduction of the mud's therapeutic effect, that is used to treat different chronic diseases of the nervous system, locomotion system, cardiovascular system, digestive system, metabolic diseases.

Lake water desalination considered as an ecological problem first of all because of the fact that in the process of therapeutic mud deposition saving of the balance of brine content is the limiting factor.

Brine from the Tambukan lake has been having consistent ion-salt composition for decades. However, in the last 5-10 years the percentage of salts had reduced from 340 g/dm³ to 24 g/dm³ [6].

This happened because of the usage of therapeutic mud in different drug production, as well as in a variety of health resorts in Caucasian Mineral Waters.

Various researchers suppose that bedded wood lines round the coast are the cause of the lake desalination. It prevents the natural evaporation of the rain wash, which lies on the lake's surface and reducing its mineralisation level. Also, the rain wash consider petrochemistry products in it from the federal highway Caucasus as well as the water wash from the nearest fields containing great amount of agricultural chemicals.

Besides the hydrological and climatic conditions, the human impact on the whole ecosystem of Tambukan has been increased. The proximity of the road with heavy traffic (exhaust gases and oil residues get into the water), unreasonable agricultural and entrepreneurial activity have a polluting effect on the quality of therapeutic mud. All this leads to a decrease in the accumulation of nutrients of a natural resource.

The source of nutrients that sludge mud of the Tambukan Lake is saturated with is blue-green algae, which have been living in the lake for more than 3.5 billion years. The mud formation process associated with the vital activity of macro- and microorganisms inhabiting it leads to the accumulation of various organic substances, which are the products of decay and synthesis. Due to its high thermal capacity, Tambukan mud retains heat for a long time.

For wider use of the mud from the Tambukan lake Karagulov Kh.G. proposed to obtain an extract of tambukan mud. Extraction refers to mechanical methods for the preparation of "non-pharmacopoeial" preparations [7]. The proposed method of two-phase extraction with ethanol and vegetable oil makes it possible to separately extract hydrophilic and lyophilic compounds. This technology makes it possible to obtain biologically active substances with high efficiency in one technological stage of extraction with a high content of complex complexes [8]. The

disadvantage of this method is the energy consumption and use of an organic solvent.

An oil solution of Tambukan therapeutic mud is recommended for prevention measures and complex therapy, as an immunomodulating, antioxidant, anti-inflammatory, stimulating and regenerative agent. The unsaturated fatty acids contained in the preparation are the main flexible material of biological membranes and have an anti-sclerotic effect.

One of the main ingredients produced by blue-green algae, providing the therapeutic effect of Tambukan mud, are carotenoids (the content of β -carotene 25 times more than in carrots) [9]. These are polyenes synthesized in plants and certain microorganisms, used by plants and animals in various physiological processes. These are natural pigments with antioxidant functions that protect against oxidative stress and malignant neoplasms.

II. EXPERIMENTAL

As a material for the research was taken a sample of the profundal mud from the Tambukan lake. The sampling was carried out by the enterprise of KMKR JSC on August 20, 2018.

For an initial assessment of the total carotenoid content or the main carotenoid content, absorption spectrometry and near-infrared reflectance spectroscopy were used [10]. The use of spectrophotometric analysis is based on a linear dependence of the absorption value on the concentration gradient. Deviation from linear dependence occurs under the influence of various factors. Substances can be in ionized and non-ionized form, because many of them have acidic, basic or amphoteric properties. Due to the fact that these forms have a difference in electronic structure, they differ in absorption spectra.

The general properties of carotenoids include their water resistance and good solubility in many organic solvents (chloroform, benzene, hexane, petroleum naphtha, tetrachloride, etc.). Hydroxyl-containing carotenoids are better soluble in alcohols (methanol, ethanol). The solutions of carotenoids in organic solvents during spectrophotometric studies give typical absorption bands mainly visible, and stereoisomers show them also in the ultraviolet. This is one of the most accurate indicators used to identify these substances.

The main advantages of using ultrasound are the speed of the extraction process and an increase in the yield of the target product (increase of the concentration of active components). Ultrasound reduces the operating temperature, which allows to extract the thermolabile compounds.

The mathematical design of the experiment involves studying the influence of a large number of factors, but the influence of most factors has already been studied. To simplify the experiment, constants were determined for some factors:

1. Extractant used in the extraction process. Based on the chemical composition and already available data on the determination of carotenoids in Tambukan mud, sunflower oil was selected, since carotenoids, which are a component of silt mud, are water resistant.

Extraction temperature. Based on a literature review of the chemical composition and already available data on the preparation of Mumiyo extract, a temperature of 20–25 °C was chosen, due to the complex multicomponent composition of Mumiyo to prevent acceleration of chemical reactions.

2. Humidity of tambukan mud. When the water content in the native mud is 60-55%, the oil forms a finely dispersed colloidal solution with water and mud, which does not settle when standing and is not filtered through a paper filter. With a water content of 50-40%, extraction gives a clear, bright yellow oil solution. A decrease in water content of less than 40% leads to a decrease in the product yield due to its oxidation [11].

