Obtaining a Dermocosmetics from the aerial parts of Eclipta alba (L.) for the treatment of alopecia

Obtenção de um Dermocosmético das partes aéreas de Eclipta alba (L.) para o tratamento da alopecia

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ABSTRACT
Eclipta alba (L.) has been studied for years to treat various diseases amongst them, the alopecia. The objective of this work was to perform the physico-chemical and phytochemical characterization of E. alba to develop a capillary product for alopecia. The physico-chemical characterization of the aerial parts of the herbal drug was performed according to the Brazilian Pharmacopoeia 6th edition, and the phytochemical analysis was based on the literature. Then the methanolic extract obtained was subjected to spray drying for subsequent incorporation into the dermocosmetic batches. Finally, the product was subjected to quality control analyzes for 15 days and the quantification of wedelolactone was also performed. The percentage of total ashes of the E. alba powder was considered high value, when compared to a published study, where the value of the non-volatile inorganic impurities of the vegetable drug was 10.54%. According to the Brazilian Pharmacopoeia, the humidity of E. alba powder is within the specified limit, which is 8 to 14%. With the granulometric distribution, it was possible to classify it as moderately thick. The pH of the methanolic extract was within the considered ideal for the cosmetic formulations, which is between 4.5 and 6.0. The Thin Layer Chromatography of the extractive solution revealed the presence of several constituents that have pharmacological activities, amongst them it was possible to evidence the presence of wedelolactone, a coumarin that provides capillary growth. Additionally, the coumarin assay was performed by spectrophotometry at 340 nm, demonstrating the linearity of the method. Therefore, E. alba has been used in a formulation that satisfy the specific care that alopecia needs and meets the expectations of the population that has been affected by hair loss.

Keywords: Eclipta alba, Hair, Alopecia, Thin Layer Chromatography.

RESUMO
Eclipta alba (L.) tem sido estudada há anos para tratar várias doenças entre elas, a alopecia. O objetivo deste trabalho foi realizar a caracterização físico-química e fitoquímica de E. alba para o desenvolvimento de um produto capilar para alopecia. A caracterização físico-química das partes aéreas da droga vegetal foi realizada de acordo com a Farmacopeia Brasileira 6ª edição, e a análise fitoquímica baseada na literatura. Em seguida, o extrato metanólico obtido foi submetido à secagem por atomização para posterior incorporação aos lotes dermocosméticos. Por fim, o produto foi submetido a análises de controle de qualidade por 15 dias e foi realizada a quantificação da wedelolactona. A porcentagem de cinzas totais do pó de E. alba foi considerada de alto

valor, quando comparada a um estudo publicado, onde o valor das impurezas inorgânicas não voláteis da droga vegetal foi de 10,54%. De acordo com a Farmacopeia Brasileira, a umidade do pó de E. alba está dentro do limite especificado, que é de 8 a 14%. Com a distribuição granulométrica, foi possível classificar a droga vegetal como moderadamente grossa. O pH do extrato metanólico ficou dentro do considerado ideal para as formulações cosméticas, que está entre 4,5 e 6,0. A Cromatografia em Camada Delgada da solução extrativa obtida revelou a presença de vários constituintes que possuem atividades farmacológicas, entre eles foi possível constatar a presença da wedelolactona, uma cumarina que proporciona o crescimento capilar. Adicionalmente, o ensaio da cumarina foi realizado por espectrofotometria em 340 nm, demonstrando a linearidade do método. Portanto, E. alba pode ser utilizada em uma formulação que atende aos cuidados específicos que a alopecia necessita e atende às expectativas da população afetada pela queda de cabelo.

Palavras-Chave: Eclipta alba, Cabelo, Alopecia, Cromatografia em Camada Delgada.

1 INTRODUCTION

Hair, throughout history, has gone through several stages of responsibilities and functions such as protection and temperature regulation. Hair worship has crossed the barrier of time, and significantly symbolizes strength and power. Since the beginning of humanity, it has been observed that the hair beauty is related to the adaptation of peoples, cultures, religions and ethnicities [1]. Alopecia, popularly known as hair loss, is a chronic inflammatory condition that affects hair follicles and results in hair loss, afflicting men and women in different age groups.

