The Application Perspectives of Lake Clay Infused with Natural Active Agents - Essential Oils in Cosmetology and Dermatology

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Abstract. **The aim of the research is to experimentally test and scientifically substantiate the mixture of lake clay and a natural active agent – essential oil, in perspective of its application in cosmetology and dermatology. Sensory properties of lake clay and mixtures of lake clay with essential oils were tested. The presence and quantity of *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Escherichia coli*, and aerobic mesophilic microorganisms in lake clay and lake clay with essential oils mixtures were determined. Thyme (*Thymus vulgaris*), apple mint (*Mentha suaveolans*), and caraway (*Carum carvi*) essential oils, which not only improve the cosmetic value of the product but also act as antimicrobial agents and preservatives, showed the best results. They meet the requirements of Latvian Cabinet Regulation No. 354 "Procedure for Meeting the Significant Requirements for Cosmetics" and European Standard EN ISO 17516: 2014, Cosmetics - Microbiology - Microbiological limits.**

Keywords: lake clay, essential oil, microbiological quality

I. INTRODUCTION

Currently, a healthy lifestyle is gaining popularity and consumers demand for natural products - including cosmetics, is soaring. The use of clay minerals, especially in biomedical applications, is known from ancient times and they are regaining attention in recent years [1]. Clay has effectively proven itself in both cosmetology and dermatology. Clays have cleansing, moisturizing, soothing, regenerative, anti-inflammatory, sedative, anti-septic, and detoxifying effects, they rejuvenate, tone, and nourish the skin [2] - [5]. Clays can eliminate excess grease and toxins from the skin, and hence are said to be effective for dermatological diseases such as furunculosis and management of ulcers [6]. They are used to treat various cutaneous conditions such as seborrheic dermatitis, psoriasis, chronic eczema, and acne [7]. An overview of the use of clay in skin and hair care cosmetics has been published, presenting a general introduction regarding these minerals and its wide-ranging application potential in the biomedical field, which could be useful for formulating novel solid shampoo formulas [8]. Most of the clays in Latvia are brown, they can be applied as pigment in sunscreens or tonal creams. At the same time, the addition of the clay fraction increases the sun protection factor (SPF) of the product, thereby decreasing the necessary amount of synthetic UV filters to obtain a certain SPF value [9]. Latvia is one of the European countries that have the richest clay deposits per resident [10]. Latvia is rich in waters; its territory contains 2256 lakes with the water surface area over 1 ha and the total area about 1001 km2, which is 1.5% of the territory of Latvia [11]. Latvian lakes are rich in clay deposits, which is a valuable, but little studied natural resource. Lake clay effect can be different from lithospheric clay considering that lake clay has formed in different environment and is covered by sapropel layer which medical effectivity has been proven. Considering lake clay chemical parameters, granulometric content, specific surface area and adsorption capacity, lake clay is suitable for cosmetic and dermatological application [12], [13]. However, the presence of some microorganisms in the clay presents the risk of infection, spoilage of the product during storage. Therefore, in this study, control of microbiological contamination of clay and clay essential oils was carried out.

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Up to 73.3% of individuals have been affected by *Acne vulgaris* by the age of 20, furthermore, up to 12% of women and 3% of men have active acne in the age group of 25-58 years [14], [15]. Acne is associated with decreased quality of life and can sometimes lead to depression and even suicidal thoughts; additionally, inflammatory lesions tend to resolve with scarring. Therefore, prompt and effective treatment is of outermost importance [16], [17]. Clay promotes the absorption of active substances in the deeper layers of the epidermis [18]. The positive effect of the use of certain essential oils on the skin and their successful use in cosmetology and dermatology have been proven, which means that adding essential oils to clay could improve the effectiveness of the clay and the Clay - essential oil mixture could be an effective treatment aid in cosmetology and dermatology.

Study aim - to experimentally test and scientifically substantiate the perspectives of a mixture of local lake clay and essential oils for safe use in cosmetology and dermatology.

II. Materials and Methods

Z/S "Rūķīšu tēja" is a Latvian company that cultivates medicinal plants and produces essential oils. The experiments utilized 8 essential oils from Z/S "Rūķīšu tēja": thyme (*Thymus vulgaris*), caraway (*Carum carvi*), apple mint (*Mentha suaveolens*), sage (*Salvia officinalis*), lavender (*Lavandula angustifolia*), wormwood (*Artemisia absinthium*), German chamomile (*Matricaria recutita*), peppermint (*Mentha piperita*), and lake clay obtained from Lake Zeiļu at a depth of 5-6 meters.

