Maria Curie-Sklodowska University
Faculty of Chemistry

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SCIENCE AND INDUSTRY
challenges and opportunities

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STUDY OF THE RELEASE OF CHLOROPHYLLS OF THE *LAMIACEAE* FAMILY BY THE SPECTROPHOTOMETRIC METHOD

A. KUSTOVSKA[1], V. PAIENTKO[2], O. PINCHUK[1], Y. BUYAN[1], A. BRYAZUN[1], E. SKWAREK[3], [1]Dragomanov Ukrainian State University, Faculty of Natural Sciences, Department of Biology, 03164 Kyiv, Ukraine, [2]Chuiko Institute of Surface Chemistry, NAS of Ukraine, Department of Amorphous and Structurally Ordered Oxides, Gen. Naumov St. 17, 03164 Kyiv, Ukraine, [3]Maria Curie-Sklodowska University, Faculty of Chemistry, Institute of Chemical Sciences, Department of Radiochemistry and Environmental Chemistry, M. Curie-Sklodowska Sq. 3, 20-031 Lublin, Poland.

Abstract: The study is devoted to the analysis of chlorophyll content in plants of the *Lamiaceae* family, which includes many medicinal plants. Since there is a relationship between the intensity of the colour of the solution under study and the content of the substance in the solution, which is expressed by the Lambert-Beer law, the use of spectrophotometry allows us to accurately determine the quantitative indicators of chlorophyll content in plants. The results of this study may shed more light on the biochemical characteristics of the *Lamiaceae* family and its potential use in the pharmaceutical and cosmetic industries

Introduction: Phytotherapy has come a long way from occultism and primitive empirical observations and guesses of primitive man to the disclosure of certain secrets of the action of the constituent parts of individual plants. Doctors' ignorance of botany, herbal medicine, and their hostile attitude to the use of plants in medical practice have caused much damage to medical science and health care. Medicinal plants have gained great popularity in the 21st century and are attracting increasing attention from scientists in a variety of fields, from cosmetology to cooking. Plant pigments may have health benefits.

The physiological role of chlorophylls in plants is due to their participation in the absorption and transformation of energy, which is used in the synthesis of specific substances that are necessary for plant growth and development [1]. When eating green plants, humans consume a lot of chlorophyll. Its physiological effects on the human body are less well understood than those of carotenoids. However, clinical studies have shown [7- 9] that chlorophyll in the human body promotes the formation of hemoglobin and can be used in medicine as a valuable therapeutic agent that accelerates hematopoiesis and stabilizes the circulatory system. In order to control the absorption of these valuable compounds from food or medicines and dietary supplements or to nourish the skin or hair with cosmetics a combination of yellow clay and selected plants of the *Lamiaceae* family can be offered as a complex system as nutrient carriers. Yellow clay is an effective sorbent, so it is promising for use in cosmetics [3]. Also, one of the important characteristics of yellow clay as a component of cosmetic compositions is its ability to exhibit antibacterial effects against gram-positive and gram-negative bacteria *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* [5].

Clay minerals are widely used in cosmetics and are included in toothpastes, masks and shampoos because they have the ability to catalyse biochemical reactions, including: in contact with the skin, which contributes to the normalization of metabolic processes responsible for cell growth and tissue regeneration. The structure of natural aluminosilicates gives them properties such as dispersion, adsorption capacity, gelation and the possibility of being used as an abrasive material, which creates a wide range of applications in cosmetics. Particularly noteworthy is their use as functional fillers in cosmetic products and carriers of biologically active substances, including vitamins. The release kinetics of SBCs are controlled by the chemical nature of the surface and the porous structure of the carrier. By changing the surface properties and porosity by using aluminosilicate matrices with different structures as carriers, it is possible to control the release of biologically active substances, extending the period of their effective use. In addition to the fact that these carriers enable the transport of biologically active substances, they are biocompatible and bioavailable and do not cause allergic reactions. Natural minerals contain various trace elements that also influence the properties of biologically active substances, forming complex compounds with them. Currently, clays are widely used in the pharmaceutical and cosmetic industries. In the first case, they are used both as auxiliary substances in the production of oral, sublingual, transdermal and other medicinal forms, and as active ingredients in antidiarrheal and anti-inflammatory agents. The pharmacological effect of clays results mainly from their enveloping and absorbing properties. In the second case, clay minerals are part of pastes, masks, shampoos, because they have the ability to catalyse biochemical reactions, including those in contact with the skin, which contributes to the normalization of metabolic processes responsible for cell growth and tissue regeneration.The factors that determine the advantages of using clays as cosmetic products are: colour, pH, viscosity, mineralogical purity, presence of metal ions, electrolyte compatibility, cation exchange capacity and associative minerals.

