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Research Article

The use of snail slime collected by sustainable methods in cosmetic creams

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ABSTRACT

There are many snail slime (secretion) creams in the cosmetic industry. Many of them are formulated with herbal or synthetic allantoin when the cream ingredients are read. However, natural snail secretion also contains many bioactive components (glycoproteins, fatty acids, polyphenols, vitamins, etc.) other than allantoin. For this reason, cosmetic effectiveness decreases in products that do not contain natural snail secretion. It is not easy to reach the natural snail slime cream due to the limited collection of secretions from the appropriate snail species and limited sustainable methods. While the secretions are collected from snails by traditional methods (exposure to stresses such as salt and electricity application), animals are usually harmed or killed. If the method used for secretion collection is not suitable, animals are exposed to stress and release skin irritants. In addition, the secretion of each snail species does not have the suitable ingredients in the production of cosmetics. In this study, various essential oils and ozone were sprayed with cold steam into the porous chamber containing the snails in a specially designed instrument. Secretion was collected, from live snails (Helix aspersa) suitable for use in cosmetics, without being exposed to stress. Then the cream was formulated with this secretion. Microbiological and physicochemical analyzes were made in the cream produced. As the results; secretion containing important bioactive components was obtained from the appropriate snail species with sustainable methods, and the analysis results showed that the cream formulated with the addition of the secretion was qualified and suitable for use.

INTRODUCTION

Although the skin is defined as the outer surface of the human body, it is also categorized as an organ in the human body. Aging of the skin occurs with processes in which both internal and external factors play a role. Internal skin aging is induced by oxidative cellular metabolism from reactive oxygen species (ROS). External aging is related to air pollution, smoking, etc. Although it is dependent on exposure, it also occurs in the form of photo-aging. While external factors cause the skin to be dry, thin or thick-skinned, it causes wrinkles to be superficial or deep (Puizina-Ivi´c, 2008; Khavkin and Ellis, 2011). The term "nutricosmeceutical" is derived from combining the terms "nutraceutical" and "cosmeceutical". This term is used by the health and cosmetic industry to describe supplements, functional foods and beverages containing active ingredients that can enhance beauty and health (Kligman, 1993). With the improvement of economic conditions in the world and the increase in the elderly population, the nutricosmetic market is expected to show a 5.0% growth rate (CAGR) from 2017 to 2025 and this growth is estimated to be 7.93 billion USD by 2025. The nutricosmetic market is segmented by type of application (skin care, hair care, nail care) and region of sale. As the average age increases in Europe, it is expected that more cosmetics will be sold for the elderly. In the US region, herbal supplements are expected to be sold (Market, 2017).

In natural raw materials, new natural components have gained importance in the cosmetics industry with the 'green movement' that started in Europe at the end of 1960, and plant, marine, mineral, etc. originated and even organic cosmetic raw materials, mixtures and finished products have quickly found their place in the market (Grossman, 2005). The demand for natural cosmetic raw materials has increased the share of cosmetics in the global market. In addition to being a common food consumed by millions of people worldwide, snails are also a source of secondary metabolites used in cosmetics and medicine (Jess, 1998; Murphy, 2001). *Helix* members, an important snail genus in cosmetics, have a wide distribution including the south and east of Europe, Anatolia and the Caucasus, Iran and Jordan as their natural distribution area. The secretions of snails belonging to the genus *Helix* have been widely studied and described (Conte, 2016). Snail slime (*Helix aspersa*) is a mixture of active compounds such as proteins, glycoproteins, glycosaminoglycans, fatty acids, polyphenols, vitamins, glycolic acid, allantoin, minerals (Gugliandolo et al., 2021; Noothuan et al., 2021; Onzo et al., 2021) and carbohydrates (Newar et al., 2015). Because of these properties, snail mucus is used in cosmetics to promote the formation of collagen, elastin and dermal components that repair signs of photo-aging and to reduce damage caused by free radicals. It is stated that snail secretions can be an alternative antibacterial source to expensive synthetic antibacterial agents used in wound treatment (Iguchi et al., 1982; Ehara et al., 2002; Etim et al., 2016). Snails like moist places because they lose a lot of water through mucus secretions. In general, 80% humidity is sufficient for snails (Gökhan, 2003).

