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Original Article

New antioxidant lauryl-free herbal shampoo formulation with a Brazilian plant extract

Nova formulação de xampu herbal antioxidante e sem lauril com extrato de planta brasileira

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Abstract

Sodium lauryl sulfate is the main cleaning ingredient in shampoos, even though it may be potentially damaging to hair. The demand for antioxidant-rich cosmetics, on the other hand, has encouraged green cosmetics research. Brazil has vast biodiversity that can be exploited for the production of these cosmetics. This work aimed to develop a minimalist antioxidant lauryl-free shampoo formulation with leaf extracts from the Brazilian plant *Hancornia speciosa* Gomes. Two hydroethanolic extracts were prepared using different extraction methods, Soxhlet, and ultrasound. The extracts were characterized by the presence of saponins, polyphenol quantification, and HLPC chemical identification of the compounds. Antioxidant activity was determined using the DPPH method. The antioxidant lauryl-free shampoo was developed using hydroxyethyl cellulose with two concentrations of leaf extract obtained by Soxhlet, 0.125 mg/g (XP1) and 0.250 mg/g (XP2). Along with the antioxidant activity, the physical and chemical properties, cleaning potential, and foam quality were evaluated. The Soxhlet leaf extract revealed a more favorable chemical profile, including a positive result for saponins, as well as a larger quantity of polyphenols and increased antioxidant activity. The XP2 formulation showed better foam height, dirt dispersion, and antioxidant activity. Thus, the use of mangabeira leaf extract appears to be promising for the development of shampoos with antioxidant activity.

Keywords: mangabeira, phenols, flavonoids, cosmetics.

Resumo

O lauril sulfato de sódio é o principal ingrediente de limpeza em xampus, embora possa ser potencialmente prejudicial ao cabelo. A demanda por cosméticos ricos em antioxidantes, por outro lado, tem incentivado as pesquisas com cosméticos verdes. O Brasil possui uma vasta biodiversidade que pode ser explorada para a produção desses cosméticos. O objetivo deste trabalho foi desenvolver uma formulação de xampu antioxidante minimalista sem lauril com extratos de folhas da planta brasileira *Hancornia speciosa* Gomes. Dois extratos hidroetanólicos foram preparados usando diferentes métodos de extração, Soxhlet e ultrassom. Os extratos foram caracterizados quanto à presença de saponinas, quantificação de polifenóis e identificação química HLPC dos compostos. A atividade antioxidante foi determinada pelo método DPPH. O xampu antioxidante sem lauril foi desenvolvido utilizando hidroxietil celulose com duas concentrações de extrato de folha obtido por Soxhlet, 0.125 mg/g (XP1) e 0.250 mg/g (XP2). Juntamente com a atividade antioxidante, foram avaliadas as propriedades físicas e químicas, potencial de limpeza e qualidade da espuma. O extrato da folha de Soxhlet revelou um perfil químico mais favorável, incluindo resultado positivo para saponinas, além de maior quantidade de polifenóis e aumento da atividade antioxidante. A formulação XP2 apresentou melhor altura de espuma, dispersão de sujeira e atividade antioxidante. Assim, o uso do extrato da folha de mangabeira parece ser promissor para o desenvolvimento de xampus com atividade antioxidante.

Palavras-chave: mangabeira, fenóis, flavonoides, cosméticos.

1. Introduction

Hair plays an important physiological role in protecting the scalp from environmental aggressors, including ultraviolet radiation and extreme temperatures. Because hair is often part of social and cultural positioning, hair fiber care has traditionally been centered on hair beautification. Numerous hair cosmetics have been developed to enhance the appearance of hair while also keeping it soft and clean.

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Shampoos are cosmetics used to clean the hair. The most common detergent component is sodium lauryl sulfate (or sodium lauryl ether sulfate), which is responsible for hair cleansing. According to Cornwell (2018) despite its superior cleaning properties, lauryl weakens the hair fiber. As a result, other substances such as saponins may be investigated as a possible substitute for lauryl in hair formulations. Saponins are secondary metabolites found in plants that act as a detergent and emulsifier, resulting in persistent foam.

Brazil is a tropical country known for having one of the highest levels of biodiversity on the planet. Although much remains unknown, numerous investigations have been conducted to ascertain the biological activities of Brazilian plants. *Hancornia speciosa* Gomes, for instance, is a medium-sized tree, popular known as mangabeira. Mangabeira leaves have been shown to possess significant antioxidant activity in studies published in the literature (Leite et al., 2020; Panontin et al., 2022). The antioxidant activity helps hair cosmetics because it is intimately connected with the protection of the hair fiber (Kim, 2011). The antioxidant action of this plant's leaves encourages the development of hair cosmetics.