Immobilization is a set of techniques that lead to partial or complete limitation of movement by binding molecules, substances or biological materials to a carrier. Composites are excellent materials for using this phenomenon in practice. Creating new forms of delivering biologically active substances is an important and urgent problem of modern pharmacology and cosmetology. The use of carriers for biologically active substances allows to obtain dosage forms with improved solubility in biological fluids, controlled entry time into the body, high stability during storage, etc. Clays, natural micro/nanostructured materials can be used as inorganic ingredients. This makes it possible to obtain nanostructured composites based on clay with specific additives, and allows to control such practically important features as morphological, structural, textural, mechanical, abrasive, thermal and other complex materials. Clay can be easily mixed with other micro- or nanostructured materials, including silicas, diatomite's, hydroxyapatite, metal powders, bioactive (e.g. natural) materials used in medical and cosmetic applications, etc. By changing the composition and structure of such composite materials, we can influence the necessary load-bearing capacity and regulate the kinetics of the release of active substances. It should be noted that bound water and hydrogen bonds at the interfacial surfaces of nanostructured solids play an important role that strongly influences the properties of composites and natural bioactive materials. Some synergistic effects on the properties of nanostructured hybrid composites can be expected due to component interference and changes in morphological and other

properties during composite preparation and processing. Virtually all of these effects concern surface layers and interfacial phenomena in nanostructured materials.
The aim of our study was to investigate the release of chlorophylls from the composite mixtures of yellow clay and raw materials of *Lamiaceae* species with the spectrophometric method and the physicochemical properties of composite mixtures of yellow clay and raw materials of plants of the *Lamiaceae* family.

Experimental: White and yellow clay from "Mel-OK", Kyiv, Ukraine were used for experiments (Fig.1).

Fig.1. White clay and yellow clay.

The component of the crystalline phase of yellow clay is kaolinite and α-quartz, it is a composite consisting of $Al_4(OH)_8Si_4O_{10}$, SiO_2, muscovite, illite. Its average hydrodynamic radius is 1.75 mm. The component of the crystalline phase of china clay is kaolinite, it is a composite consisting of $Al_4(OH)_8Si_4O_{10}$, muscovite and illite. Its average hydrodynamic radius is 1.15 mm.
In order to mix all powders thoroughly, but without strong mechanical stress, they were processed in a Chemland knife mill (250W) for 3 min at room temperature (Fig.2).

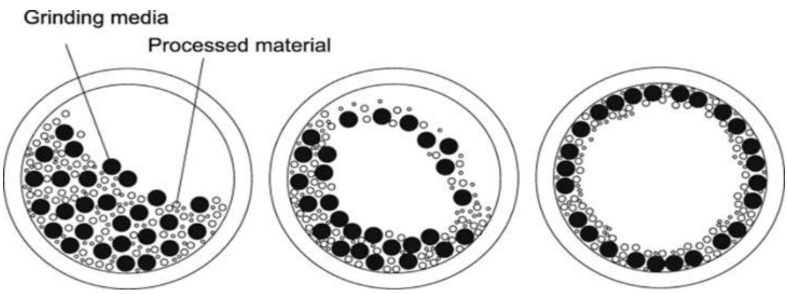

Fig.2. Scheme of mechanochemical activation.

