

MICROBIOLOGICAL CONTROL OF MOISTURIZING MASK FORMULATION ADDED OF HIBISCUS FLOWERS, ASSAI PALM, BLACK MULBERRY AND PAPA W GLYCOLIC EXTRACTS

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Received: 16 Nov 2012, Revised and Accepted: 11 Dec 2012

ABSTRACT

The microbiological control of moisturizing mask formulation added of hibiscus flowers, assai palm, black mulberry and papaw glycolic extracts, determining the number of viable microorganisms and possible presence of pathogenic. The moisturizing mask formulation was composed of zinc oxide (5.0%) and moisturizing cream constituted of tricetareth-4 phosphate (and) cetyl alcohol (and) stearyl alcohol (and) sodium cetearyl sulfate (and) oleth-10 (qs 50g). To this formulation was added hibiscus flowers glycolic extract (2.5%), assai palm glycolic extract (1.5%), black mulberry glycolic extract (1.5%) and papaw glycolic extract (2.0%). The formulation was stored in aseptically clean recipients, away from humidity and light, in fresh and airy places. The results of the microbiological analysis on the counting of aerobic mesophilic microorganisms (bacteria and fungi), of the above mentioned formulation, revealed a bioburden < 10 CFU/mL in all samples. Such data indicate adequate microbiological quality of the tested products, according to official recommendations. Furthermore, it was not detected the presence of pathogenic microorganisms, assuring the harmlessness of the formulation. The results lead us to conclude that the formulation and raw materials analyzed did not present microbial contamination, evidenced for estimating the number of viable microorganisms (<10 UFC/g) and for researching pathogens.

Keywords: Microbiological control, Moisturizing mask formulation, Glycolic extracts. Aerobic mesophile microorganisms

INTRODUCTION

The control of microbial contamination of cosmetics is of fundamental importance, related to public health as the quality and stability of the product [1,2]. Practically all cosmetic products are subject to contamination with microorganisms. The growth of fungi and bacteria in the products depend of physicochemical factors such as water availability, product composition which provide nutrients for the microorganisms, storage temperature, among others [3]. The growth of microorganisms in cosmetic products may lead to constituents degradation, and increase the chances of risk to the health of the consumer if the contamination by pathogenic microorganisms [4,5].

The low microbial quality of cosmetics may occur mainly due to microbial contamination resulting from poor hygiene in the manufacture and of the low stability of the constituents of the formulation. During manufacturing it is important to prevent microorganisms from invading the product, which is an substrate with excellent substances for their growth, causing modifications apparent or not in the final product, such as color change, odor, viscosity, sensory characteristics, degradation of the formulation components. and could cause toxic reactions to the user, depending on the type of microorganism present, the route of administration used and the condition of the product user [6].

Microbiologists working in the field of cosmetics are frequently required to design preservative systems that provide good protection of cosmetic products against microbial contamination [7]. Cosmetic preservatives are molecules used in pharmaceutical and cosmetic formulations that are effective against both prokaryotic and eukaryotic cells, as, unlike antibiotics, they do not act against a defined target cell [8,9]. Some of the cosmetic preservatives like phenols, paraben and benzoic acids are some of preservatives used in cosmetics, but exhibit toxic effects, such as parabens that accumulate in the body over exposure [10].

The hibiscus flowers, assai palm, black mulberry and papaw glycolic extracts has been added to several cosmetic products for its riche moisturizing action, preventing the ageing of the skin [11-14].

The hibiscus glycolic extract (*Hibiscus rosa-sinensis*) is obtained from the flower of the hibiscus, contains flavinoids and anthocyanins, proteins, alpha-hydroxy acids (malic, oxalic, citric and tartaric acids) and uric acid has antioxidants like vitamins C, E and A (β -carotene) and has an anti-free radicals and moisturizer, being used in some cosmetics such as face creams and tonics [11,14,15].