Natural cosmetics are widely available, and the number of brands and variations containing plant actives continues to grow in response to consumer demand for eco-friendly products (Cervellon and Carey, 2011). This article aims to present the step-by-step process for the development of an antioxidant shampoo obtained from mangabeira leaf extract.

2. Material and Methods

2.1. Plant material

The leaves of *H. speciosa* were collected in Palmas-TO, Brazil (10°18'00" S, 48°31'41" W) in the morning. The registration in SISGen was carried out under the number A8853F4 and the exsiccate was deposited and identified by the herbarium of the State University of Tocantins (UNITINS), under the registration number 7278.

2.2. Extract collection and preparation

The leaves were dried and ground in a Willye-type knife mill (Fortinox STAR FT 50), on a 20 mm mesh and stored in amber-type flasks. Hydroethanolic extracts of *H. speciosa* leaves were prepared using extraction in the Soxhlet apparatus (SOX) and ultrasound-assisted extraction (US), obtaining respectively, L_SOX and L_US extracts.

To obtain the extracts by ultrasound, 5 g of leaves were mixed with 80 ml of 70% ethanol and placed in an ultrasound bath at a frequency of 40 kHz (135 W) for 1 h at room temperature. For extraction with the Soxhlet apparatus, 5 g of the powder of each plant material was used in 200 ml of 70% ethanol for 5 cycles. After extraction, the solvent was removed by rota-evaporation and the extracts were lyophilized in a Benchtop Lyophilizer L101 (LIOTOP).

2.3. Phytochemical tests of extracts from the leaves of H. speciosa

2.3.1. Phytochemical screening

In the phytochemical screening assay, specific assays were performed for the detection of saponins through Rossol, Mitchell, Rosenthalen reactions, reaction with sulfovanillic reagent, and Liebermann-Buchard reaction, according to Sociedade Brasileira de Farmacognosia (2022).

2.4. Determination of total phenols content

Total phenol content was determined using the Folin-Ciocalteu method, proposed by Soares et al. (2014), using tannic acid as standard. The absorbances were measured in a BEL-Photonics SP 2000 UV spectrophotometer at 760 nm. Distilled water was used as blank.

The phenolic content was determined using the equation of the line $y = 0.0776x + 0.0083$, with $R^2 = 0.9981$, with tannic acid as standard. The result was expressed as mg of tannic acid equivalents (TAE) per gram of lyophilized extract (mg TAE/g).

2.5. Determination of total flavonoids content

The quantification of total flavonoids was performed using the method proposed by Soares et al. (2014). The solutions were kept under the protection of light for 30 minutes and the absorbances were measured at 460 nm, in a BEL-Photonics, SP 2,000UV spectrophotometer. Distilled water was used as blank.

For flavonoid analysis, a rutin calibration curve was obtained with a straight line equation $y = 0.0229x + 0.0005$, with $R^2 = 0.9967$. The result was expressed as mg of rutin equivalents (RE) per gram of lyophilized extract (mg RE/g).

2.6. Chemical characterization by High Performance Liquid Chromatography (HPLC)

The extracts obtained were analyzed by high performance liquid chromatography (HPLC) on a Shimadzu® LC-10 Chromatograph, equipped with a pump (LC-10AD), degasser (DGU-14A), UV-VIS detector (SPD - 10A), column oven (CTO-10A), manual injector (20 µL loop) and Shimadzu Class-VP software integrator, according to Panontin et al. (2022). The identification of compounds was performed by comparing the retention times of the samples and the authentic standards gallic acid, catechin, syringic acid, chlorogenic acid, vanillic acid, p-coumaric acid, naringin, vitexin, rutin, isorhamnetin, hesperidin, myricetin, morin, rosmarinic acid, quercetin, luteolin and apigenin (Sigma®). The extracts were dissolved in the elution solvent and the standards in methanol. All were filtered through Millipore® membrane (0.45 µm).

2.7. Determination of antioxidant activity

The evaluation of antioxidant activity was determined using the DPPH • (2,2-Diphenyl-1-picrylhydrazyl) radical method, according to the methodology proposed by Peixoto Sobrinho et al. (2011). 3 ml of the DPPH• solution (40 µg/ml of DPPH in methanol), 0.5 ml of the methanol extracts (20, 40, 60, 80, 100, 120, 140 µg/ml) were added.

The samples remained in the dark, for 30 minutes and then the absorbances were determined at 517 nm. Measurements were compared to negative control, using 0.5 ml of methanol in 3 ml of DPPH• solution. The percentage of antioxidant activity was calculated according to Equation 1.

$$\%AA = \frac{Abs\ sample - Abs\ control}{Abs\ control} \times 100 \quad (1)$$

Where %AA is percentage of antioxidant activity and Abs is Absorbance.