Microbiological analysis and sensory properties – aroma, color, and consistency were determined on the 5th day and after 4 months of mixing clay and essential oils.

Sample preparation: The experiments utilized a 0.3% mixture of essential oil and clay. To prepare it, 100 grams of clay were weighed in a sterile container, 0.3 grams of oil were added, and mixed with a mixer for 5 minutes. In Regulation No. 354 of July 2, 2013, of the Cabinet of Ministers, the microbiological cleanliness criteria for cosmetics are specified, including the quantity of aerobic mesophilic microorganisms (mesophilic bacteria, yeast, and mold), *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Escherichia coli*. Therefore, samples of lake clay and clay with essential oil mixtures were tested for the presence and quantity of *C. albicans, P. aeruginosa, S. aureus,* aerobic mesophilic microorganisms.

Sample preparation for the microbiological testing: 10 g of sample + 90 mL of 2% Tryptone water + LP80, mix the sample in a stomacher for 30 seconds, let it rest for 10 minutes, prepare dilutions of 1:100, 1:1000, 1:10 000, which are then plated on 3 parallel Petri dishes.

Determination of mesophilic aerobic microorganisms:

1 mL from each dilution is spread on Tryptone soya broth (TSB) agar in a volume of 12–15 mL, at a temperature of 45°C. After pouring the agar, the sample is gently mixed in the container.
Incubation at 32.5°C ± 2.5°C for 72 hours ± 6 hours.

Determination of *Staphylococcus aureus*:
1 mL from each dilution is spread on Petri dishes with Baird Parker agar, leveled with a spatula.
Incubation at 32.5°C ± 2.5°C for 48 hours.

Determination of *Pseudomonas aeruginosa*:
1 mL of the sample is spread on Nutrient agar.
Incubation at 32.5°C ± 2.5°C for 24 hours.

Determination of *Candida albicans*:
1 mL of the sample is spread on Sabouraud dextrose chloramphenicol agar.
Incubation at 32.5°C ± 2.5°C for 48 hours.

Determination of *Escherichia coli*:
1 mL of the sample is spread on MacConkey agar.
Incubation at 32.5°C ± 2.5°C for 24 hours.
After incubation, colonies are counted on Petri dishes.
The number of microorganisms per 1 mL of the sample is calculated using the formula:

$$X = a \times b / c \quad (1)$$

where a - number of colonies grown on Petri dishes; b - dilution factor; c - number of Petri dishes plated; X - number of colony-forming units per 1 mL of the sample (CFU/1mL).

III. Results and Discussion

A. Sensory properties of lake clay and mixtures of lake clay with essential oils

The sensory properties of clay and clay with essential oils are summarized in Table 1. The smell of clay samples is characteristic of clay, with a consistency that is plastic, soft, and smooth, and a color that is greenish gray. When oils were added to the clay, the color of the mixture remained unchanged, except for the clay-chamomile mixture, where the chamomile oil has a bluish hue, resulting in a bluish gray color of the mixture. The criteria for aroma of the mixtures were categorized as - unnoticeable, vague, strong, very strong. For all samples, the smell remained unchanged and pleasant even after 4 months of storage (see Table 1).

B. Microbiological properties of lake clay and mixtures of lake clay with essential oils

None of the samples were found to contain *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans*, or *Escherichia coli*. The quantity of mesophilic aerobic microorganisms (bacteria, yeasts, and molds) in the lake clay is $\leq 1x10^3$ CFU per 1 mL. Therefore, based on the conducted microbiological analysis, the lake clay meets the requirements of Latvian Cabinet Regulation No. 354 “Procedure for Meeting the Significant Requirements for Cosmetics” and European Standard EN ISO 17516: 2014, Cosmetics - Microbiology - Microbiological limits.

Thyme (*T. vulgaris*) essential oil, which not only improved the cosmetic value of the product but also acted as antimicrobial agent and preservative, showed the best results.

In the clay without additives, the quantity of mesophilic aerobic microorganisms was 7.1 x 10^2 CFU/1mL, but in the clay and thyme oil mixture on the 5th day, the number of microorganisms decreased to 7.4 x 10 CFU/1mL. After 4 months, the number of microorganisms in the clay sample increased to 9.0 x 10^2 CFU/1mL, while in the clay-thyme oil mixture, it increased to 1.1 x 10^2 CFU/1mL (see Table 2). Thus, the thyme oil possesses antimicrobial properties.