Alopecia can present several alterations and characteristics, being classified into cicatricial and non-cicatricial alopecia, both triggered by several different reasons [2]. The main types of non-cicatricial alopecia are alopecia androgenetic (AAn), alopecia areata (AAr), telogen effluvium (TE) and trichotillomania [3]. The main cause of AGA is a genetic problem that usually attacks only the follicles in the upper part of the head, where hair miniaturization occurs, with consequent decrease in its normal size. The hair bulb matrix contains an enzyme, 5α-reductase (5αR), which transforms the hormone testosterone into dihydrotestosterone (DHT) and penetrates the follicle, transforming its metabolism, weakening it and, consequently, accelerating hair loss. This microsomal enzyme is responsible for the reduction of steroid-3-oxo-delta 4 compounds, such as testosterone, progesterone, and corticosterone.

For those who suffer from the so-called hair loss, the first measure is to find a cosmetic product that will restore hair health. The intense search for effective products that induce hair growth has led to an attempt to establish new resources that would

alleviate or correct the intensity of hair loss. According to Barsanti [4], it has been possible to use plant extracts such as the palm extract Serenoa repens, green tea extract, ho-show-wu extract and soy in order to obtain similar or even better aesthetic results, to those obtained with synthetic drugs, such as minoxidil and finasteride. In addition to these extracts, some authors suggest that Eclipta alba (L.) extract has a potential factor for hair growth [5, 6].

E. alba, also known as Eclipta prostrata, is a weed that belongs to the Asteraceae family. It is commonly known as “false daisy”, a plant which is originally from Asia, classified by the Ayurvedic pharmacopoeia of India as having hepatoprotective function [7]. In Brazil, it can be found nearly in the entire territory, being very frequent in the wet areas of the North and Northeast [8]. This plant material contains a wide variety of secondary metabolites including coumarins, flavonoids, glycosides, polyacetylenes, triterpenoids [9], which can be found in different parts of the plant material.

E. alba is a species used to promote the growth of healthy hair. This effect was confirmed in a study developed with the use of its methanolic extract to induce the anagen phase with stimulation of hair follicles [5]. Therefore, this paper addresses the pharmaceutical development of a dermocosmetic for the treatment of alopecia.

2 MATERIALS AND METHODS

2.1 COLLECTION, IDENTIFICATION, AND PROCESSING OF RAW MATERIAL

The plant material made up of the aerial parts of Eclipta alba was collected during the morning, in Recife (Pernambuco - Brazil), with the following geographical coordinates: 8°4'45"S latitude and 34°54'36"W longitude. An exsiccate of the species was identified by Dr. Rita de Cássia Pereira, from the Agronomic Institute of Pernambuco (IPA), under n. 91177. Additionally, the material was registered in the National Management System for Genetic Heritage and Associated Traditional Knowledge (Sisgen) under number A74E617.

After collection, the fresh plant material was stabilized: washed with running water to remove dirt and sprayed with 70% ethyl alcohol (v/v). Then, it was submitted to a previous drying, at a temperature of 25ºC for 2 days. After this drying, the plant material was put in a kiln with forced air circulation (400-TD, Ethik Technology®), at a temperature of 40 ºC for 14 h. The dry plant material was crushed in a knife mill (SL-31, Solab®) using a 30-mesh sieve, obtaining the herbal drug [10].

2.2 PHYSICO-CHEMICAL CHARACTERIZATION OF THE HERBAL DRUG

The physicochemical analysis of the E. alba powder was carried out according to the Brazilian Pharmacopoeia 6th edition [11]. The tests for determining moisture, total ash and particle size distribution were performed in triplicate, and the values were expressed as mean ± standard deviation.

2.3 EXTRACTION

The vegetable drug was subjected to the extraction process by Soxhlet for 12 h at a temperature between 80-85 ° C, using the ratio 1:5 (w/v) and methanol as solvent, according to Savita et al. [12]. After obtainment, the methanolic extract was characterized in terms of pH and density. The pH analysis was performed in a pHmeter (PG 1800, Gehaka®) previously calibrated with pH 4.0 and 7.0 buffers (Certipur®). The analysis of the relative density was performed using a 25 mL pycnometer, previously calibrated, being calculated according to the ratio of the mass of the liquid sample to the mass of the water [11].