The assai palm glycolic extract (*Euterpe oleracea*) is obtained from the fruit of açaí originally from the northern South America and contains vitamins C, B1, B2 and B3, carbohydrates, flavonoids (anthocyanins), proteins, 19 kinds of amino acids, lipids and minerals. Because it contains minerals act as remineralizing, and also has moisturizing and emollient properties for skin and hair [12,14,16], besides anthocyanins possess high antioxidant capacity, antiproliferative, anti-inflammatory and cardioprotective with an action confirmed by *in vitro* and *in vivo* assays [17-19].

The black mulberry (*Morus nigra*) has alpha-hydroxy acid such as malic and citric acid, methyl salicylate, tannin, pectin, phenolics, anthocyanosides, sugars, vitamin C and mucilaginous matter. Because it contains AHA has the effect of micro-exfoliation of the stratum corneum, providing a normalization of cell renewal. It also has properties: moisturizing, refreshing, astringent and soothing the skin [12,13,20].

Papaya (*Carica papaya*) is rich in vitamin C, carotenoids (Lycopene, β -cryptoxanthin, and β -carotene) and B complex, besides possessing minerals (Fe, Ca, P), proteins, amino acids and an enzyme called papain. It has properties: moisturizing, softening, remineralizing and renewing cells of the epidermis, primarily through the action of the enzyme papain and vitamins [11,12,21].

The study of stability of cosmetic formulations is important because provides indications about the behavior of the product at any given time interval, compared to the environmental conditions that can be submitted from manufacturing until the end of expiration, helping to guide the development of formulation and packaging material appropriate, providing subsidies for the improvement of formulations; estimate the shelf life and also to assist in monitoring of the organoleptic, physicochemical and microbiological stability, producing information about the reliability and safety of the products [22,23]. The objective of this work was to accomplish the microbiological control of moisturizing mask formulation added of hibiscus flowers, assai palm, black mulberry and papaw glycolic extracts, determining the number of viable microorganisms and possible presence of pathogenic.

METHODS

Preparation of the formulation

A moisturizing mask formulation was composed of zinc oxide (5.0%) and moisturizing cream constituted of tricetareth-4 phosphate

(and) cetyl alcohol (and) stearyl alcohol (and) sodium cetearyl sulfate (and) oleth-10 (qs 50g). To this formulation was added hibiscus flowers glycolic extract (2.5%), assai palm glycolic extract (1.5%), black mulberry glycolic extract (1.5%) and papaw glycolic extract (2.0%).

Microbiological quality control

This analysis provides tests for qualitative and quantitative estimation of mesophile aerobic microorganisms present in the samples. It includes assays for total viable aerobic count and test for the detection of specified microorganisms such as *Salmonella* sp., *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [24,25]. These four species of aerobic microorganisms are relevant for the evaluation of microbial contamination not only in the finished pharmaceutical and cosmetic products, but also in the bulk product. They are representative of the microorganisms which should not exist in these kinds of products [24].

Aliquots of the formulations were added to specific culture environments, thus determining the total number of microorganisms and the presence of *Salmonella* sp. (ATCC 19196), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923). In these analyses, representative samples of the product content were analyzed, in accordance with methods announced on the United States Pharmacopeia [24]. The analyses were made in triplicates for each formulation. The formulations subject to microbiological control were stored in aseptically clean containers, far from humidity and light, in cool and aired places, according to the Brazilian Pharmacopeia [26].

Estimating the number of viable microorganisms

The viable mesophile bacteria and fungi in the formulas were determined by the pour-plate method. One-mL aliquots of 1:10 and 1:100 dilutions of the product in a saline solution were transferred to two series of Petri dishes, in triplicate. The first set was homogenized with 15 mL of soybean-casein digest agar (TSA) and the other with 15 mL of Sabouraud dextrose agar (SDA) (Difco®). Plates were incubated at 32 ± 2.5 °C and 22 ± 2.5 °C for bacteria and fungi, respectively. Finally, the counting of the colonies grown in these ways of culture was performed, then calculating the number of microorganisms per gram of the product, multiplying it by the used dilution, which was expressed in CFU/g [24].

