Synthesis and determination of the Sun Protection Factor of a cosmetic gel-cream based on norbixin

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ABSTRACT
This work aimed to determine the Sun Protection Factor (SPF) of a cream-gel based on norbixin through spectrophotometry. Samples of gel-cream A and B were prepared at a concentration of 0.2 µL/mL and the absorbances were measured in the UVB region and the SPF was calculated using the equation used in the Mansur method. The absorption spectrum of each cream-gel was obtained using the origin program. Gel-creams A and B showed absorption in the UVA and UVB region and also in the long UVA region, therefore, they presented photoprotective characteristics. Regarding the Sun Protection Factor - SPF, both the cream-gel A and the cream-gel B were considered photoprotective by ANVISA. Norbixin increased the Sun Protection Factor - SPF and this potentiation was proportional to its concentration. Therefore, the use of norbixin in photoprotective products proved to be effective, favoring an increase in the photoprotection of the formulations.

Keywords: Norbixin, sun protection factor, spectrophotometry.

1 INTRODUCTION

Ultraviolet radiation is a source of energy needed by humans; it is responsible for the synthesis of Vitamin D in the skin. This vitamin acts on bone repair and proper cellular and neuromuscular functioning, however, too much exposure to these ultraviolet radiations can bring harm to health (Lima; Shah; Lima, 2020).

According to Sonda (2011) factors such as genetic characteristics and excess solar radiation, as well as lack of photoprotection, lead to the development of melanoma (cancer with a high mortality rate).

According to the National Cancer Institute (INCA) it is estimated that in Brazil, skin cancer, being the most common, is responsible for 30% of the recorded cases of tumors. It is estimated for

each year of the triennium 2020 – 2022, the emergence of 177 thousand new cases of non-melanoma skin cancer (INCA, 2020).

In this sense, the application of sunscreens is indispensable to protect the skin against the harmful effects of ultraviolet radiation. One of the alternatives is the production of sunscreens based on natural plant products (Santos *et al*., 2021).

Several natural products have been used against the increase of the deleterious effects of the sun, through natural topical formulations associated with chemical filters, with the intention of protecting the skin in the Ultraviolet region (Munhoz, 2012).

Norbixin is a carotenoid derived from annatto. Some natural antioxidants, such as vitamins, polyphenols and carotenoids, are being used in the area of cosmetics in photoprotective formulations due to their photoprotective and antioxidant characteristics (González; Fernandez; Gilaberte, 2008).

Given this context, the present work describes the synthesis and determination of the sun protection factor (SPF – UVB) *in vitro* of a cosmetic gel-cream based on norbixin, obtained by means of spectrophotometry.

2 DEVELOPMENTS

2.1 ULTRAVIOLET RADIATION

The sun's rays are essential to humans, since they are responsible for the activation of vitamin D and melanin production. However, too much exposure to this solar radiation can trigger many skin diseases, such as cancer and premature photoaging. The effects on the skin come from the formation of free radicals which, in turn, are formed through the interaction between UV radiation and reactive oxygen species (Lodyga *et al*., 2015; Wang *et al*., 2017).

Ultraviolet radiation is divided into three regions, according to the position occupied in the electromagnetic spectrum. The first region is the UVA (400 – 320 nm) which is responsible for the production of skin pigmentation and formation of free radicals, in addition to being able to induce skin cancer. It is subdivided into UVA I – long (400 – 340 nm) and UVA II – short (340 – 320 nm) (Nichols; Katiyar, 2010).

The second region is UVB (320 290 nm), which is associated with premature aging of cells, DNA damage as well as sunburn. Finally, the UVC region (290 – 100 nm), which have shorter wavelengths and high energies, are therefore extremely harmful to humans. However, most of it is absorbed by the atmosphere, so almost no UVC radiation reaches the Earth's surface (Mbanga *et al*.; 2014).

2.2 SUNSCREENS

To slow down or prevent damage to the skin induced by ultraviolet rays is necessary for its photoprotection. Sunscreens are topical formulations made with the purpose of protecting the skin against ultraviolet radiation and have as their main active ingredient sunscreens. These are classified into inorganic and organic (Santos *et al*., 2018).

