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FORMULATION AND MICROBIOLOGICAL EVALUATION OF A COSMETIC CREAM WITH SEA BUCKTHORN EXTRACT AND WITHOUT PRESERVATIVES

BY

CLAUDIA COBZARU[1], OANA-NICOLETA ŞCHIOPU[1], MĂLINA-MARIA CERNĂTESCU[2], NICOLAE APOSTOLESCU [1] and CORINA CERNĂTESCU[1,*]

[1]“Gheorghe Asachi” Technical University of Iaşi, “Cristofor Simionescu” Faculty of Chemical Engineering and Environmental Protection, Iaşi, Romania
[2] “Grigore T. Popa” University of Medicine and Pharmacy Iași, Romania

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Abstract. The study focuses on the formulation and microbiological evaluation of a cosmetic cream with sea buckthorn extract and without preservatives. The microbiological evaluation was carried out by determining the total number of viable microorganisms (aerobic bacteria, yeasts and filamentous fungi) and identifying pathogenic microorganisms such as: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. The results obtained showed that from a microbiological point of view, the product corresponds since after the incubation period no microbial growth was observed.

Keywords: cosmetic cream, sea buckthorn extract, preservatives, microbiological evaluation.

1. Introduction

A number of cosmetic products such as creams, powders, shampoos, lotions, etc., are extremely vulnerable to the multiplication of microorganisms

*Corresponding author; *e-mail*: corina.cernatescu@academic.tuiasi.ro

due to the ingredients used in their preparation that can create an appropriate environment for infestation. Moreover, the appearance of microorganisms in cosmetic products, especially those intended for skin care, poses a very high risk to the health. A cosmetic product that is not microbiologically compliant is distinguished from other products by the changings of its color, odor and viscosity. In general, the vast majority of cosmetic creams are water-based, which is a favorable environment for the development of microorganisms (Lungu and Merica, 2000). Also, cosmetic creams with plant extracts can be exposed to such a risk due to the nature of the plants or/and plant extracts that are incorporated into them.

Thus, the critical parameters that affect the microbiological stability of creams are the nature of the plant or extract, the ratio of raw materials, the method of obtaining and the method of preservation (Mungwari *et al*., 2025). It is worth mentioning that microorganisms develop in conditions of humidity, heat and darkness (Dao *et al*., 2017). However, there are a number of plants or plant extracts that can contribute to the microbiological stability of the creams in which they are introduced. One of these plants is the sea buckthorn (*Hippophae Rhamnoides L*.) which grows spontaneously in different regions of the world, on different kind of soils and various temperatures. Moreover, it is an excellent source of bioactive and nutritional substances and is very often used in the preparation of food supplements or even radioprotective creams for astronauts. Over time, the importance of this plant has grown, so that in recent years, more and more countries have become aware of the therapeutic potential and its processing is carried out at an industrial level (Gupta and Upadhyay, 2011; Ciesarová *et al*., 2020; Kumar *et al*., 2022; Wang *et al*., 2022).

Literature studies show that the valuable bioactive substances present in sea buckthorn, namely: vitamins, carotenoids, phenolic compounds, minerals, amino acids, essential fatty acids and organic acids, are the basis of the numerous properties of the plant such as: antioxidant, anti-inflammatory, emollient, antimicrobial, antibacterial, antiviral, antitumor, cardioprotective, hepatoprotective and tissue regenerator (Ciesarová *et al*., 2020; Criste *et al*., 2020; Zeb, 2004). In practical applications, the plant can be used fresh, dried or in the form of extracts: powder, macerate, infusion, decoction, syrup and oil (Koçyiğit and Haspolat, 2023). Among these, the most well-known and applied extract is sea buckthorn oil with a rich content of vitamins (A, E, C, complex B), minerals (Ca, Mg, Fe) and phytosterols which has a nourishing and regenerating effect on the skin, stimulates collagen restoration and prevents depigmentation caused by aging (Gâtlan and Gutt, 2021; Stanciu *et al*., 2022; Chen *et al*., 2024).