The IC_{50}% was calculated based on the linear regression resulting from the plotting of %AA by the concentrations analyzed. The percentage of maximum antioxidant activity was determined at a concentration of 140 µg/ml.

2.8. Shampoo development

The components listed in Table 1 were employed to produce the shampoo with the fewest raw materials. The formulation was based on Formulário Nacional (ANVISA, 2012). The extract was added at two different concentrations to determine whether the amount of extract impacted the formulations' quality.

To obtain the shampoo control (XPC), phenoxyethanol and EDTA were solubilized in a sufficient amount of purified water under manual stirring and mild heating (45 °C). This step was reserved. Hydroxyethyl cellulose was added to the remaining purified water and heated to 60 ºC, followed by agitation (2000 rpm) until the formulation was swollen. The speed was reduced to 200 rpm and the first step was added. Then, cocoamidopropyl betaine and sodium lauryl sulfate were added, and the mixture was slowly stirred until it was completely incorporated.

The shampoo base (XPB) was obtained in the same way, only without the addition of Sodium lauryl sulfate (SLS). To obtain the formulations XP1 and XP2, the lyophilized extract of the *H. speciosa* leaf was pre-solubilized with a small amount of purified water and Polysorbate 80 (a few drops just to moisten and solubilize the extract before incorporation) and then the incorporation was performed in the formulation of the shampoo base on a geometric scale. The system was subjected to slow agitation at 200 rpm in a mechanical shaker. For the XP1 formulation, the extract concentration was 0.125 mg/g and for the XP2 formulation, the concentration was 0.250 mg/g.

2.9. Foam volume determination and maintenance

Approximately 50 mL of the 1:10 solution of the formulation in distilled water were transferred into a 250 mL graduated cylinder, then the capped cylinder was manually shaken 10 times at a 90º angle. At the end of the agitation, the indicated volume was recorded at the maximum height of the foam formed. After 10 minutes, the foam height was recorded again (Badi and Khan, 2014). The volume of the foam was determined by subtracting the indicated volume from the maximum height of the volume immediately after stirring, and the maintenance of the foam was verified after 10 minutes.

2.10. Determination of solid waste

In a previously weighed porcelain capsule, 4 g of each shampoo formulation were transferred. The capsule was placed on a hot plate for evaporation of the liquid portion of the shampoo, cooled in a desiccator, and weighed again. The percentage of solid content was calculated (Badi and Khan, 2014), according to Equation 2.

$$\%solid\ content = \frac{starter\ shampoo\ mass}{final\ shampoo\ mass} \times 100 \quad (2)$$

2.11. Determination of dirt dispersion

A solution of one percent (1%) of each shampoo and a drop of India ink were added to a 250 ml beaker and shaken ten times. The amount of ink in the foam was estimated to be none, light, moderate, or severe. The shampoo base and the shampoos containing the extracts were analyzed. XPC was used as control (Badi and Khan, 2014; Kumar and Mali, 2010).

2.12. Determination of the antioxidant activity of shampoo formulations

The evaluation was carried out using the DPPH• method at 515 nm, according to the methodology proposed by

Table 1. Raw materials and their concentrations of shampoo formulations.

Raw material	Concentration (%)			
	XPC	XPB	XP1	XP2
Leaf extract	-	-	0.125 mg/g	0.250 mg/g
Sodium lauryl sulfate	35.0	-	-	-
Cocoamido propylbetaine	4.0	4.0	4.0	4.0
Hydroxyethyl cellulose	0.7	0.7	0.7	0.7
EDTA	0.1	0.1	0.1	0.1
Phenoxyethanol	1.0	1.0	1.0	1.0
Polysorbate 80	-	-	2 drops	2 drops
Deionized water (up to)	100.0	100.0	100.0	100.0

EDTA: ethylenediamine tetraacetic acid; XPC: shampoo control; XPB: shampoo base; XP1: shampoo with 0.125 mg/g; XP2: shampoo with 0.250 mg/g of *H. speciosa* leaf extract.

Leite et al. (2019), with adaptations. In this test, DPPH• was prepared in 70% ethanol to solubilize the formulation. 2.5 ml of the DPPH• solution (40 µg/ml) and 0.5 g of the formulation solubilized in 0.5 ml of 70% ethanol were added. The samples remained at rest in the absence of light for 30 minutes, and then their absorbances were determined at 515 nm. Measurements were compared to the negative control, using 1 ml of 70% ethanol in 2.5 ml of DPPH• solution.