Inorganic sunscreens act by reflecting and scattering ultraviolet radiation. They have low irritability and are widely used in products for sensitive and children's skin. This provides safe photoprotection (O'donoghue, 2007).

In turn, organic filters act by absorbing ultraviolet rays, transforming them into radiations with lower energies and that are not harmful to the body. They are formed mainly by aromatic compounds (Flower; Davolos; Correa, 2007).

For a sunscreen to be effective, it is necessary that it presents photostability, is not toxic and broad-spectrum, that is, it must act in the UVA and UVB region. However, some actives used in sunscreens can cause undesirable side effects such as thyroid alteration and endocrine disorders (Ruszkiewicz *et al*., 2017).

Another worrying factor about the photodegradation of organic sunscreens is the effect of their derivatives on the environment (Santos *et al*, 2012). According to studies done by Fent *et al* (2010) some substances resulting from the photodegradation of sunscreens have been found in the aquatic environment.

With this, the study for substances that cause less damage to health has grown. In addition to having photoprotective characteristics, they may also have antioxidant activity. In this way, natural actives have stood out in the cosmetics market in sunscreens (Saewan; Jimtaisong, 2015).

2.3 DETERMINATION OF THE SUN PROTECTION FACTOR

The Sun Protection Factor -SPF is one of the many ways used to analyze the safety of a photoprotector. Through the SPF it is possible to verify the absorption in the UVB region (Brasil, 2012). There are *in vitro* methods that are simple, low cost and fast.

Mansur *et al* (1986) proposed a methodology based on spectrophotometric methods for the *in vitro determination* of the Sun Protection Factor (SPF). This methodology, in addition to being used to determine the Sun Protection Factor of formulations, also helps in the quality control of products with photoprotection (Zocoler *et al,* 2019).

2.4 UVA/UVB RATIO AND CRITICAL WAVELENGTH ΛC

Another way to examine the photoprotection capacity of a sunscreen is through the FP-UVA, which checks the absorption of radiation in the UVA region. The *in vitro method* used for the evaluation

of FP-UVA is spectrophotometry. By means of spectrophotometry it is possible to determine the UVA/UVB Ratio and the Critical Wavelength (λc) (Diffey, 1994).

According to Donglikar; Deore (2017), the UVA/UVB Ratio corresponds to the ratio between the integrated areas of the curve in the UVA and UVB range and aims to evaluate whether the protection provided by the photoprotector is broad spectrum.

According to Brasil (2012), The critical wavelength (λc), is the wavelength that originates in 290 nm and refers to 90% of the area of the integrated curve between 290 and 400 nm, is also determined in *vitro* by scanning spectrophotometry.

3 METHODOLOGIES

3.1 RAW MATERIAL AND BASE FORMULATION

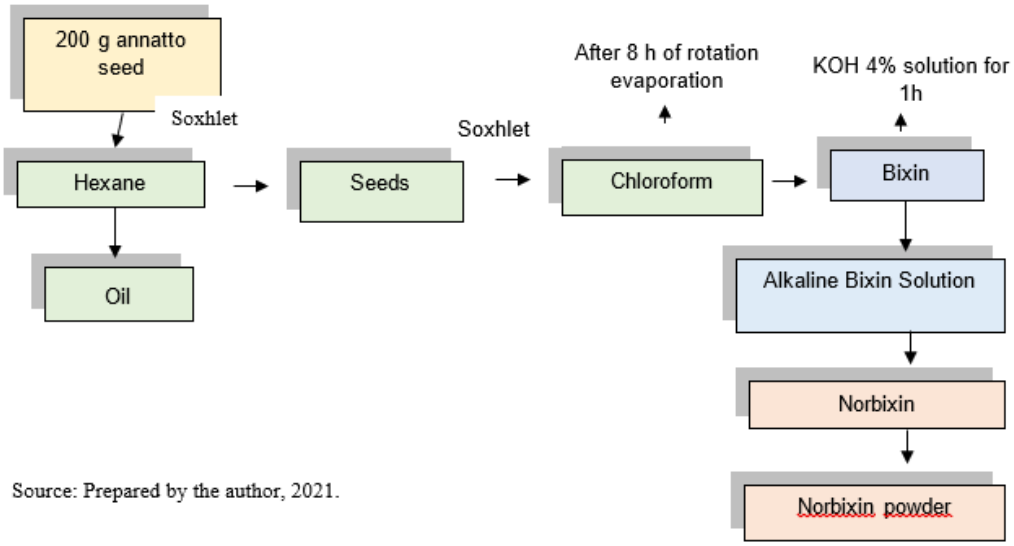
The annatto seeds were purchased in the central market and the basic formulation for the incorporation of chemical filters and norbixin was acquired in a compounding pharmacy in the city of Teresina-PI.