Validation of the method for estimating the number of viable microorganisms

This assay was performed in triplicate using the microorganisms *Salmonella* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*. One mL aliquots of 1:2 and 1:10 dilutions of the formulations in a sterile saline solution, and 0.5 mL of microbial suspension containing less than 100 CFU (colony forming units) were transferred to Petri dishes. Then, the aliquots of these formulations were mixed with 15 mL of TSA. The assay sequence was the same as the one described for determining viable microorganisms. The control assays were also performed in parallel, by transferring 0.5 ml of each microbial suspension to Petri dishes using 15 mL of TSA [27]. Petri dishes were incubated at 32 ± 2.5 °C and 22 ± 2.5 °C for bacteria and fungi, respectively.

Research of *Salmonella* spp. and *Escherichia coli*

Ten grams of sample were transferred to 90 mL of Lactose broth for the research on *Salmonella* sp. and *E. coli*. The culture was incubated at 36 ± 1 °C for 24 hours. After this period, the broth was observed regarding its growth.

Research of *Salmonella* spp.: 1 mL of Lactose broth was transferred to Petri dishes containing tetrathionate broth base and selenite cystine enrichment broth. Plates were incubated at 36 ± 1 °C, for 24 hours. After this period, samples of the tetrathionate broth base were transferred to Petri dishes containing brilliant green agar, xylose lysine desoxycholate agar (XLD) and bismuth sulfite agar. The same procedure was taken for the sample inoculated in the selenite cystine enrichment broth. Plates were incubated at 36 ± 1 °C, for 24

hours. The growth and characteristics of the colonies were observed. The suspicious colonies were sown with a straight handle in Petri dishes containing triple sugar iron agar (TSI) and incubated at 36 ± 1 °C, for 24 hours.

Research of *E. coli*: 1 mL of the Lactose broth was transferred to Petri dishes containing MacConkey agar. Plates were incubated at 36 ± 1 °C, for 24 hours. The growth and characteristics of the colonies were observed. The suspicious colonies were sown with platinum handles in Petri dishes containing Levine EMB agar. Plates were incubated at 36 ± 1 °C, for 24 hours.

Research of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Ten grams of sample were aseptically transferred to 90 mL of tryptic soy broth for the research of *P. aeruginosa* and *S. aureus*. The broth was incubated at 36 ± 1 °C for 24 hours. After this period, the environment was observed regarding its growth. It was sown in Petri dishes containing Vogel Johnson agar for the research of *S. aureus*, and, for the research on *P. aeruginosa*, dishes of cetrimide agar were used. It was incubated at 36 ± 1 °C, for 24 hours. Later on, the growth and characteristics of the colonies were observed.

RESULTS AND DISCUSSION

A cosmetic formulation generally provides important requirements for microbial growth such as water, various minerals and vitamins, besides being an environment with oxygen, pH and temperature favorable [28-30]. Another aspect that makes cosmetics targets for the development of microorganisms is that in their production there are several possibilities for contamination, since the microorganisms inhabit easily the air, water and has great affinity with the raw materials and equipments used in preparatory processes and with packaging, and also during use of the product [9,31,32]. The water contaminated with dissolved salts, gases and microorganisms have the capability to corrode and deteriorate industrial equipment used in the cosmetics preparation contributing to the contamination and possibly for generating skin problems to consumer [33].

The microbiology contamination of cosmetic by use repeated doses is great, since contact with the formulation occurs repeatedly, by inadequate cleaning of hands, incorrect use of the cream, high exposure of air, temperature and humidity of each environment, it contributes to the proliferation of microorganisms [34,35].

The creation of the guide of Good Manufacturing Practices (GMP) was developed considering the preservation of quality, consumer safety ensuring the efficacy of the product [34]. The United States Pharmacopeia proposes limits of microorganisms in products cosmetics. The major contaminants of the formulations are *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp. and *Staphylococcus aureus* [5]. The microbiological control of raw materials was analyzed separately in accordance with GMP, ensuring that there were no contamination outside the limit [24] and hence its quality was confirmed. The formulations with and without glycolic extract were also analyzed.