The percentage of antioxidant activity was calculated according to equation 01

2.13. Preliminary stability of formulations

The criteria for the preliminary stability analysis were followed according to Guia de Estabilidade de produtos cosméticos (ANVISA, 2004).

Approximately 5 g of each sample of the selected formulations were centrifugated at 3000 rpm for 30 minutes. The samples were submitted to the preliminary stability test, exposed to six cycles of 24 hours in a freezer (−5 ºC ± 2 ºC) and 24 hours in an oven (50 ºC ± 2 ºC). The analysis of organoleptic parameters (color, odor, and appearance), density, viscosity, and pH were performed at time zero and at the end of the test.

For color analysis, homogeneity and clarity were observed. The symbols +, ++, +++, and ++++ were used to show how the intensity of the colors changed. The symbols + represent the lowest intensity, and the symbols ++++ represent the highest intensity. The odor was verified by olfactory perception only in order to characterize the formulations. Initially, the samples were exposed to air for 15 minutes, and each odor was recorded using the expressions: odorless; practically odorless; and slight characteristic odor.

In the analysis of the aspect, it was observed macroscopically changes in precipitation or turbidity. In general, the product must maintain its initial appearance under the different conditions tested, except when subjected to high temperatures, freezer or cycles in which small changes are acceptable.

Density was evaluated by the pycnometer method (ANVISA, 2019). The viscosity of the formulations was determined by an orifice viscometer (Gehaka Mod. VG 200), using orifice number 4 to carry out the analysis. The result, in centistokes, was automatically obtained from the first interruption of the flow and printed on an auxiliary printer.

The pH was analyzed by diluting the formulation in purified water in the proportion 1:10 with a digital pH meter. Shampoo's ideal pH ranged between 5.5 and 6.5 (ANVISA, 2004).

2.14. Accelerated stability of formulations

For preliminary stability analysis, formulations were subjected to 90 days of exposure under different temperature conditions: high temperature (45 °C) and low temperature (5 °C). The parameters of organoleptic characteristics, pH, density, viscosity, as well as the specific analysis of each formulation, dirt dispersion, foam height, and antioxidant activity of the shampoos were investigated.

Analyses were performed at time zero (t0), right after production, and at times 15, 30, 45, 60, and 90 days and compared to a reference sample kept at 15 °C (ANVISA, 2004).

2.15. Statistical analysis

Statistical analysis was performed to evaluate the initial parameters of the formulations. One-way ANOVA was used, followed by Tukey's test ($p < 0.05$). In Principal Component Analysis (PCA), the R software with the Stats package was used. The data matrix has 4 x 10 dimensions, with 4 shampoo formulations (control - XPC, shampoo base - XPB and shampoo formulations obtained from the extract of the *H. speciosa* leaf - XP1 and XP2) and 10 properties analyzed (color, odor, appearance, dry residue, density, viscosity, pH, foam height, foam maintenance, and foam dirt). Because there is a great variation in the responses of the different variables, that is, they differ in order of magnitude, the data were previously self-scaled and centered before being submitted to the principal component analysis.

3. Results and Discussion

3.1. Phytochemical tests of H. speciosa leaf extracts

The results obtained for saponins screening in extracts from the leaves of *H. speciosa,* obtained by Soxhlet (L_SOX) and ultrasound (L_US) were systematized in Table 2 and the results found for the phenolic compounds and antioxidant activity are shown in Table 3.

The extract obtained by Sohxlet showed more satisfying findings than the extract obtained by ultrasound as it contained a phytochemical profile of saponins (which was positive in all tests), and a phenolic profile with a higher quantity of phenols and flavonoids.

In the HPLC analysis, different types of substances were found in each type of extraction (Figure 1).

The compounds identified in the leaf, catechin and quercitin, were also identified by Santos et al. (2016), Leite et al. (2020) on the leaves and bark of *H. speciosa. p*-coumaric acid, isorhamnetin and morin were identified in the leaf extract obtained by Soxhlet and only in the leaf extract obtained by ultrasound. Catechin was identified in both extracts. Quercitin and rosmarinic acid were identified in the leaf extracts obtained by ultrasound.

Isorharmmetin has anti-inflammatory and antioxidant (Kim et al., 2019) activity, while morin has anti-inflammatory and antiallergic activity (Yu et al., 2017).

Table 2. Profile of saponins present in *Hancornia speciosa* leaf extracts, obtained by Soxhlet (L_SOX) and ultrasound (L_US).