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Plant essential oils have been used for many years for different purposes, especially in many scientific and commercial areas. At the beginning of these usage areas are cosmetics, medicine, food industry, aromatherapy and phytotherapy. Essential oils show strong expectorant (accelerating mucus flow), antimicrobial and antiviral effects when used by inhalation in a censer or a container filled with water in a closed environment. Essential oils that increase mucus secretion are seen as primarily medicinal mint and eucalyptus, rosemary, lemon, chamomile, etc. Standard methods of secretion extraction from live snails are very inadequate, both analytically and industrially. The available literature shows that most of the methods tried for secretion extraction resulted in the death of snails (Das et al., 2022). For this reason, it is very important to obtain secretions without harming the snails while they are alive and will be valuable in terms of sustainable production methods and technologies.

In this study, a special instrument was designed that allows to obtain secretions without harming the snails and this instrument was used to collect the snail secretion. The snails (*Helix aspersa maxima*) with the secretion known to have skin regenerative properties and which have been identified were obtained from Burdur Mehmet Akif Ersoy University Snail Farm. The snails taken into the porous chamber of the secretion collection device were exposed to cold steam carrying ozone and essential oils, the secretion was collected and this secretion was used in the production of snail cream. Microbiological and physicochemical (colorimetric, rheological, pH) analyzes were performed on the cream.

MATERIALS and METHODS

Snails, essential oils and ozone

Snails (*Helix aspersa maxima*), identified from the Burdur Mehmet Akif Ersoy University Snail Farm, were obtained. Lavandin essential oil was obtained by distillation from Lavandin (*Lavandula x intermedia*) collected from Burdur Mehmet Akif Ersoy University campus area. 500 g stemless dry Lavandin flowers were weighed. Then the flowers were submitted to hydro distillation with a clevenger-type apparatus. The essential oil was extracted over a period of 120 min. using a clevenger apparatus. (Aytaç, 2020). Peppermint and eucalyptus essential oils and ozone to be applied to snails are commercially available. It was aimed to increase mucus secretion with sprayed ozone, peppermint and eucalyptus oil, and to have an anti-stress effect on snails with lavandin oil with cold steam.

Designing the secretion device and collecting snail secretions

The instrument used in the world to collect snail secretion without harming the living thing and working on an industrial scale were researched and examined, and a special device was designed at laboratory scale and built by a local company. The images of the instrument are given in Figure 1. The system includes an ozone and steam generator and sprays cold steam into the snail chamber at an adjustable flow rate.

The snails provided for secretion are placed in the chamber in the instrument. Each of the essential oils was added to the cold steam generator at a concentration of 0.5 mL/L. The

Figure 1. a. Snail secretion collection device b. Snail chamber c-d. Ozone and cold steam generators and secretion collection chamber

snails were exposed to cold vapor containing ozone and essential oils for 20 minutes (Figure 2). The secretion obtained after the application was filtered through a 0.45 micrometer filter, purified from possible bacterial load and solid particles, and kept at -20°C until the end of cream production and analysis (Das et al., 2022). The snails that were alive and unharmed after the procedure were returned to the Snail Farm (Figure 3-c).

Figure 2. Treatment of snails with cold steam containing ozone and essential oil in a specially designed device

Preparation method of snail slime cream

A new cream formulation containing natural snail slime was made in the R&D laboratories of Burdur Mehmet Akif Ersoy University Cosmetics Application and Research Center (KOZAM), and the analysis/tests were carried out on this product (Figure 3-b). In the cream production process (Figure 3), which

is carried out in accordance with the cosmetics legislation, thickeners, oils, emulsifiers, moisturizers and preservatives are included in the formulation together with snail secretion. The oil phase and water phase of the creams were first mixed and heated up to 80°C, then cooled to 40 °C and mixed for another 15 minutes (Şenses, 2007). Afterwards, it was passed through a homogenizer and the cream took its final shape. The secretion rate added to the cream was determined as 1%.