3.2 NORBIXIN EXTRACTION

200 g of annatto seeds were weighed and kept in reflux for a period of 8h in hexane in a Soxhlet system. The oily fraction obtained was separated from the seeds. Then the seeds were placed in reflux with chloroform for a period of 8 h, thus obtaining bixin (Barbosa-Filho, 1998).

A 4% KOH solution was added to bixin in constant agitation at 700C for 1 h. Subsequently, HCl was added for the precipitation of norbixin. The solution was washed with water and vacuum filtered and dried in the oven for 24 hours.

Figure 1: Norbixin Obtaining Flowchart



Source: Prepared by the author, 2021.

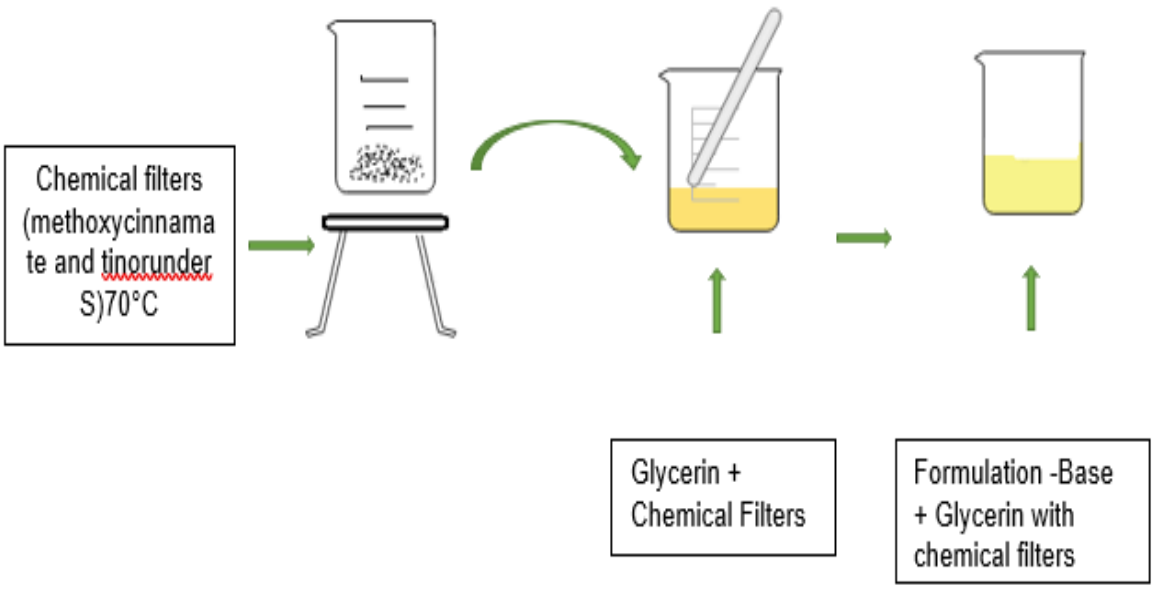
The spectrophotometric evaluation of absorption of the cosmetic formulation occurred in the spectrum of ultraviolet radiation following the method of Mansur *et al* (1986). Thus, scans from 290 to 320 nm were performed, at intervals of 5 nm, and absorbances were read at the end of time.

The UV Spectrophotometer UV-1800 SHIMADZU equipment (Figure 3) was used with quartz cubettes of 1 cm optical path to obtain the reading. The values obtained were multiplied by a correction factor, and by the normalized weight values that are constant (Table 1) and applied in the equation of Mansur *et al (*1986) to determine the in vitro sun protection factor *(*Figure 4).