Extracts	Assays for qualitative detection of Saponins				
	Rossol	Mitchell	Rosenthalen	Sulfo-Vanílico	Liebermann-Burchard
L_SOX	+	+	+	+	+
L_US	-	-	-	-	-

Table 3. Phenolic profile and antioxidant activity of *Hancornia speciosa* leaf extracts, obtained by Soxhlet (L_SOX) and ultrasound (L_US).

Extract	Total Phenolics (mg TAE/g)	Total Flavonoids (mg RE/g)	Antioxidant Activity IC_{50} (μg/mL)	Antioxidant Activity (%) (140μg/ml)
L_SOX	72.61 ± 1.73 ^{C}	157.64 ± 2.62 ^{A}	41.22 ± 9.02^{D}	91.93 ± 0.15^{A}
L_US	59.11 ± 1.36 ^{D}	144.25 ± 1.01 ^{B}	100.24 ± 3.53^{B}	67.74 ± 1.64^{B}
Rutin	-	-	21.64 ± 0.98^{E}	98.86 ± 0.14^{A}

Means that do not share the same letter are significantly different ($p < 0.05$).

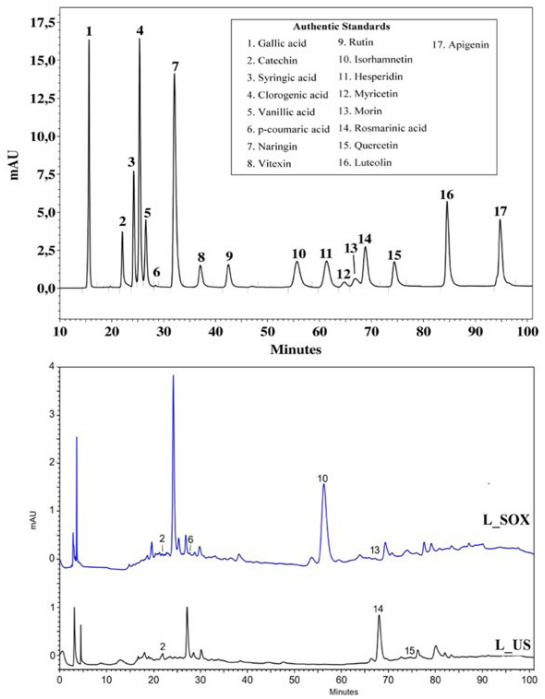

Figure 1. Chemical characterization by HPLC of *H. speciosa* leaf extracts obtained by Soxhlet (L_SOX) and ultrasound (L_US).

Rosmarinic acid (L_US), isorhamnetin (L_SOX) and morin (L_SOX) were identified for the first time in *H. speciosa* leaves, highlighting that the chemical composition may be related to the influence of extrinsic factors.

3.2. Shampoo development

Many factors were considered when developing the formulation. Sodium lauryl sulfate (SLS) is a widely used surfactant in shampoos due to its high detergency and excellent foam formation, but it has the potential to weaken the hair fiber (Cornwell, 2018). In the formulations (XP1 and XP2) the LSS was replaced by the extract, which showed positivity for saponins in the phytochemical screening. Thus, it was necessary to replace the sodium chloride thickener with hydroxyethyl cellulose, since sodium chloride has the ability to increase the viscosity of preparations containing sodium lauryl sulfate (Donaldson and Messenger, 1979).

During the development of the formulations, the concentration of hydroxyethyl cellulose that produced good viscosity was evaluated, not exceeding the limit of 1.5%. Higher amounts might create dandruff-like remnants on the hair, which is undesirable (Ferreira, 2010). Following several testings, it was determined that a concentration of 0.9 percent was the optimal concentration for the thickening agent.

3.3. Aspects analyzed in the formulations obtained

The results of the properties found in the formulations developed are listed in Table 4.

Regarding the macroscopic analysis of the color of the formulations, it was possible to verify that the extract changed the color of the base formulation from colorless to slightly yellow. This change is due to the natural color of the lyophilized dry extract. The XP2 formulation, as it contains twice as much extract as the XP1 formulation, showed more intense coloring than the XP1 formulation.

The coloring potential of the extract should be regarded as a positive characteristic of the formulation. It can be used instead of synthetic dyes, which can raise the cost and toxicity of the formulation (Yang et al., 2018).

Although the color of the formulation changed with the addition of the extract, all samples remained translucent and odorless. The absence of odor in the formulation is important because it allows the developer to choose between a hypoallergenic, fragrance-free formulation or any scent they desire, as there are no odor interferences from the formulation to conceal.

In the statistical analysis of the percentage of dry residues, it was possible to observe a statistical difference between the samples ($p < 0.05$), so that the XPC was statistically different from the formulations that did not contain lauryl. The low amount of solid residues presented in these formulations is probably due to the fact that the formulations contain few raw materials.