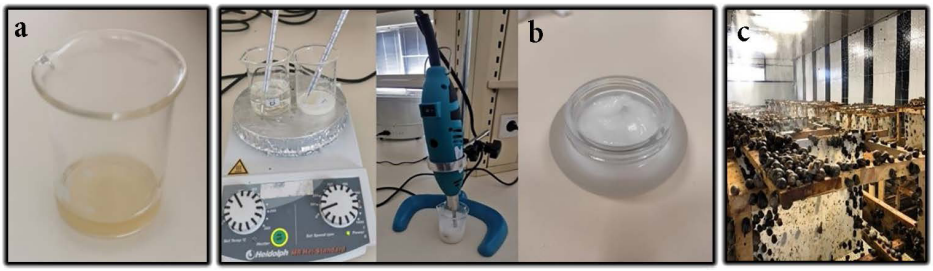

Figure 3. a. Secretion from snails b. Cream making processes (R&D laboratories of KOZAM) c. Burdur Mehmet Akif Ersoy University Snail Farm

Measurements/analysis of the snail slime cream

Rheological, colorimetric and pH measurements of the cream as well as microbiological analyzes were made. Measurements and analyzes were performed in three replications of the cream on "the 1st day (sample 1), the 30th day (sample 2) and the 60th day (sample 3)". The cream was stored in a frosted glass cream jar at room temperature (20°C±2) until the analysis was completed.

Rheology is a measure of the resistance of a fluid to deform under surface tension. It can also be defined as the internal resistance of the fluid to flow. Rheological measurement results are given in Table 1. Brookfield RV/DV-II+Pro Extra Viscometer Device (Brookfield Engineering Laboratories, Inc., Middleboro MA, USA), Spindle No: RV 6 was used for the measurements. Analysis was carried out in a 50 mL falcon tube (plastic tube with a centrifugal cap) at room temperature (Bovey, 1965; Erbil, 2000).

Konika Minolta (Chroma Meter CR-400/410) was used for colorimetric (CIELab) measurements. L*, a* and b* measurements were made by calibrating the color device with a white plate and using the D65/10 light source in 3 repetitions. The whiteness value of the samples was determined by Sarbon et al. (2015)'s method is calculated according to the formula below.

$BI = 100 - [(100 - L^{\square})^{2} + a^{\square 2} + b^{\square 2}]^{1/2}$ (Metin, 2020).

Mettler Toledo brand and S20K model pH meters were used for pH measurements of creams. Measurements were made with a calibrated pH meter. For each formulation, the measurement was repeated 3 times and the average was recorded (Oğuz, 2019).

"Total mesophilic aerobic bacteria count" and "total yeast-mold count" analyzes were performed on the cream sample. In the cream sample, 1/10 dilution was carried out in suspension containing 0.09% NaCl. For total mesophilic aerobic bacteria count, PCA (Plate Count Agar) and for Yeast-Mold count PDA (Patato Dextrose Agar) agar medium, the cream sample diluted with 3 repetitions was inoculated with 1 mL drop and inoculated as a smear (Gök et al., 2019). Incubation was carried out at 35°C for 48 hours for total mesophilic aerobic bacteria count, and for 72 hours at 25°C for yeast-mold counting. Colony count was performed after incubation.

RESULTS

In all parameters, depending on the day the snail slime cream was prepared, three results were given as the 1st day (sample 1), the 30th day (sample 2) and the 60th day (sample 3). The results of all measurements and analyzes studied in the cream formulated with snail secretion are given in Table 1-4.

The rheological results of the prepared snail cream are given in Table 1. Rheological measurements were carried out at different rpm (revolution per minute) values (10-100 rpm). When all results were examined, the first measurement was found 34200-5120 mPa.s, the 30th day as a result of 28100-4000 mPa.s and the 60th day as a result of 27834-2260 mPa.s.