3.3 PREPARATION OF COSMETIC FORMULATION

Initially, the chemical filters methoxycinnamate (3.5 %) and tinorsob s (2.5 %) were heated to 70^0 C. Then they were added to a beaker with glycerin (6%) and homogenized. To this mixture of chemical filters and glycerin was incorporated the gel-cream and norbixin at 2% and 4%.

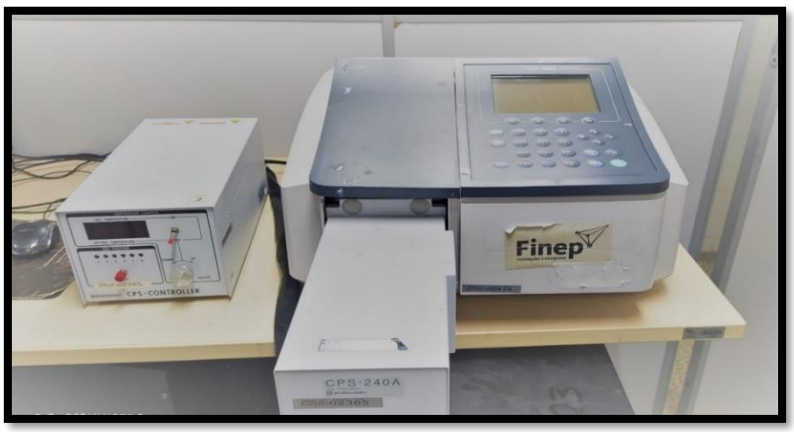
Figure 2 – Preparation of gel-creams



Source: Author, 2021. EdrawMax Program

3.4 DETERMINATION OF THE SUN PROTECTION FACTOR (SPF) OF THE COSMETIC FORMULATION

Figure 4- Equation used in the Mansur met



Source: Prepared by the author, 2021.

Table 1 - Erythemogenic effect (EE) versus radiation intensity (I) relationship according to wavelength λ (nm)

Wavelength λ (nm)	EE (L) x I (L)
290	**0,0150**
295	**0,0817**
300	**0,2874**
305	**0,3278**
310	**0,1864**
315	**0,0839**
320	**0,0180**

Fonte: Velasco *et al.,* 2011.

Figure 4- Equation used in the Mansur method

$$FPS\ espectrofotométrico = FC \cdot \Sigma_{290}^{320}\, EE(\lambda).Abs\ (\lambda)$$

Source: Prepared by the author, 2021.

Where:

HR = Correction factor (equal to 10);

EE = Erythematogenic effect of wavelength radiation (λ);

I = Intensity of the sun at wavelength (λ);

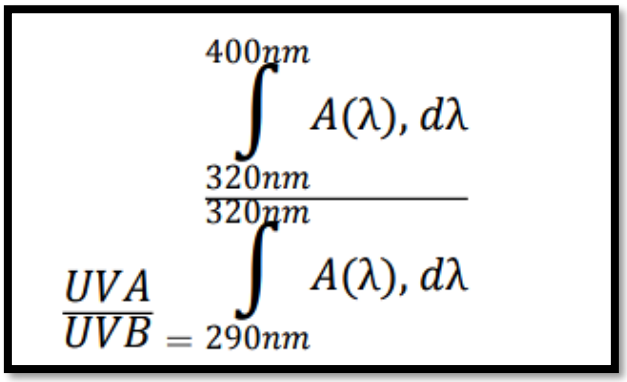
Abs = Absorbance of the sample in solution at wavelength (λ).

The tests were performed in triplicate, using pyrrolidone as white.

3.5 UVA/UVB RATIO AND CRITICAL WAVELENGTH (ΛC)

The UVA/UVB ratio was calculated through the ratio of the areas under the UVA curve in relation to the UVB curve (Springsteen *et al*., 1999), according to the equation shown in Figure 5 and with the aid of the Origin 7 program. The two gel-creams were classified according to the Boot's Star Racing System related to the UVA/UVB ratio as shown in Table 2.

Figure 5 – Equation for the calculation of the UVA/UVB Ratio



Source: Springsteen *et al*., 1999.