This is related to the ease with which hair products can be rinsed. Formulations containing a high proportion of solid residues (greater than 30%) are more difficult to be removed from the hair (AlQuadeib et al., 2018).

Regarding density, no sample of the XP1 (d = 1.005 ± 0.003 mg/ml) and XP2 (d = 1.002 ± 0.008 mg/ml) shampoos showed a statistical difference ($p < 0.05$) from the control (XPC, d = 1.0136 ± 0.005 mg/ml) and base (XPB, d= 1.0061 ± 0.000 mg/ml), which demonstrates that the addition of extracts in the specified amounts did not change the initial density of the base, and that they do not differ from to

Table 4. Results obtained for the analyzes carried out in the formulations of shampoo controls (XPC), shampoo base (XPB) and shampoo formulations obtained from the extract of the leaf of *H. speciosa* (XP1 and XP2).

Analyzed properties	Formulations			
	XPC	XPB	XP1 (0.125mg/g)	XP2 (0.250 mg/g)
Color	Colorless	Colorless	Light yellow	Yellow
Odor	odorless	odorless	odorless	Odorless
Aspect	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Dry residue	9.33±0.29^{A}	2.85±0.014 ^{B}	3.09±0.039 ^{B}	3.016±0.014 ^{B}
Density (g/ml)	1.013±0.005 ^{A}	1.006±0.000 ^{A}	1.005±0.003 ^{A}	1.002±0.008 ^{A}
Viscosity (Cp)	1,996.02±2.34^{A}	,1421.78±2.52^{B}	1,032.80±0.71^{D}	1,072.44±0.27^{C}
pH	5.33±0.06^{A}	4.53±0.06 ^{B}	4.33±0.12 ^{BC}	4.43±0.06 ^{C}
Foam height (ml)	153.00±5.20 ^{A}	71.00±1.00 ^{D}	91.33±1.16 ^{C}	111.00±1.73 ^{B}
Foam maintenance (%)	80.33±2.52 ^{A}	65.00±1 ^{C}	68.67±0.58 ^{C}	75.00±1.00 ^{B}
Foam dirt	None	None	None	None

Means that do not share the same letter are significantly different ($p < 0.05$).

conventional shampoo. According to Ferreira (2010) the results found are close to those recommended, between 1.010 and 1.020 g/ml. Similar density values were found by Moghimipour et al. (2021) for the shampoo control (d = 1.017 g/ml) and for the herbal shampoo with 15% saponins (d = 1.033 g/ml).

The viscosity of the shampoo control, XPC (1,996.02 ± 2.34 cP) is significantly higher ($p < 0.05$) than the other samples. The shampoo base, XPB, had lower viscosity (1,421.78 ± 2.52 cP) when compared to the XPC. Sodium lauryl sulfate, present only in the XPC formulation helped to increase the viscosity of the formulation that already contained hydroxyethyl cellulose, resulting in a higher viscosity than the other samples tested.

When comparing the viscosity of the shampoo samples containing extract, XP1 and XP2 with the shampoo base formulation, the shampoo containing a greater amount of extract (XP2) had a higher viscosity (1,072.44 ± 0.27 cP) than the shampoo containing the smallest amount of extract (XP1) (1,032.80 ± 0.71 cP). This oscillation, although numerically significant, was not macroscopically perceptible.

All samples had a pH below 6.5, as recommended by Formulário Nacional (ANVISA, 2012). Through the statistical analysis (Table 4) the difference presented between the samples ($p < 0.05$). The pH of the shampoo control was higher among the analyzed samples (pH 5.33 ± 0.06) and with the removal of sodium lauryl sulfate, the shampoo base (pH 4.53 ± 0.06) presented a pH statistically lower than the shampoo control. As the extracts were added, a slight decrease in pH was observed for both the XP1 formulation (pH 4.33 ± 0.12) and the XP2 formulation (pH 4.43 ± 0.06); however, only the XP1 formulation showed a statistical difference from the XPB base formulation. The two herbal shampoos showed no statistical difference in pH between them.

In the foam height test, the formulations showed lower foam formation than the control, but higher than the shampoo base, which indicates that the increase in foam formation was due to the addition of the extract.

Although foam is desirable in shampoos (Draelos, 2000), it is not responsible for cleaning. Instead, it is related to the sensory appeal of the formulation (Halal, 2016). Thus, one of the requirements of a good shampoo is to obtain good foam formation (Donaldson and Messenger, 1979).