The estimate of the number of viable microorganisms the obtained as a result of the microbial analyses for counting aerobic mesophile microorganisms (bacteria and fungi) for the studied formulation and their raw materials can be observed in Table 1 the evaluation revealed a bioburden (number of contaminating microorganisms on a certain amount of material prior to that material being sterilized) < 10 CFU/mL. Such data indicate adequate microbiological quality of the tested products, according to official recommendations [24]. The products did not present microbial contamination on the three essays (<10 CFU/g). Considering the possibility of some contamination during the manipulation of the products, the microorganisms did not survive, in result of a proper composition on the formulations.

Validation of the method for estimating the number of viable microorganisms

In the test validation, a high index of microorganism recovery in the adopted conditions was observed. Data of over 80% were obtained in both employed dilutions. In accordance to the official

recommendation [24] high index of microorganism recovery (higher than 75%) in tests of microbiological validation shows absence of

antimicrobial activity. Hence, the selected method for enumerating challenging microorganisms can be considered validated.

Table 1: Counting of viable mesophile microorganisms (bacteria and fungi) in formulation and raw materials.

Formulation and raw materials	Number of microorganisms (CFU/G)*	
	Bacteria	Fungi
Formulation with extracts	< 10	< 10
Formulation without extracts	< 10	< 10
Zinc oxid	< 10	< 10
Triceteareth-4 phosphate (and) cetyl alcohol (and) stearyl alcohol (and) sodium cetearyl sulfate (and) oleth-10	< 10	< 10
Hibiscus flowers glycolic extract	< 10	< 10
Assai palm glycolic extract	< 10	< 10
Black mulberry glycolic extract	< 10	< 10
Papaw glycolic extract	< 10	< 10

*3 assays with 2 repetitions

Research of *Salmonella* spp. and *Escherichia coli*

After performing the essays for *Salmonella* spp. and *E. coli*, any growth of these pathogens was not observed, ensuring, thus, the quality of the formulations.

Research of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

After performing the essays for *S. aureus* and *P. aeruginosa*, any growth of these pathogens was not observed, ensuring, thus, the safety of the formulations. The results obtained for all the formulations suggest that they are approved for use regarding their microbiological aspects.

Cosmetic products are divided into two different categories (5): (i) products specifically intended for children under 3 years or to be used in the eyes area and on mucous membranes and (ii) other products [4]. For products in category 1, the total viable counts for aerobic mesophilic microorganisms must not exceed 100 CFU/g in 0.5 g of the product, and furthermore, the pathogenic microorganisms' *P. aeruginosa*, *S. aureus*, *E. coli* and *Salmonella* spp. must not be detectable in 0.5 g of the product. For products in category 2, total viable counts must not exceed 1000 CFU/g in 0.1 g, and the pathogens mentioned above must not be detectable in 0.1 g of the product [24].

In this research, the formulation was classified as other products. It was observed that the formulations remained below the limits praised for the USP 35 (2012) and Brazilian legislation (2010) [24,26]. Also they did not present growth of pathogens in the assays, confirming the quality of the formulations from the microbiological point of view.

The use of hibiscus may have contributed to the potentialization of antimicrobial formulations, since the antimicrobial activity of the extract through the *Hibiscus rosa-sinensis* flowers, this is due to plant produces substances for self-defense against invading natural of the microorganisms, and it produces components such as tannins, flavonoids, alkaloids, triterpenes and phenolic compounds that possess antimicrobial and antioxidant activity [36-38].

CONCLUSIONS

It was concluded that the formulation and raw materials analyzed did not present microbial contamination, evidenced for estimating the number of viable microorganisms (<10 UFC/g) and for researching pathogens. The use of the hibiscus flowers, just as the assai palm, black mulberry and papaw glycolic extracts, which also possess substances of self-defense against invading may also be contributing against the contaminations of the formulations.

ACKNOWLEDGMENTS

PADC-FCF-UNESP and CNPQ.

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