A selection and analysis of literature related to the topic of this study was carried out. The study was carried out using the spectrophotometric method, which is based on measuring the absorption of light at a certain wavelength (monochromatic radiation), which corresponds to the maximum absorption for the substance under study. The optical density was determined at λ=649 nm and λ=665 nm for chlorophyll using a spectrophotometer SF-46 (LOMO, Russia) in cuvettes with a layer thickness of 1 cm. For comparison, a solution containing 96% ethyl alcohol was used.

Results: In general, the highest concentration of released chlorophyll (A+B) was observed for the systems based on *Ocimum basilicum L.* (15.79) and *Thymus vulgaris L.* (14.92), the lowest for the systems based on *Lavandula angustifolia Mill* (2.55) and *Ocimum basilicum L*. (2.77). The concentration of extracted compounds was calculated according to Equations [6]:

$$C_a=13.70\cdot A665-5.76\cdot A649$$

$$C_b=25.80\cdot A649-7.60\cdot A665$$

$$C_{tot}=6.10\cdot A665+20.04\cdot A649$$

where: A649 is the absorbance measured at 649 nm, A665 the absorbance measured at 665 nm, C_a is the concentration of chlorophyll a (mg/g), C_b is the concentration of chlorophyll B (mg/g), C_{tot} is the total chlorophyll concentration (sum of chlorophylls a and B) (mg/g) [6].

The kinetic release may be related to the following factors: the structure of the pigment molecules being released, temperature, pH, or light availability. Another important issue is the choice of the type and proportion of composite phases. Each composite formulation can provide different results in the kinetic release of chlorophylls due to the different structure and stability of the resulting systems (Tables 1 and 2).

Table 1. Results of the study of chlorophyll release in *Lamiaceae* raw materials/yellow clay.

Yellow clay/Plant name	λ 665 nm	λ 649nm	C_a	C_b	C_{tot}
Lavandula angustifolia Mill	0.129	0.088	1.26	1.29	2.55
Melissa officinalis L.	0.507	0.520	3.95	9.56	13.51
Mentha piperita L.	0.527	0.226	5.91	1.82	7.73
Ocimum basilicum L.	0.139	0.096	1.35	1.42	2.77
Ocimum purpura L.	0.770	0.554	7.35	8.44	15.79
Origanum vulgare L.	0.225	0.407	0.73	8.79	9.52
Salvia officinalis L.	0.621	0.491	5.67	7.94	13.61
Thymus vulgaris L.	0.674	0.540	6.12	8.80	14.92

Table 2. Results of the study of chlorophyll release in *Lamiaceae* raw materials/white clay.

Yellow clay/Plant name	λ 665 nm	λ 649 nm	C_a	C_b	C_{tot}
Lavandula angustifolia Mill	0.062	0.037	0.63	0.48	1.11
Melissa officinalis L.	0.091	0.048	0.97	0.54	1.51
Mentha piperita L.	0.39	0.171	4.35	1.44	5.8
Ocimum basilicum L.	0.111	0.053	1.21	0.52	1.73
Ocimum purpura L.	0.257	0.132	2.76	1.45	4.21
Origanum vulgare L.	0.108	0.058	1.14	0.67	1.82
Salvia officinalis L.	0.215	0.097	2.38	0.86	3.25
Thymus vulgaris L.	0.026	0.016	0.26	0.21	0.47

Conclusions: The kinetics of chlorophyll release (chlorophylls a and B and their sum) from white clay/plant and yellow clay/plant composites of different composition was studied and the data on chlorophyll concentration were presented. Different release of compounds may be associated with different composition and degree of dispersion of the composite components. The latest approaches in phytotherapy and biochemistry, particularly, the creation of composite nano-mixtures from plant materials, open many interesting opportunities in the search for effective therapeutic and care products and means, so the obtained composites require a more detailed study and are promising for use in cosmetology.

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