2.4 PHYTOCHEMICAL SCREENING OF THE EXTRACT BY THIN LAYER CHROMATOGRAPHY (TLC)

For the identification of the classes of metabolites of E. alba extract, the Thin Layer Chromatography (TLC) was employed, using silica gel F_{254} plates (Macherey-Nagel®) as stationary phase. The methanolic extract and standards were applied manually to the plates. Then, the plates were placed in vats previously saturated with the mobile phase, according to the metabolite of interest. At the end of the elution, the plates were dried at room temperature and then derivatized with specific developers, being observed at 254 and 366 nm when appropriate. The observation of the plates under UV light and image acquisition was performed using a MultiDoc-It® imaging system (model 125), with a Canon® camera (Rebel T3, EOS 1100 D) and UVP® software. The developers, mobile phase systems and standards for each class of metabolite used were listed in Table 1.

Table 1 - Standards, systems and developers used to identify metabolites.

Metabolite	Standard	System	Developer
Hydrolysable Tannins	Gallic acid and Ellagic acid	ethyl acetate: formic acid: water (90: 5: 5)	NEU + UV 366 nm
Flavonoids	Quercetin and Rutin		
Cinnamic Derivatives	Caffeic Acid and Chlorogenic Acid		
Condensed Tannins	Catechin		Hydrochloric

			vanillin
Terpenes and Steroids	β-Sitosterol	toluene: ethyl acetate (70:30)	Liebermann-Burchard +Δ 366 nm
Coumarinn	Coumarin and Wedelolactone	ethyl alcohol: toluene: acetic acid (10%) (50:50:50)	KOH + Δ + UV 366 nm
Saponins	Aescin	ethyl acetate: acetic acid: formic acid: water (100:11:11:26)	Liebermann-Burchard +Δ
Reducing sugars	D-fructose	ethyl acetate: methanol: water (50:20:10)	Thymol + H_2SO_4 10% + Δ
Alkaloids	Pilocarpine nitrate	ethyl acetate: methanol: water (50:6.75:5)	Dragendorff
Anthraquinones	Senoside A		HNO_3 + KOH 10% 366 nm

NEU: 1% ethylborylaminoester acid in ethanol; Δ: Heating; H2SO4: sulfuric acid; HNO3: nitric acid; KOH: potassium hydroxide.

2.5 WEDELOLACTONE HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

The HPTLC analysis was performed on a Linomat V (Camag®) equipment. Aliquots of 10 µL of the methanolic extract of E. alba and the standard of Wedelolactone (> 95.5%, Sigma-Aldrich®) were applied on a silica gel plate F_{254} (Macherey-Nagel®), with the aid of a syringe and semi-automatic applicator. The sample and the standard were applied in bands with 10 mm width and spacing between bands of 5 mm. The chromatogram was developed in a double vertical glass chamber (10 cm × 10, Camag®), after saturation for 30 min with the mobile phase consisting of the mixture toluene: ethyl acetate (50:50 v/v). After elution, the plate was dried at room temperature and derivatized with KOH, with subsequent observation at 366 nm, as recommended by Savitta and Prakashchandra [12]. The observation of the plates under UV light and image acquisition was performed using a MultiDoc-It® imaging system (model 125), with a Canon® camera (Rebel T3, EOS 1100 D) and UVP® software.

2.6 OBTAINING DRY EXTRACT BY SPRAY DRYER

The determination of dry residue was carried out transferring 2 g of previously desiccated extract to weighing bottles. The weighing bottles were left in a water bath until complete evaporation. Then, they were dried in an oven at 105 °C for 3h and, subsequently, it was left to cool in a desiccator containing phosphorus pentoxide. Finally, the weighing bottles were weighed, and the dry residue was calculated in percentage [11]. The tests were performed in triplicate.

After determining the methanolic extract dry residue, the drying was performed using colloidal silicon dioxide 30% as an adjuvant, stirring until complete dissolution. To

the volume of 50 mL of extract, 200 mL of purified water were added. After stabilization, the solution was subjected to the drying process in Mini Spray Dryer (B-290, Buchi®), using 120 °C as inlet temperature and outlet temperature 86 °C.

2.7 QUANTIFICATION OF WEDELOLACTONE BY UV-VIS SPECTROPHOTOMETRY

For the analytical curve, 5 mg of wedelolactone standard were transferred to a 25 mL volumetric flask by measuring the volume with methyl alcohol (Qhemis®). Successive dilutions were performed, using water as a solvent, to obtain seven concentrations between 1.0-7.0 µg/mL [10]. The absorbance readings were performed on a UV-Vis spectrophotometer (Vankel® 50), using a wavelength equal to 275 nm [13].