In free-lauryl formulations, although foam is not abundant, LSS replacement can prevent scalp irritation and hair follicle damage (Monselise et al., 2017). It can also prevent swelling of skin and hair proteins, which leads to possible degradation and flaking (Cornwell, 2018). Thus, a market study is necessary to determine whether the consumer would accept a formulation containing natural surfactants at the cost of foam formation and hair fiber degradation.

When the foam was analyzed for dirt dispersion, it was discovered that it remained white in all samples, indicating that there was no ink present. The ink must remain in the aqueous portion of the shampoo, along with the white foam, in order for it to be considered of high quality (AlQuadeib et al., 2018).

3.4. Statistical analysis

In PCA analysis (Figure 2), the components CP1 and CP2 describe 99.8% of the total variation of the data and provide discriminatory information about the samples. The first principal component (CP1) describes 95.1% of the total variation, and the second (CP2) only 4.7%. PC2 presents the relationship of the analyzed properties (eigenvectors) on the shampoo formulations (eigenvalues).

The odor, foam dirt, appearance, density, viscosity, pH, and color properties are found to have a significant effect on the XPB formula. Regardless of the formulation, the foam height property has no effect. It is possible to observe discrimination between formulations, that is, a difference in behavior between shampoo control (XPC) and shampoo base (XPB) formulas and shampoo formulations derived from *H. speciosa* leaf extract (XP1 and XP2), as

explained by CP1 (95.1%). We observe a similar pattern of discrimination between control and shampoo bases (XPC and XPB), but this is explained by PC2 (4.7%). The analyzed dry residue property is the best at differentiating the XPC formulation from the XP1 formulation, while the foam maintenance property is the best at differentiating the XP1 formulation from the XPC formulation.

3.4.1. Determination of antioxidant activity

XP1 e XP2 showed pronounced antioxidant activity with values for XP1 of 51.15 ± 0.35% and for XP2 79.60 ± 0.14%. These results are promising, especially for the XP2 formulation, which showed antioxidant activity superior to XP1. This result indicates that the antioxidant action of this extract may be dose-dependent.

Similar results for antioxidant activity were found in the study developed by Joshi et al. (2018) in the development of a polyherbal shampoo, with extracts from guava and betel leaves. However, the authors of the presented study used a larger amount of extract to achieve the same antioxidant activity. In a dose-dependent antioxidant activity, the extract of mangabeira leaves appears to be more effective for increasing the antioxidant activity, as it produced similar activity with a lower concentration.

Other studies have suggested that the use of antioxidant compounds is an important strategy for retaining cellular youth (Dyshlyuk et al., 2024; Silva et al., 2021), which can be linked to the enhancement of hair fiber. Other papers have also highlighted the utilization of antioxidant compounds is a crucial strategy for preserving cellular young.

3.5. Preliminary stability of formulations

In the centrifugation test, all formulations maintained their initial characteristics, which indicates that they can be conducted for the stability test.

After analyzing the 6 cycles (Figure 3), it was possible to verify that the samples remained unchanged until the 4th cycle. In the 5th cycle there was a slight color change of the XP2 sample and in the 6th cycle to XP1.

The macroscopic stability indicates robustness of the formulations, being able to follow for the stability studies.

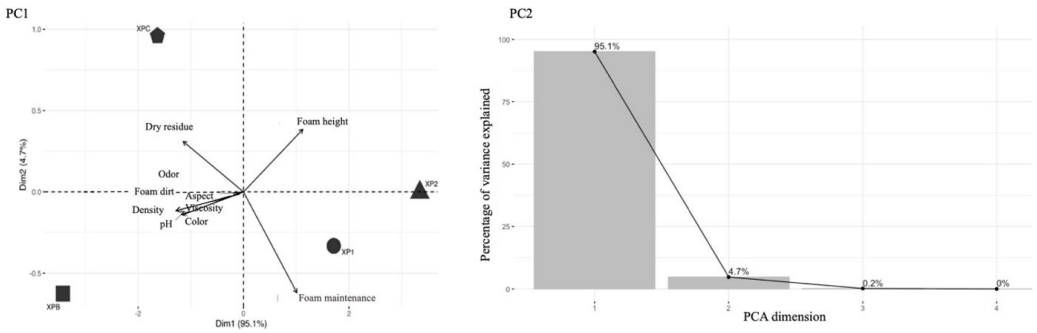

Figure 2. Principal Component 1 (PC1) and Principal Component 2 (PC2).