Table 1. The Viscosity Measurements (RPM) of Snail Slime Cream

Sample	10	20	30	40	50	60	70	80	90	100
1	34200± 305	18400± 684	13600± 723	10700± 532	8960± 380	7800± 256	6914± 345	6150± 510	5555± 340	5120± 278
2	28100± 942	12800± 1230	9989± 441	7875± 761	6733± 804	6294± 587	7062± 720	5117± 404	5393± 284	4000± 524
3	27834± 2753	10284± 1411	4100± 295	3500± 349	4980± 180	4544± 323	4550± 959	3339± 330	2576± 33	2260± 187

The results of the colorimetric measurements of the cream are given in Table 2. The measurement results of the three

samples are close to each other, no significant change detected. L values were found very close to 100 in all samples.

Table 2. The Colorimetric Measurements of Snail Slime Cream

Sample	L*	a*	B*
1	85.61	3.37	-4.43
2	84.83	3.39	-4.41
3	84.85	3.35	-4.42

The pH results are given in Table 3. When the results of the cream pH measurements were examined, no significant difference was found in all samples. pH values were measured between 5.43-5.49.

Table 3. The pH Measurements of Snail Slime Cream

Sample	pH
1	5.46
2	5.43
3	5.49

The results of microbial analysis in the cream are given in Table 4. As a result of the analysis, no contamination was detected in 3 samples.

metic manufacturers generally use herbal or synthetic allantoin instead of snail secretion and market it only as a snail slime cream. Natural snail secretion contains important bioactive components, especially allantoin, but if the method used for secretion collection is not suitable, animals experience stress and secrete skin irritants. In addition, not all snail species are suitable for cosmetic use. When the secretion collected by sustainable methods, using snail species suitable for cosmetics, is added to cosmetic formulations, it increases the added value and quality of the product considerably. Creams are one of the most frequently used products among cosmetics. Creams, in the simplest terms, are systems called emulsions, which are formed as a result of the dispersion of two immiscible liquids such as water and oil in one another with the help of a third component, an emulsifier. Apart from these three basic ingredients, in creams; There are lubricants, thickeners or viscosity increasers, auxiliary emulsifiers, moisturizers, preservatives and essences (Şenses, 2007).

In this research, secretion was collected from *Helix aspersa maxima*, which is known to be rich in bioactive components (Gugliandolo et al., 2021; Noothuan et al., 2021; Onzo et al., 2021), with sustainable methods, without harming the living thing, and was used in the production of snail slime cream. Rheological, colorimetric and pH measurements of the produced cream as well as microbiological analyzes were made (Table 1-4).

Table 4. The Antimicrobial Activity of Snail Slime Cream

Sample	Total Mesophilic Aerobic Bacteria Count	Yeast-Mold Count
1	- (Not found)	- (Not found)
2	- (Not found)	- (Not found)
3	- (Not found)	- (Not found)

DISCUSSION

The word cosmetics in the Cosmetics Regulation published by the Ministry of Health in 2005; "*It is prepared to be applied to the outer parts of the human body, the epidermis, nails, hair, lips and external genitalia, or the teeth and oral mucosa, for the sole or main purpose of cleaning these parts, giving them fragrance, changing their appearance, protecting them, keeping them in good condition or it refers to all substances or mixtures of substances with the purpose of correcting their odor*" (Ministry of Health, 2005).