To obtain the E. alba dry extract curve, 50 mg were weighed and transferred to a 100 mL volumetric flask, measuring the volume with methanol: water (80:20 v/v), constituting the stock solution (SS). From the SS, successive dilutions were performed to obtain reading solutions with concentrations between 4.0–11.0 µg/mL. The readings were performed in triplicate, using a spectrophotometer at 340 nm.

2.8 DEVELOPMENT OF E ALBA DERMOCOSMETIC

Two batches of 20 mL each were obtained, containing the excipients and concentrations according to the data in the literature. After obtainment, the physicochemical control (appearance, color, and odor) of the formulations was carried out, and then, the best formulation was selected to be submitted to the preliminary stability test. For better identification, the batches were listed as I and II. The components and concentrations in percentage (%) of the lots are shown in table 2.

Table 2 - Composition of batches manipulated from the dermocosmetic.

Components	**Batch I (%)**	**Batch II (%)**
E. alba Extract	6.0	6.0
Novamit	0.2	0.2
Propylene glycol	2.0	2.0
Panthenol	1.0	1.0
Green tea fragrance	0.2	0.2
Cereal ethyl alcohol	5.0	10.0
Distilled water	85.6	80.6
Total	**100.0**	

3 RESULTS AND DISCUSSION

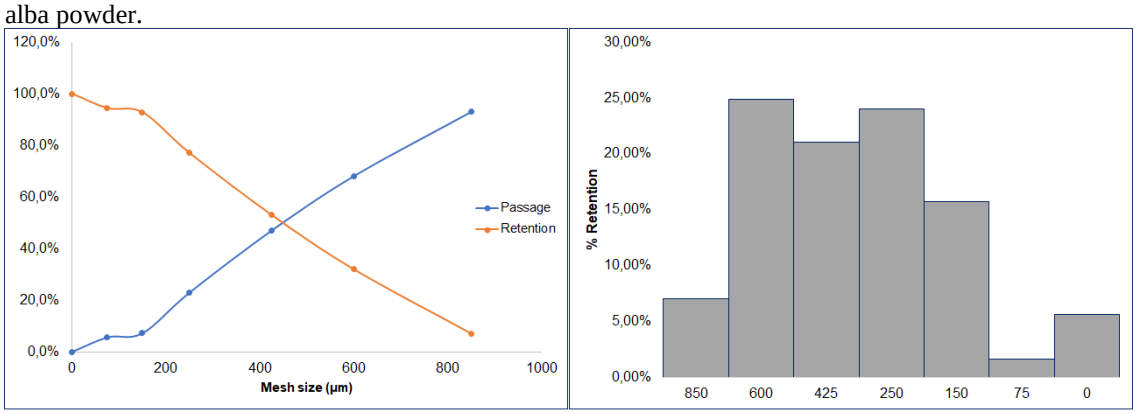
3.1 PHYSICOCHEMICAL ANALYSIS

After collecting 4.5 kg of fresh plant material of E. alba, kiln drying and grinding it, 2.440 kg of dry plant powder were obtained, with a yield equal to 54.22% of dry plant in relation to the fresh plant. From the moisture content of raw materials, it is possible to infer the microbiological and chemical stability of plant drugs, since high values can lead to the development of fungi and bacteria, hydrolysis and enzymatic activity with consequent deterioration of chemical constituents [14]. The obtained results indicate that the drying process was considered adequate for the vegetable raw material under study, with an average value of 8.38% ± 0.3004 (3.58%). In studies carried out by Arantes [15], the moisture content found for the sample of E. alba was 8.36%, showing conformity between the results.

The total ash content obtained was equal to 14.44% ± 0.1900 (1.32%), considered a slightly higher value when compared to other studies in the literature. According to Arantes [15], the percentage of ash obtained for the sample of E. alba that he evaluated was 10.54%. The value obtained in our study, slightly higher, may be associated with the urban area habitat in which the plant drug was collected.

When comparing the result of the granulometric distribution of the powder with the classification of the Brazilian Pharmacopoeia 6th edition, it was observed that the obtained powder was classified as moderately thick, and its average particle size was around 416 µm (Figure 1).

Figure 1 - Curves of retention and passage. Histogram of particle size distribution of the aerial parts of E. alba powder.



