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BRENNA COUTO NICARETTA
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FACIAL BIOCOSMETICS BASED ON NATURAL DYES FROM AMAZON WOOD RESIDUES

SANTARÉM-PA
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Trabalho de Conclusão de Curso apresentado ao curso de Bacharelado em Farmácia, da Universidade Federal do Oeste do Pará, Instituto de Saúde Coletiva, como requisito para a obtenção do grau de Bacharel em Farmácia.
Orientador (a): Kariane Mendes Nunes

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RESUMO

A ocorrência de diversos flavonoides com potencial antioxidante e antiinflamatório na madeira pode ter uma aplicação viável na indústria de biocosméticos. Este trabalho estuda a viabilidade técnica de redirecionamento dos resíduos madeireiros das principais espécies amazônicas, *Dipteryx odorata* (cumaru), *Manilkara huberi* (maçaranduba), *Hymenaea courbaril* (jatobá) e *Handroanthus serratifolius* (ipê amarelo), para obtenção de corantes naturais para obtenção de biocosméticos faciais. Para cada amostra de corante, foi determinado o perfil fitoquímico de compostos fenólicos e o teor de flavonoides totais expresso em rutina e quercetina (ug mL–1). O rendimento dos extratos de resíduos de madeira foi 6,5% para cumaru, 8,1% para maçaranduba, 16,3% para jatobá e 8,8% para o ipê amarelo. As propriedades físico-químicas estavam de acordo com os compêndios oficiais. A análise por TLC revelou a presença de flavonoides (quercetina) e taninos hidrolisáveis (ácido gálico). A concentração do teor total de flavonoides expressos em rutina e quercetina variou entre 0,106 a 7,5 e 0,1 a 3,12ug.mL–1, respectivamente, para todas as espécies. Obteve-se formulações biocosméticas faciais com propriedades organolépticas (aparência, cor e odor) adequadas para uso facial a partir dos corantes de maçaranduba, jatobá e ipê-amarelo. Portanto, o reaproveitamento de resíduos madeireiros de espécies amazônicas é uma proposta tecnológica inovadora e sustentável para agregar valor aos bioprodutos da biodiversidade amazônica, contribuindo para o desenvolvimento socioeconômico da região.

Palavras-chave: Aproveitamento de resíduos madeireiros. Corantes naturais. Biocosméticos faciais.

ABSTRACT

The occurrence of several flavonoids with antioxidant and antiinflammatory potential properties in wood may have a viable application in the biocosmetic industry. This paper studies the technical viability of redirecting wood residues of the main commercialized Amazonian species, *Dipteryx odorata* (cumaru), *Manilkara huberi* (ironwood), *Hymenaea courbaril* (Brazilian cherry) and *Handroanthus serratifolius* (yellow ipe), to obtain natural dyes for facial biocosmetics development. For each dye sample, phytochemical profile of phenolic compounds and the total flavonoids content expressed in rutin and quercetin (ug mL–1) was determined. Wood residues extract yield was 6.5% for cumaru, 8.1% for ironwood, 16.3% for Brazilian cherry and 8.8% for yellow ipe. The physical-chemical properties were compliant with the official compendia. TLC-analysis revealed the presence of flavonoids (quercetin) and hydrolyzable tannins (gallic acid). Concentration of total flavonoids content expressed in rutin and quercetin varied between 0.106 to 7.5 and 0.1 to 3.12ug.mL–1, respectively, for all species. Facial biocosmetic formulations with organoleptic properties (appearance, color and odor) suitable for facial use could be obtained from ironwood, Brazilian cherry and yellow ipe dyes. Therefore, Amazon species wood residues reuse is an innovative and sustainable technological strategy to add value to bioproducts of Amazon biodiversity, contributing to the region's socio-economic development.

Keywords: Wood residue reuse. Natural dyes. Facial biocosmetics.