T. vulgaris essential oil prevents premature hair loss and dandruff [19] - [21]. Due to its antiseptic properties, thyme essential oil prevents wound infections. The oil improves acne and scar healing; it can be used to treat eczema, dermatitis. Thymol, the most prevalent terpene in thyme oil, has antimicrobial properties [22]. One study found that thyme oil was effective in treating bacterial infections, especially caused by *Staphylococcus spp.* and treatment-resistant strains like methicillin-resistant *S. aureus*. Thyme essential oil has also shown to be effective against bacterial strains *Enterococcus* and *Escherichia* [23]. Thyme oil has strong antibacterial activity against *Propionibacterium acnes* [24]. Along with action against bacteria, thyme essential oil also has anti-fungal properties which might be effective against *Candida albicans,* a type of yeast commonly found in and on the body [25].

Clay masks have been historically used as auxiliary treatment measures for acne [26]. Acne is a chronic inflammatory condition that involves the formation of non-inflammatory lesions known as comedones. Comedones are formed due to the hyperkeratosis of the follicular unit that causes retention of sebum produced by the sebaceous gland [27]. Overgrowth of the commensal Gram - positive bacteria *Propionbacterium acnes* and rupture of the follicular wall promotes inflammation that clinically manifests as inflammatory lesions such as papules and pustules [28]. Local tissue and systemic oxidative stress have also been demonstrated in acne patients [16]. Acne prone skin has impaired epidermal barrier function that is associated with increased TEWL, furthermore, common medication used to treat acne, including benzoyl peroxide and retinoids, can cause skin irritation and even damage the skin barrier [29] – [31]. Administration of appropriate skin care is crucial to improve the epidermal barrier [32]. In long–term tests lake clay applications showed a statistically significant impact on skin elasticity and hydration with no significant changes in skin pH. Even though statistically insignificant, TEWL had a tendency to decrease [33]. Clay has been reported to remove excessive sebum and hyperkeratosis from the acne-prone skin, thus impeding the formation of comedones [26]. Zhang et al. [34] study demonstrated the clay mask's efficacy in managing acne and oily skin, improving hydration and texture. Therefore, the combination of clay and thyme oil could be an effective aid in acne treatment.

The mesophilic aerobic microorganism count also decreased in the clay mixture with apple mint and caraway essential oil (see Table 2). Apple mint (*Mentha suaveolens*) essential oil possesses cell regenerative, antioxidant, antiseptic, and insecticidal properties. The oil's anti-aging activity has been demonstrated [35] – [36]. Therefore, this oil and clay mixture could also be an effective aid in acne treatment.

In clay mixtures with oils such as chamomile, lavender, peppermint, and thyme, the count of mesophilic aerobic microorganisms increased on the 5th day already and exceeded the requirements of Latvian Cabinet Regulation No. 354 after 4 months (see Table 2)

TABLE 1 SENSORY PROPERTIES OF LAKE CLAY AND MIXTURES OF LAKE CLAY WITH ESSENTIAL OILS

<table>
<tr><th>Parameter</th><th colspan="2">Smell</th><th colspan="2">Consistency</th><th colspan="2">Color</th></tr>
<tr><th>Time</th><th>1*</th><th>2*</th><th>1</th><th>2</th><th>1</th><th>2</th></tr>
<tr><td>Clay</td><td>Unnoticeable</td><td>Unnoticeable</td><td>Applies easily on the skin</td><td>Applies not so well on the skin</td><td colspan="2" rowspan="8">Greenish gray</td></tr>
<tr><td>Clay + T. vulgaris</td><td>Strong</td><td>Strong, pleasant</td><td colspan="2" rowspan="8">Slightly softer, applies easier on the skin</td></tr>
<tr><td>Clay + C. carvi</td><td>Very strong</td><td rowspan="7">Vague, pleasant</td></tr>
<tr><td>Clay + M. suaveolen</td><td>Vague</td></tr>
<tr><td>Clay + S. officinalis</td><td rowspan="6">Strong</td></tr>
<tr><td>Clay + L. angustifolia</td></tr>
<tr><td>Clay + A. absinthium</td></tr>
<tr><td>Clay + M. piperita</td></tr>
<tr><td>Clay + M. recutita</td><td colspan="2">Bluish gray</td></tr>
</table>