Currently, on the market there are numerous cosmetic products that contain sea buckthorn extracts in their formulation, namely: shampoos and hair conditioning products; aftershave lotions; face and body lotions; mouthwash; moisturizing creams for treating eczema; sunscreen creams and cosmetic products for skin regeneration. Among these, the demand for cosmetic creams

intended for skin care is very high, due to their benefits such as cleansing, moisturizing and protecting the skin against bacterial and fungal infections, as well as its restoration in the case of cuts, burns and wounds. However, all products contain preservatives in order to be usable for longer periods of time.

Based on these aspects, we aimed to formulate and microbiologically evaluate a cosmetic cream with sea buckthorn extract and without preservatives, prepared in the laboratory. We believe that the active substances in the sea buckthorn extracts, one of the basic ingredients, improve the qualities of the product and contribute to its microbiological stability.

2. Experimental

2.1. Material selection

For this study, dried sea buckthorn was purchased from a health food store. In order to obtain the sea buckthorn extract by maceration, extra virgin olive oil was purchased from the supermarket. The beeswax necessary to obtain the cosmetic cream was purchased from a health food store, and the surfactant (sodium dodecyl sulfate) and pH regulator (borax) were purchased from Merck.

2.2. Production of sea buckthorn extract

Sea buckthorn extract was obtained by maceration at room temperature. So, the dried sea buckthorn (previously coarsely chopped) and olive oil were placed in a glass container in a ratio of 1:10 w/w. The container was tightly closed and kept at room temperature for 14 days, periodically shaking it (Fig. 1a). The obtained mixture was filtered, and the resulting sea buckthorn extract was subsequently stored in glass containers with a tight closure, at a temperature of 10-15°C and protected from light (Fig. 1b).

Fig. 1 – Obtaining sea buckthorn extract by maceration in olive oil.

2.3. Formulation of the cosmetic cream using the sea buckthorn extract

The cosmetic cream was obtained according to the recipe described in the literature (Cernătescu, 2016). Thus, the olive oil and beeswax were placed in a laboratory flask, and the mixture was heated on a hotplate, with magnetic stirring to T= 67-68°C. Then, under vigorous stirring, the surfactant and borax dissolved in the amount of water prescribed in the recipe were added. The cream was then gradually cooled to room temperature under continuous stirring. Finally, the obtained cosmetic cream with sea buckthorn extract was transferred to a glass container and kept at room temperature for analysis (Fig. 2).

Fig. 2 – The cosmetic cream with sea buckthorn extract.

3. Results and discussions

The evaluation of cream quality, from a microbiological point of view was carried out according to the specialized literature (Bernauer *et al*., 2023; European Pharmacopoeia, 2021a; European Pharmacopoeia, 2021b), pursuing two objectives.

3.1. Determination of the total number of viable microorganisms

For the quantification of total viable microorganisms (aerobic bacteria, yeasts, and filamentous fungi), the procedure described below was followed: Under the laminar flow hood, the sample to be analyzed was weighed and then suspended in a buffer solution containing phosphate NaCl peptone with polysorbate 80, (dilution 1:10). The sample thus prepared was homogenized for 30 min, the pH of the sample being adjusted to 7 with a solution of HCl or NaOH 1M depending on its acidity or basicity. From the 1:10 dilution, another 1:100 dilution was prepared, using the same buffer solution. Petri dishes with a diameter of 9 cm were used in which 1 mL of sample from the 2 dilutions was brought. Two Petri dishes were used for each dilution. In 2 boxes, approx. 15-20 mL of

culture medium (agar) liquefied and cooled to 40°C (for bacteria, Fig. 3), and in the other 2, approx. 15-20 mL of culture medium (Sabouraud agar) liquefied and cooled to 40°C (for yeasts and fungi, Fig. 4). The Petri dishes with agar were incubated at 30-35°C for 3-5 days, while those with Sabouraud agar were incubated at 20-25°C for 5-7 days.

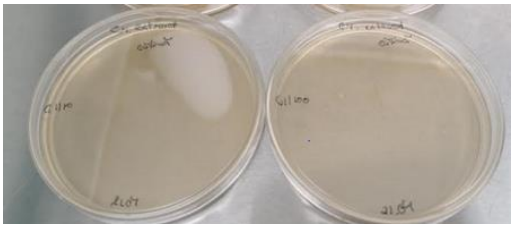

Fig. 3 – Petri dishes with agar for bacteria.