Table 2 - Boot's Star Rating System related to UVA/UVB ratio

UVA/UVB Stars Ratio Description
0.0 to < 0.2 - very low
0.2 to < 0.4 * moderate
0.4 to < 0.6 ** Good
0.6 to < 0.8 *** Superior
0.8 to < 0.9 **** Maximum
≥ 0.9 ***** Ultra

Source: Adapted from Velasco *et al,* 2011.

The critical wavelength was measured *in vitro* by scanning spectrophotometry and quantified using the Origin 7 program. It originates in 290 nm and corresponds to 90% of the area of the integrated curve in the region between 290 and 400 nm (Brasil, 2012).

4 RESULTS AND DISCUSSION

4.1 DETERMINATION OF THE SUN PROTECTION FACTOR - SPF

The Sun Protection Factor and the absorption spectra of gel-cream A and gel-cream B and are shown in Table 3 and Figure 6, respectively. It is observed that both the gel-cream A and the gel-cream B present absorption in the UVA and UVB region, thus indicating photoprotective activity of the ultraviolet region.

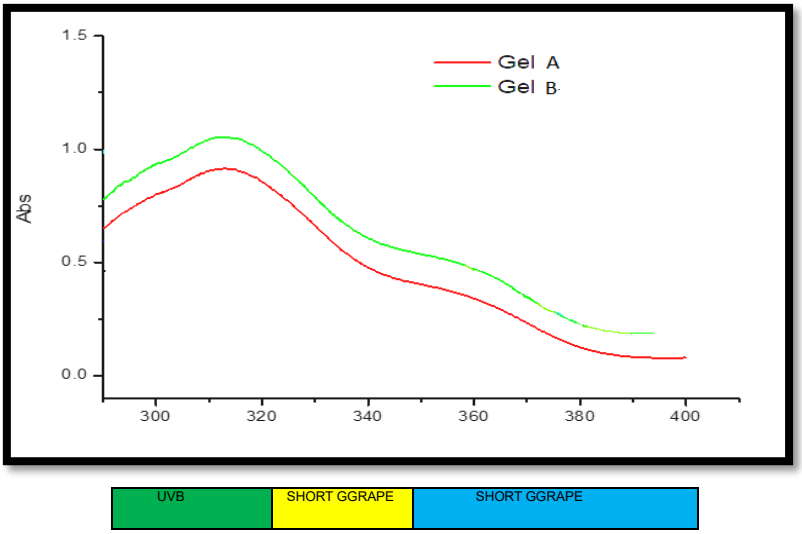
According to Rai *et al* (2012), a photoprotector is efficient when it absorbs both in the UVA region and in the UVB region indicating a product with broad-spectrum protection.

Table 3 – SPF of gel-cream A and B

Cosmetic Formulation	Gel-cream A (2% norbixin)	Gel-cream B (4% norbixin)
FPS	8,3	9,6

Source: Prepared by the author, 2021.

Figure 6 – UV/Vis absorption spectrum of gel-cream A and B



Source: Prepared by the author, 2021.

Figure 6 shows that the highest absorption peaks for the two gel-creams are between the UVB and short UVA regions. However, there is also significant absorption in the long UVA region. According to Ribeiro (2010), this region of the long UVA is directly associated with photoaging and skin cancer, being a difficult region to be reached by some chemical filters.

Regarding the Sun Protection Factor – SPF, it was found in Table 3 an increase in SPF in gel-cream B indicating that the SPF boost effect was intensified with the increase in norbixin concentration.

Considering the resolution RDC º 30 of June 1, 2012 of the National Health Surveillance Agency - BRAZIL (2012), for a product to be classified with photoprotector it must present the minimum value of SPF of 6 (six). Therefore, observing the results of Table 3, it can be said that gel-cream A and B fall into the category of photoprotectors.

Currently there is a tendency to use natural products in sunscreens in order to intensify the effect of synthetic chemical filters and thus increase the Sun Protection Factor (SPF) (Munhoz *et al*, 2012).