1* - 5th day after mixing
2* - 4 months after mixing

TABLE 2. MICROBIOLOGICAL PROPERTIES OF LAKE CLAY AND MIXTURES OF LAKE CLAY WITH ESSENTIAL OILS

<table>
<tr><th>Types of microorganisms</th><th colspan="2">Stafilococcus aureus</th><th colspan="2">Pseudomona aeruginosa</th><th colspan="2">Candida albicans</th><th colspan="2">Escherichia coli</th><th colspan="2">Total Aerobic Mesophilic Microorganisms (AMC)</th></tr>
<tr><th>Measurement unit</th><th colspan="8">CFU/1 mL</th></tr>
<tr><td>Time</td><td>1*</td><td>2*</td><td>1</td><td>2</td><td>1</td><td>2</td><td>1</td><td>2</td><td>1</td><td>2</td></tr>
<tr><td>Clay</td><td colspan="8">Not detected; < 10</td><td>7.1 x 10^2</td><td>9.0 x 10^2</td></tr>
<tr><td>Clay + T. vulgaris</td><td colspan="8">Not detected; < 10</td><td>7.4 x 10</td><td>1.1 x 10^2</td></tr>
<tr><td>Clay + C. carvi</td><td colspan="8">Not detected; < 10</td><td>6.9 x 10</td><td>6.1 x 10^2</td></tr>
<tr><td>Clay + M. suaveolen</td><td colspan="8">Not detected; < 10</td><td>1.1 x 10^2</td><td>8.0 x 10^2</td></tr>
<tr><td>Clay + S. officinalis</td><td colspan="8">Not detected; < 10</td><td>1.2 x 10^2</td><td>1.0 x 10^4</td></tr>
</table>

Clay + *L. angustifolia*	Not detected; < 10	3.1×10^3	4.7×10^3
Clay + *A. absinthium*	Not detected; < 10	3.4×10^3	6.7×10^5
Clay + *M. recutita*	Not detected; < 10	3.5×10^3	1.1×10^4
Clay + *M. piperita*	Not detected; < 10	2.3×10^5	2.8×10^5

1* - 5th day after mixing
2* - 4 months after mixing

Cellulite is a condition that affects 85-95% of post-pubertal females and manifests as skin dimpling in specific body areas such as pelvis, thighs, and abdomen. The skin in these areas can sometimes resemble an orange peel. There is a gender predilection and men usually do not develop cellulite. Diet rich in carbohydrates, sedentary lifestyle, pregnancy and increased body mass index usually facilitate development of the condition. Cellulite forms due to protrusions of subcutaneous fat tissue between fibrous septae. Subcutaneous tissue in women prone to cellulite differs from men and women without cellulite in a structural level. Women with cellulite have a higher number of thinner perpendicularly oriented hypodermal septae, whereas in men there is a higher number of fibrous septae organized in a criss-cross pattern [37]. At the moment there is no single method for the treatment of cellulite that is completely effective. Therefore, a combination of topical therapies and procedures such as massages, laser therapy, ultrasound, and radiofrequency are frequently administered due to the possible synergistic effect of several methods. The goal of these procedures is not only to decrease the amount of subcutaneous fat tissue, but also to tighten the skin and enhance the fibrous septae in the subcutaneous layer and in the reticular dermis [38]. To our knowledge topical clay applications have not been widely studied as an auxiliary for the treatment of cellulitis. However, clay has shown promotion of collagen synthesis in vivo and even improves wound healing [39], [40].

CONCLUSIONS

The mixtures of lake clay with essential oils of thyme (*T. vulgaris*), apple mint (*M. suaveolans*), and caraway (*C. carvi*) showed the best compatibility, enhancing the cosmetic value of the product and acting as antimicrobial agents and preservatives.

According to the performed microbiological analysis, lake clay and clay-thyme *(T.vulgaris)*, clay- apple mint (*M. suaveolans*) and clay-caraway (*C. carvi*) essential oil mixtures meet the requirements of Latvian Cabinet Regulation No. 354 "Procedure for Meeting the Significant Requirements for Cosmetics" and European Standard EN ISO 17516: 2014, Cosmetics - Microbiology - Microbiological limits.

According to the performed microbiological analysis and literature data, the lake clay – thyme *(T.vulgaris)* essential oil mixture has a high potential as an acne vulgaris treatment aid.

Chamomile, lavender, peppermint, wormwood essential oil mixtures with lake clay exceed the requirements of Latvian Cabinet Regulation No. 354 and European Standard EN ISO 17516: 2014, Cosmetics - Microbiology - Microbiological limits, therefore these oils are not suitable for combination with clay.

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