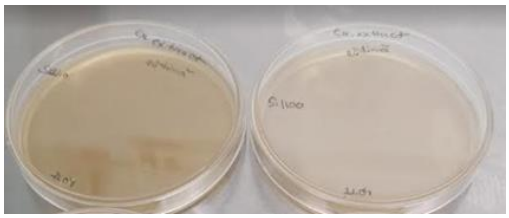

Fig. 4 – Petri dishes with Sabouraud agar for yeasts and fungi.

At the end of the incubation time for the analyzed sample, no colonies of bacteria and yeasts/fungi were detected on the culture media studied.

3.2. Identification of pathogenic microorganisms

3.2.1. Identification of *Escherichia coli*

1 mL of the 1:10 dilution of the sample taken for work was inoculated with 10 mL of nutrient broth in a Petri dish (Fig. 2). This was incubated at 30-35°C, for 18-24 hours.

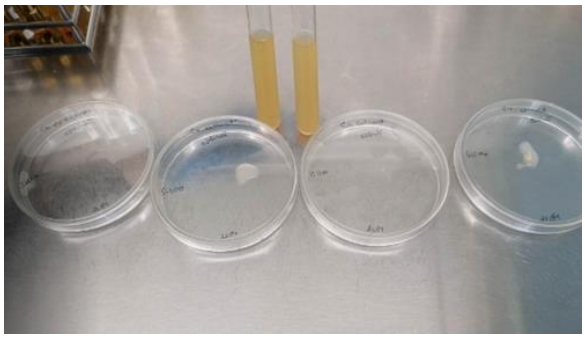

Fig. 5 – Seeding Petri dishes with nutrient broth.

After incubation, 1 mL of broth from the Petri dish was transferred to 100 mL of Mac Conkey bullion culture medium (Fig. 6) and incubated at 42-44ºC, for 24-48 hours. After this interval, a subculture was performed on agarized selective medium: Mac Conkey agar by the sterile swab plating technique and incubated at 30-35°C for 18 – 72 hours.

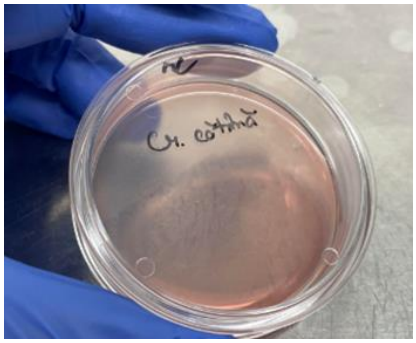

Fig. 6 – Specific culture medium for identification of *Eschericia coli*.

After the incubation period, no microbial growth was observed.

3.2.2. Identification of *Pseudomonas aeruginosa*

1 mL of the 1:10 dilution of the sample taken for work was inoculated with 10 mL of nutrient broth in a Petri dish, and was incubated at 30-35°C, for 18-24 hours. After incubation from the Petri dish with broth, passages were made on agarized selective medium: Cetrimide agar (Fig. 7) by the sterile swab plating technique and incubated at 30-35°C, for 18-72 hours. The negative result confirmed that after the incubation period no microbial growth was observed.

3.2.3. Identification of *Staphylococcus aureus*

1 mL of the 1:10 dilution of the sample taken for work was inoculated with 10 mL of nutrient broth in a Petri dish. This was incubated at 30-35°C, for 18-24 hours. After incubation from the Petri dish with broth, passages were made on agarized selective medium: Mannitol salt agar (Fig. 7) by the sterile swab plating technique and incubated at 30-35°C for 18-72 hours. In this case too, the negative result confirmed that after the incubation period no microbial growth was observed.

3.2.4. Identification of *Candida albicans*

1 mL of the 1:10 dilution of the sample taken for work was inoculated with 10 mL Sabouraud broth in a Petri dish. The Petri dish with Sabouraud broth

was incubated at 30-35°C for 3-5 days. After incubation from the Petri dish with Sabouraud broth, passages were made on agarized medium: Sabouraud agar (Fig. 7) by the sterile swab spreading technique and incubated at 30-35°C for 24-48 hours.