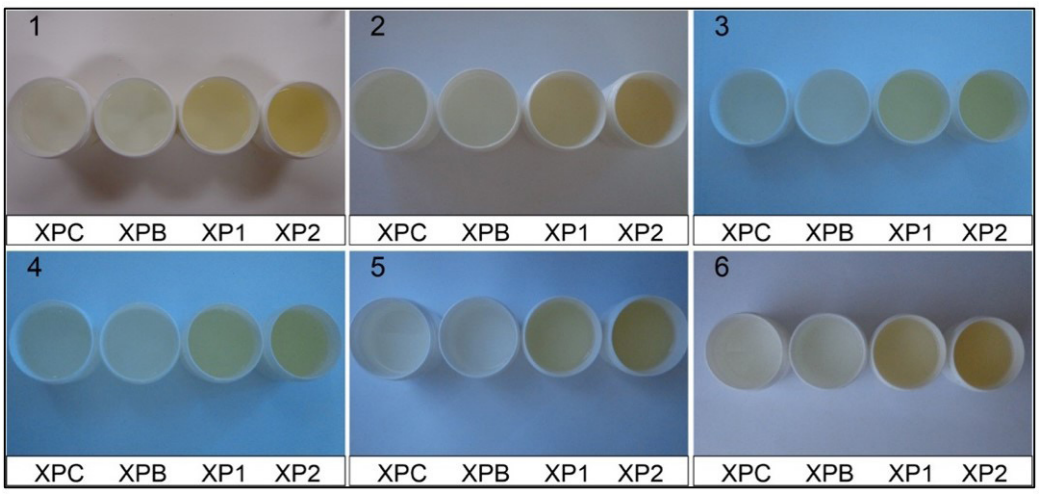

Figure 3. Color and appearance parameters of the formulations of shampoo control (XPC), shampoo base (XPB), shampoo 1 (XP1) containing 0.125 mg/g of *H. speciosa* leaf extract (L_SOX) and shampoo 2 (XP2) containing 0.250 mg/g of L_SOX found in the 6 cycles of the preliminary stability test.

3.6. Accelerated stability of formulations

For XPC, XPB and XP1, the parameters of color, odor and appearance did not change over the 90 days at the two temperatures evaluated. Density, viscosity, pH and foam height showed a slight decrease, especially after the 60th day of evaluation. During the entire test, no dirt was observed in the foam. The variation of parameters was more intense for the formulations stored at 45 ºC than at 5 ºC.

In the XP1 formulation, the antioxidant activity remained more preserved at 5 ºC (45.23 ± 0.40%) than when compared to the sample submitted to 45 ºC (36.18 ± 0.58%). It was observed that on the 90th day of the stability study, the XP1 shampoo at a temperature of 45 ºC showed a slight presence of dirt in the foam, showing that it was no longer effective for cleaning the hair.

In the XP2 formulation, there was an increase in the coloring intensity on the 45th day of storage at 45 ºC. From the analysis on the 60th day, it was possible to observe light precipitation in XP2 at a temperature of 45 ºC. The parameters of odor, appearance, density, pH, foam height and dirt dispersion showed small fluctuations. The viscosity, as with the other formulations, decreased over time at the two temperatures tested.

The XP2 formulation presented a more intense coloring than XP1, possibly due to the fact that XP2 presented twice the amount of incorporated extract, which is also related to the XP2 formulation presenting antioxidant activity superior to XP1. The XP2 formulation showed an initial antioxidant activity of 79.60 ± 0.14% and ended the 90th day with 61.79 ± 0.63% at 5 ºC and 56.77 ± 1.27% at 45 ºC.

The data collected in this test suggest that the milder temperature helps to maintain the antioxidant activity of the formulations. The XP2 formulation was the one that obtained the best result, with greater antioxidant activity and better maintenance of the analyzed parameters than XP1.

Hair cosmetics with antioxidant activity are beneficial for all types of hair, particularly dyed hair, since the antioxidant activity inhibits lipid peroxidation, improves mechanical properties, and maintains color and shine. Additionally, it protects against ultraviolet radiation and improves the hair fiber's integrity (Fernández et al., 2012), while also reducing hair loss due to breakage (Kim, 2011). As a result, the use of antioxidants in hair cosmetics appears to build a relationship with the hair fiber in terms of protection, resistance, and shine.

4. Conclusion

The L_SOX extract had the highest concentration of polyphenols and antioxidant activity. This made it the optimal extract for use in the product's formulation. The XP2 formulation demonstrated favorable organoleptic, physical, and chemical properties, as well as excellent cleaning properties and high antioxidant activity.

Because foam creation is a sensory component to consider when choosing a shampoo, the low foaming power must be evaluated. These are preliminary results and further research may be conducted using different concentrations of the extract, as well as the inclusion of a main surfactant to boost the foaming power. A free-lauryl shampoo from mangabeira leaves is an innovative and ecologically sustainable (eco-friendly) option.

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