The use of cosmetics is one of the indispensable routines of people of all ages, regardless of gender. This increases the economic growth and mobility that falls on the share of the cosmetics industry (Market, 2017). The demand for cosmetics that include natural raw materials and important bioactive components in its formulation is increasing in the industry. Snail slime cream, serum, etc. cosmetic products are also in high demand in this context. However, reaching the natural snail secretion is not sustainable as it harms the living thing. However, reaching the natural snail secretion is limited because it harms the snails and it is difficult to obtain it continuously and sustainably. For this reason, some of the cos-

Brookfield viscometer has limited sensitivity; The change in the behavior of the Non-Newtonian fluid depending on the temperature change or temperature change creates a constant difference in the viscosity measurement. Measurements should be made with the same spindle at constant temperature and constant rotational speed (Bovey, 1965; Erbil 2000). In the prepared cream; In the measurements made with the same spindle at constant temperature, it was determined that the rheology of the snail cream decreased at a statistically insignificant level from the day it was produced to the 60th day (Table 1). However, it was observed that the cream was spreadable at all three measurement times, including the rheological values after 60 days (Kwak, 2015). It is thought that the decrease in rheology may be due to time-dependent deterioration and segregation under stabilization conditions, and this situation is in parallel with similar literature (Oğuz, 2019). It is anticipated that this situation can be prevented by storing the cream at +4°C, which has already been stored at room temperature.

Whiteness value is an important criterion for industrial applications. In cosmetic products/creams, it is of great importance not to change the color of the formulation and to

obtain the light color that is generally preferred by consumers (Metin, 2020). CIE L*, a*, b* The color system contains three coordinates. These; (L*) brightness (0,black ;100,white), (a*) red to green (+a,red; -a,green), and (b*) yellow to blue (+b,yellow; b,blue) and (ıy*) represents the jaundice index (Snell et al., 2002). Accordingly, L* value ranges from 0 (black) to 100 (white), a* value indicates red-green and b* value indicates yellow-blue scale (Üren, 1999). In the study, the formula given in the method was applied and color measurements depending on the day (1st, 30th and 60th days) were determined. Accordingly, it was calculated as 84.6, 83.8 and 83.9, respectively, and the color is quite white (Table 2). Accordingly, the color change in the cream on a daily basis (1st, 30th and 60th days) is negligible. The whiteness value of the cream meets the expectation in all three measurements.

The pH of the skin is normally acidic and the pH value varies between 4.1-5.8 (Proksch, 2018; Sethi et al., 2016). When the pH values obtained from the prepared cream were examined, no significant change was observed at the end of the 1st, 30th and 60th days, and this shows that this formulation is stable in terms of pH value. All pH measurement results were found between 5.43-5.49 (Table 3). As a result, all creams are compatible with the skin in the desired pH range.

As a last parameter, as a result of microbiological analysis performed on snail slime cream, no microorganism growth was found in the cream in 3 analyzes performed on the 1st, 30th and 60th days (Table 4).

It is thought that this is due to the fact that the snails are processed after they are cleaned with water, the ozone sprayed on the snails and the presence of peppermint-eucalyptus essential oils containing menthol, which are known to have antimicrobial effects (Bocci, 2006; Turcheniuk et al., 2015), the preservative added to the cream formulation does not allow contamination, and the cream formulation is prepared in an aseptic environment.

CONCLUSION

In the research study, snail slime cream was prepared with sustainable methods and without harming the snails, then rheological, colorimetric, pH measurements and microbial analyzes were made in the cream at the end of the 1st, 30th and 60th days. When the results were evaluated, it was seen that the snail slime cream produced was compatible with the skin acidity. Color and spreadability meet user expectations. In addition, it was concluded that the absence of microbial activity in the product for 60 days is important for product safety. Furthermore, due to the snail secretion rich in bioactive components in the cream formulation, it has an important care-repair and renewal feature. It may be an important formulation that can be put on the market after more detailed and long-term studies are done.

DECLARATIONS

Ethics Approval

Since the snails, which are the study material, are invertebrates, the approval of the ethics committee is not required according to the regulation on the working principles and procedures of the animal experiments ethics committees published in the official newspaper dated 15.02.2014 and numbered 28914.

Conflict of Interest

The author declare that they have no conflict of interests.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: ACT

Data collection and analysis: ACT

Drafting of the manuscript: ACT

Critical review: ACT

Data Availability

The data used to prepare this manuscript are available from the corresponding author when requested.

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