Also, in this case, the negative result confirmed that after the incubation period no microbial growth was observed.

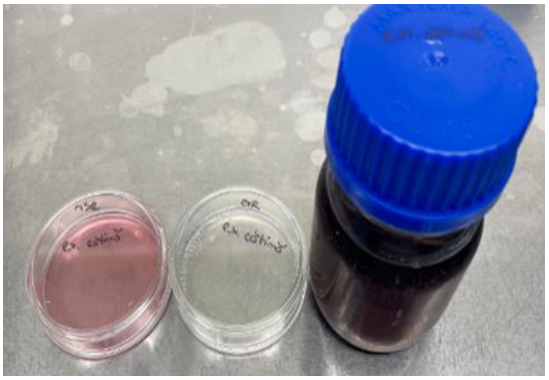

Fig. 7 – Specific culture media for identification of *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans*.

Microbiological tests performed on the samples of sea buckthorn extract cream without preservatives showed that there was no microbial growth (Fig. 8) and therefore it is qualitative from a microbiological point of view.

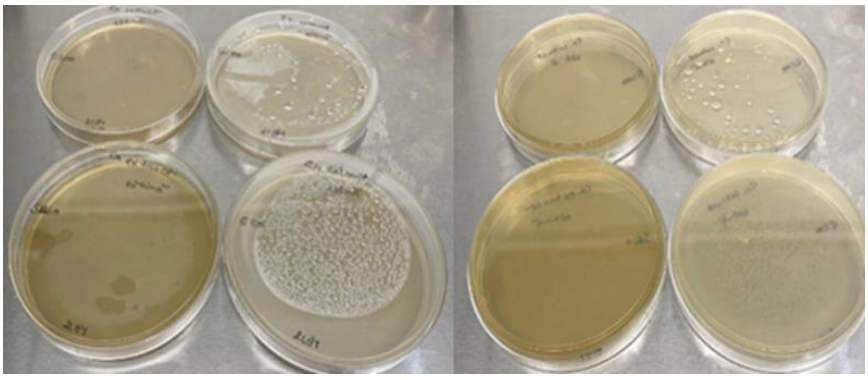

Fig. 8 – Microbiological analysis results for sea buckthorn extract cream.

4. Conclusions

This study consists of the formulation and microbiological evaluation of a cosmetic cream with sea buckthorn extract and without preservatives. The

microbiological evaluation was carried out by determining the total number of viable microorganisms (aerobic bacteria, yeasts and filamentous fungi) and identifying pathogenic microorganisms such as: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. The microbiological tests performed showed that the product complies with the microbiological standard, since after the incubation period no microbial growth was observed.

REFERENCES

Bernauer U., Bodin L., Chaudhry Q., Coenraads P.J., Dusinska M., Ezendam J., Gaffet E., Galli C. L., Panteri E., Rogiers V., Rousselle Ch., Stepnik M., Vanhaecke T., Wijnhoven S., Benfentati E., Cabaton N., Corsini E., Koutsodimou A., Louro H., Uter W., Von Goetz N., Kratke R., *The Scientific Committee on Consumer Safety Notes of Guidance for Testing of Cosmetic Ingredients and Their Safety Evaluation*, 1-167, https://health.ec.europa.eu/publications/sccs-notes-guidance-testing-cosmetic-ingredients-and-their-safety-evaluation-12th-revision_en (2023).

Ciesarová Z., Murkovic M., Cejpek K., Kreps F., Tobolková B., Koplík R., Belajová E., Kukurová K., Daško Ľ., Panovská Z., Revenco D., Burčová Z., *Why is sea buckthorn* (*Hippophae rhamnoides L.*) *so exceptional*? *A review*, Food Research International, **133**, 1-18, 10.1016/j.foodres.2020.109170 (2020).

Cernătescu C., *The technology of cosmetic products. Laboratory Guide (Tehnologia produselor cosmetic. Îndrumar de lucrări practice)*, Pim, Iași, 2016.