At the first stage of the experiment, the sludge mass of the Tambukan mud was dried in an oven at a temperature of 60 °C for 5-6 hours to a moisture content of 57%.

The second stage of the research was to obtain an oil extract of Tambukan mud by ultrasonic extraction.

Carotenoids were determined in dried and fresh mud.

We have prepared the compositions:

- A. 0.5 kg of dried Tambukan mud - 1.5 kg of refined sunflower oil;
- B. 0.5 kg of fresh native Tambukan mud - 1.5 kg of refined sunflower oil.

After vigorous mixing, the mixtures were subjected to ultrasonic extraction in max mode (3000 W) for 3 hours. Ultrasound has reduced extraction time without affecting the chemical structure.

The quantitative content of the sum of carotenoids was estimated by the UV spectrophotometric method. An exact sample 1,0 g was dissolved in 25 ml of hexane in a 50 ml volumetric flask, the solution was adjusted to the mark with the same solvent and mixed. After settling, 5 ml of the transparent upper layer was selected for further testing. The optical density of the resulting solution was measured on a PromEcoLab PE-5400V spectrophotometer at a wavelength of 450 nm in a tray with a layer thickness of 1 cm.

Meanwhile, the optical density of a solution of a working standard sample (WSS) of potassium dichromate was measured. An appropriate solvent (hexane) was used as a comparison solution. The optical density of the solution of the working standard sample (WSS) of potassium dichromate is 0.23.

The content of the amount of carotenoids in terms of β -carotene in milligrams (X) was calculated by the formula (1):

$$X = \frac{D_1 \cdot 0.00208 \cdot 50}{D_2 \cdot 5} \cdot 100 \quad (1)$$

Where D_1 - optical density of a testing solution;

D_2 - the optical density of the working standard sample (WSS) of potassium dichromate;

0.00208 - the amount of β -carotene in milligrams in 1 ml of a solution corresponding to the color of a solution of potassium phosphate dichromate; 50 and 5 - dilutions.

The testing samples are oil solutions - light brown oily liquids with a faint specific odor of hydrogen sulfide.

III. RESULTS AND DISCUSSION

According to the above formula, the concentration of carotenoids in the studied samples was calculated (2,3).

Composition A: $A = 0.302$

$$X = \frac{0.302 \cdot 0.00208 \cdot 50}{0.23 \cdot 5} \cdot 100 = 2.7 \text{ mg\%} \quad (2)$$

After the centrifugation process (1000 rpm), *ceteris paribus*, the amount of carotenoids decreased to 1.9 mg%.

Composition B: $A = 0.138$

$$X = \frac{0.138 \cdot 0.00208 \cdot 50}{0.23 \cdot 5} \cdot 100 = 1.25 \text{ mg\%} \quad (3)$$

After 10 days of extraction without exposure of foreign forces in a dark place, the amount of carotenoids increased to 1.36 mg%.

The proposed method for extraction and determination of the amount of carotenoids in Tambukan mud is less resource- and energy-demanding. However, it provides an optimal yield of the sum of carotenoids. So, for example, in the Tambuil oil solution of the Tambukan therapeutic mud, the amount of carotenoids is 3.2 mg% [3]. Fig. 1 schematically shows the percentage of carotenoids in the analyzed samples of Tambukan mud and the previously proposed drug "Tambuil".

The final yield of carotenoids from fresh native Tambukan therapeutic mud is 1.5 times lower than the quantitative content of carotenoids in previously dried mud; centrifugation reduces the final amount of carotenoids; extraction without exposure (insisting in a dark place) for 10 days provides a more complete yield of extractable substances.

In the course of the research, previously known patented methods [12] were reproduced, in contrast to them we used our modified method. Test results indicate a decrease in the amount of carotenoids in the mud due to obvious environmental problems with Tambukan lake. In recent years, desalination of Tambukan lake, a federal highway passing alongside and an increase in the number of drains from fields containing the remains of chemical fertilizers, has a negative effect on the microflora of the lake. Reduced water mineralization leads to a loss of the healing properties of Tambukan mud.

IV. CONCLUSION

Thus, as a result of the research, the possibility of optimizing the technological process of ultrasonic extraction of carotenoids from the mud of Tambukan lake has been shown. Test results indicate a decrease in the amount of carotenoids in the mud due to obvious environmental problems with Tambukan lake.

Carotenoids amount comparison



Fig. 1. Diagram of comparison of the amount of carotenoids in the compositions.

The ability to optimize the process of peloid extraction from the Tambukan mud by the ultrasound-assisted extraction has been proven. Offered method for extraction and determination of carotenoid amount in Tambukan mud is less resource- and energy-demanding.

Alongside with that in provides best possible carotenoid amount in the outcome.

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