The granulometric distribution determines the contact surface available for interaction with the solvent used to obtain the extract [10]. Therefore, larger size powders favor the extraction process, since very fine particles can adhere to the larger particles,

increasing the viscosity of the means and creating a barrier that prevents the penetration of solvents, preventing extraction and decreasing the extraction efficiency [16, 17].

3.2 OBTAINING THE METHANOL EXTRACT

The preparation of crude extracts of plant species is the starting point in the stage of isolation and purification of the fixed chemical constituents of plants [18]. The extractive process in question was based on what was proposed by Savita et al. [12], who tested several extractive techniques to obtain better extraction efficiency.

In this study, 50 mL of E. alba extract were obtained, which represents a 20% yield. The pH of the methanolic extract of E. alba was 5.95, corroborating what is recommended in the literature for the development of a hair cosmetic, since the ideal pH of a topical use formulation for alopecia must range between 4.5 and 6.0, which is the natural pH of human hair, and the use of products with high levels of pH alkalinity causes alteration or damage to the fiber [19]. In addition, the skin has a slightly acid pH between 4.6 and 6.0, which contributes to bactericidal and fungicidal protection on its surface [20].

Density or specific mass can be used in the identification and quality control of a given product, as well as be related to the concentration of solutions. The value obtained for density of methanolic extract of E. alba was 0.875 g/mL.

3.3 PHYTOCHEMICAL SCREENING OF THE EXTRACT BY THIN LAYER CHROMATOGRAPHY (TLC)

The analysis through TLC, after application of developers, allowed to verify that in the extract of aerial parts of E. alba, no condensed tannins and alkaloids were found. However, it was found the presence of hydrolysable tannins, flavonoids, cinnamic derivatives, reducing sugars, anthraquinones, saponins, coumarins, terpenes and steroids (Table 3), corroborating with some authors who have mentioned the presence of these metabolites. It was possible to evidence the presence of these metabolites by the color of the bands through comparative analysis with the bands corresponding to the standards used.

Table 3 - Results of phytochemical analysis of methanolic extract of E. alba obtained by TLC.

Metabolite	Result
Hydrolysable Tannins	+
Flavonoids	+
Cinnamic Derivatives	+
Condensed Tannins	-
Terpenes and Steroids	+

Coumarins	+
Saponins	+
Reducing sugars	+
Alkaloids	-
Anthraquinones	+

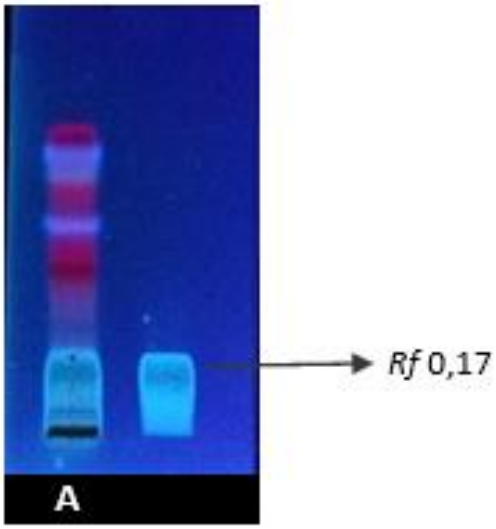
From TLC, it was also possible to verify the presence of wedelolactone, one of the main coumestanes found in E. alba, which, in addition to hair growth [5], has antihepatotoxic [21], antibacterial [22], and antiophidic [23] activity.

The presence of this coumestane was determined from the determination of the Rf of the sample, which was compared to the Rf of the wedelolactone standard, once it is possible to view it in ultraviolet light. The finding of the presence of wedelolactone in the extract of the aerial parts of the plant drug is quite interesting for the cosmetics industry, that needs an effective formulation which can be proven by constant quality of the extract.

The analysis through HPTLC, as well as TLC, is a powerful analytical tool to obtain chromatographic information of complex mixtures of inorganic, organic and biomolecules [24]. However, HPTLC has improvements aimed at increasing the resolution of the compounds to be separated, being considered as a flexible, reliable, and efficient technique in the ideal separation for the analysis of plant and herbal materials [25]. It was also possible to verify that the consumption of solvents in HPLTC was lower than in conventional TLC, moreover, in HPTLC plate, a better resolution of wedelolactone was found in the sample.

From optimization of the methodology employed by Savita and Prakashchandra [12], for analysis in HPTLC of wedelolactone (Figure 2) in methanolic extract, it was possible to verify the presence of this cousmestane by determining the Rf of the sample that was compared to the Rf of the wedelolactone standard, justifying its use in dermocosmetic to treat alopecia, in order to meet the expectations of people who dream of having an increase in the number of hair strands, by increasing the capillary density of the areas affected by alopecia.