4.2 DETERMINATION OF UVA/UVB RATIO AND CRITICAL WAVELENGTH

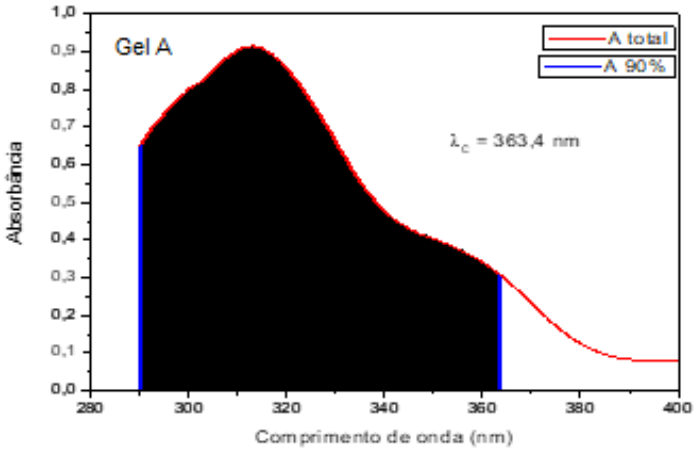
Based on the data obtained in Table 4 and shown in graphs in Figure 7 and 8, it can be said that the two gel-creams showed a good UVA/UVB ratio, and the gel-cream B had the best rate. They presented a UVA/UVB ratio ≥ 0.9 and according to the Boots Star Rating System they are considered as five stars and with ultra protection in relation to UVA radiation (Boots the chemists, 2004).

Table 4 – UVA/UVB Ratio and Critical Wavelength (λc) of gel-cream A and B

Formulations	UVA/UVB ratio	λc (nm)
Gel-cream A	1.12	363.4
Gel-cream B	1.3	370.6

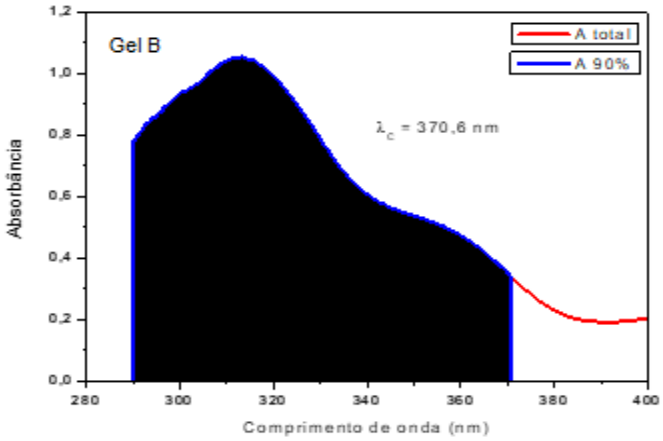
Source: Prepared by the author, 2021.

Figure 7 - Graph of the critical wavelength (λc) of the gel-cream A



Source: Prepared by the author, 2021.

Figure 8 - Graph of the critical wavelength (λc) of the gel-cream B



Source: Prepared by the author, 2021.

Table 4 showed an increase in the UVA/UVB ratio proportional to the concentration of norbixin. This result shows that norbixin favors the absorption of radiation in the UVA region, increasing the area under the curve that corresponds to the UVA region. According to Donglikar; Deore (2017) a UVA/UVB ratio closer to 1 demonstrates more effective protection against ultraviolet rays.

The gel-cream B (4% norbixin) showed the best results both in relation to the UVA/UVB ratio (1.3) and the critical wavelength λc (370.6 nm) achieved all parameters defined by ANVISA.

According to the Resolution of the Board of Directors, RDC No. 30 of June 1, 2012 of ANVISA, a product is considered photoprotector if it presents a critical wavelength λc of at least 370 nm and the longer this wavelength, the better its efficiency in photoprotection (Brasil, 2012).

5 CONCLUSIONS

In this work two gel-creams with different concentrations of norbixin that presented photoprotective properties were synthesized.

Gel-cream A and B have demonstrated absorption in the UVA and UVB region and also in the long UVA region that is difficult to achieve by synthetic chemical filters.

Regarding the Sun Protection Factor – SPF, both gel-cream A and gel-cream B are considered photoprotectors by ANVISA. For the critical wavelength (λc), only the gel-cream B can be considered photoprotective.

Norbixin increased the Sun Protection Factor – SPF and this potentiation was proportional to its concentration.

Thus, the use of norbixin in photoprotective products has been shown to be effective, favoring the increase of photoprotection of formulations.

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