Chen Y., He W., Cao H., Wang Z., Liu J., Wang B., Wang C., *Research progress of sea buckthorn (Hippophae rhamnoides L.) in prevention and treatment of cardiovascular disease*, Frontiers in Cardiovascular Medicine, 1-16 https://doi.org/10.3389/fcvm.2024.1477636 (2024)

Criste A., Urcan A.C., Bunea A., Furtuna F.R.P., Olah N.K., Madden R.H., Corcionivoschi N., *Phytochemical Composition and Biological Activity of Berries and Leaves from Four Romanian Sea Buckthorn (Hippophae Rhamnoides L.)*, Molecules, **25**, 1170-1191, https://doi.org/ 10.3390/molecules25051170 (2020).

Dao H., Lakhani P., Police A., Kallakunta V., Ajjarapu S.S., Wu K-W, Ponkshe P., Repka A.M., Narasimha Murthy S., *Microbial Stability of Pharmaceutical and Cosmetic Products*, AAPS PharmSciTech, 249-257, https://doi.org/ 10.1208/s12249-017-0875-1 (2017).

Gâtlan A-M., Gutt G., *Sea Buckthorn in Plant Based Diets. An Analytical Approach of Sea Buckthorn Fruits Composition: Nutritional Value, Applications, and Health Benefits*, Int. J. Environ. Res. Public Health, **18**, 8986-9009, https://doi.org/ 10.3390/ijerph18178986 (2021).

Gupta A., Upadhyay N.K., *Nuts and Seeds in Health and Disease Prevention*, 1011-1019, ISBN 978-0-12-375688-6 (2011).

Kumar A., Kumar P., Sharma A., Sharma D. P., Thakur M., *Scientific insights to existing know-how, breeding, genetics, and biotechnological interventions pave the way for the adoption of high-value underutilized super fruit Sea buckthorn*

(Hippophae rhamnoides L.), South African Journal of Botany, **145**, 348-359, https://doi.org/10.1016/j.sajb.2021.11.045 (2022).

Koçyiğit M., Haspolat Y.K., *Medicinal and Aromatic Plants*, Orient Publications, Çankaya, Ankara, 2023, e-ISBN: 978-625-6598-40-9.

Lungu M., Merica E., *Rheology of cosmetic products*, Corson, Iași, 2000.

Mungwari P.C., King'ondu K.C., Sigauke P., Obadele B.A., *Conventional and modern techniques for bioactive compounds recovery from plants: Review*, Scientific African **27**, 1-23 (2025).

European Pharmacopoeia, *Microbiological examination of non-sterile products: Microbial enumeration tests*, 4765-4769, https://www.ema.europa.eu/ (2021a).

European Pharmacopoeia, Microbiological examination of non-sterile products: Test for specified microorganisms, 4769-4773, https://www.ema.europa.eu/ (2021b).

Stanciu I., Popa E.E., Popa M.E., *The content of bioactive substances in sea buckthorn and the functional potential of its waste*, Journal of Agroalimentary Processes and Technologies **28**(1), 96-103 (2022).

Wang Z., Zhao F., Wei P., Chai X., Hou G., Meng Q., *Phytochemistry, health benefits, and food application of sea buckthorn (Hippophae rhamnoides L.): A comprehensive review*, Front Nutr., **6**(9), 1036295, https://doi.org/10.3389/fnut.2022.1036295 (2022).

Zeb A., *Important Therapeutic Uses of Sea Buckthorn (Hippophae): A Review*, Journal of Biological Science **4**(5), 687-693, 10.3923/jbs.2004.687.693 (2004).

FORMULAREA ȘI EVALUAREA MICROBIOLOGICĂ A CREMEI COSMETICE CU EXTRACT DE CATINĂ ȘI FĂRĂ CONSERVANȚI

(Rezumat)

Studiul constă în formularea și evaluarea din punct de vedere microbiologic a unei creme cosmetice cu extract de cătină și fără conservanți. Evaluarea microbiologică s-a realizat prin stabilirea numărului total de microorganisme viabile (bacterii aerobe, levuri și fungi filamentoși) și identificarea microorganismelor patogene precum: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* și *Candida albicans*.

Rezultatele obținute au arătat că produsul corespunde din punct de vedere microbiologic, deoarece după perioada de incubare nu s-a observat nici o creștere microbiană.