Figure 2 - Chromatographic profile obtained by HPTLC for the analysis of Wedelolactone in the methanolic extract of E. alba.



3.4 OBTAINING DRY EXTRACT BY SPRAY DRYER

The dry residue is a parameter considered important for the evaluation of the drying processes, as this test implies in quantification of substances extracted from the plant through the elimination of the extracting solvent, being an indicator of the concentration of the extract [26]. In this study, the dry residue of the methanolic extract of E. alba was equal to 9.74%. The acceptable values for the percentage of total dry residue for the methanol extract of E. alba have not yet been established in any study. The experimental conditions using temperature of 120 °C and outlet 93 °C, flow rate 7.0 mL/min and pressure of 600 mmHg, were favorable to the drying process, as there was no retention of dust in the main drying chamber.

In the drying process, adjuvant agents that improve the characteristics of the final product are used. Some examples of these agents are cited by Vasconcelos, Medeiros and Moura [27] and Silva-Júnior et al. [28]: starch, cyclodextrins, colloidal silicon dioxide, tricalcium phosphate, gelatin, gum arabic, lactose and maltodextrin. Upon drying 50 mL of the methanolic extract, using the conditions described above and with 30% Aerosil® (colloidal silicon dioxide), a dry extract was obtained appearing to be a clear, homogeneous powder and apparently with low hygroscopicity. The result of the weight obtained after drying was 1.58 g, which corresponds to a yield of 28.37%.

3.5 QUANTIFICATION OF WEDELOLACTONE THROUGH UV-VIS SPECTROPHOTOMETRY

The analytical curve obtained for wedelolactone standard ($y = 0.074x + 0.266$, $R^2 = 0.9961$) demonstrated a linear relationship between concentration and absorbance evaluated for this standard, in accordance with Brazil [29] which determines that the coefficient correlation (r) has a minimum value of 0.99.

All analyzes were carried out in an environment without light due to the wedelolactone photosensitivity and measured on the same day. Additionally, with the absorbance and analytical curve determination ($y = 0.0695x - 0.0085$; $R^2 = 0.9921$) of dry extract of E. alba, it was estimated that the dosage of wedelolactone in dry extract is 96.53%.

3.6 DEVELOPMENT OF E ALBA DERMOCOSMETIC

The manipulated sample presented a fluid aspect characteristic of suspensions, cloudy color and odor characteristic of the fragrance used in the formulation. In addition to E. alba, the formulation has D-Panthenol, which in the International Nomenclature of Cosmetic Ingredient (INCI) is called Panthenol, a skin regenerator which has a healing and anti-seborrheic action to the hair follicle, besides moisturizing and stimulating epithelial metabolism. It is used especially in cosmetic formulations for hair, skin and nails treatment [30]. In addition, the formulation also features cereal ethanol (INCI NAME: Alcohol), a product of plant origin and high purity, whose raw material is corn, soy or rice and is widely used for consumption in beverage, pharmaceutical, cosmetics and food industries, and handling pharmacies.

Out of the two batches handled, the batch II was considered the best, as it had a higher percentage of cereal alcohol, facilitating the solubilization of the extract. The Brazilian Pharmacopoeia 6th edition suggests that there is no recommended range for its use [11]. However, it was more stable after being selected and submitted to the preliminary stability test.

From the definition of the analytical curve in which the equation $y = 0.0695x - 0.0085$ with $R^2 = 0.9921$ was obtained and the concentration of coumarin in methanolic extract 60 mg/mL was estimated, such concentration was confirmed and x = 5.79 mg/mL was obtained. This resulted in 96.53%, which in fact is a good result, since the Brazilian Pharmacopoeia 5th edition defines the acceptance limit as 95% to 105%.

4 CONCLUSION

The hair lotion obtained from E. alba remained stable during the preliminary stability study through quantitative planning of an excipient, which was important for better solubilization of the extract to obtain a quality product. We achieved evidence to the presence of the compound that promotes hair growth. Therefore, after optimizing the formulation and completing the stability tests, it is interesting to evaluate the pharmacological activity of the product for extensive production of a formulation that provides the specific care that alopecia needs and meets the expectations of the population that has been affected by hair loss.

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