SUMÁRIO

Introduction

Changes in paradigms in public health and consumers preferences increase demand for bioproducts from sustainable production chains in the food, pharmaceutical and especially cosmetic sectors (Amberg & Fogarassy, 2019; Annunziata & Mariani, 2018; Ng et al., 2020; Perito et al., 2020). The scientific community has been alerting health authorities to the risks to public health caused by various substances and synthetic excipients found in various cosmetic products, often at levels higher than allowed (Bilal & Iqbal, 2019; Borowska & Brzóska, 2015). Inorganic dyes with a high heavy metals content, like lead (Pb), aluminum (Al) and cadmium (Cd), which can be hyper–allergenic, carcinogenic, and toxic, are among the potentially toxic but extensively used components in the production of facial cosmetics (Bocca et al., 2014; Liu et al., 2013; Perito et al., 2020; Zakaria & Ho, 2015). Cosmetic products that follow the new market trend of green consumers should be free of excipients of synthetic origin (Bilal & Iqbal, 2019).

The search for innovative and sustainable alternatives for obtaining safer cosmetics has made obtaining natural dyes a strategic axis of studies that aims to meet the current technological bottleneck in the cosmetics industry, which is the use of dyes and excipients synthetics and their public health problems (Bilal & Iqbal, 2019; Borowska & Brzóska, 2015). Natural, organic or biocosmetics are products made from 100% natural ingredients extracted directly from a plant, animal, microbe, enzyme, insect and organic crops (Goyal & Jerold, 2021). The occurrence of several compounds with antioxidant and antiinflammatory potential properties in wood (Da Costa et al., 2021; Costa et al., 2014) may have a viable application in the bio cosmetic industry.

The Amazon rainforest has the largest biodiversity reserve on the planet and provides multiple products. However, its management and use must be done in a sustainable way to minimize ecological impacts. Forest management and the wood industry are among the most relevant economic activities in the Amazon region. Nevertheless, these operations produce a high volume of residues, constituting a great opportunity for its use.

Forest management activities produced at least 0.3 m3 residues for each cubic meter extracted, which can negatively affect the natural regeneration of the site (Ribeiro et al., 2019, 2016). In the Amazon wood industry, about 0.6 m-3 of residue is generated for each cubic meter of a sawed log. Considering that around 3.1 million m-3 of logs are extracted, traded and sawed per year only in the state of Pará, the largest producer in the Amazon region (F.W. C. Andrade et al., 2022), we have almost 3 million m3 of forest and industrial wood residues that can be used. In addition, the inadequate disposal and inefficient management of wood residues have caused serious socio-environmental impacts in the Amazon region (Braz et al., 2014), as air quality risk due to inadequate combustion, fire hazard, emission of greenhouse gases, potential water contamination, harboring of dangerous pests and vectors (Dias-Robles et al., 2014; Adhikari & Ozarska, 2018; Ogunwusi, 2014; Owoyemi et al., 2016).

Therefore, with the increased harvest and sawing of native Amazonian wood, it is necessary to adopt measures regarding the use of residue generated by the processing of these industries (M. C. N. Andrade et al., 2013). This paper studies the technical viability of redirecting wood residues for reuse by the cosmetic industry to obtain natural dyes. The acquisition of these natural dyes becomes an innovative alternative of sustainable character in synergy with the approaches of the circular bioeconomy consequently, fostering the biobusinesses chain based on the fair-trade policy in the region.

We used the wood residues of the four most commercialized species in the Brazil Lower Amazon mesoregion, *Dipteryx odorata* (cumaru), *Manilkara huberi* (ironwood), *Hymenaea courbaril* (Brazilian cherry) and *Handroanthus serratifolius* (yellow ipe), to obtain dyes and use them in the development of facial biocosmetics. This strategy could subsidize the creation of favorable environments for innovation, sustainability and enhancement of the value chains of Amazonian socio–biodiversity, promoting socioeconomic and environmental benefits to the Lower Amazon region.

Material and methods

Collection of plant material

The material used in this research comes from forest harvest residues in a management area, close to the “Cachoeira do Aruã” community, located in Santarém (Figure 1). Five trees were sampled for each species selected among the four most commercialized in the region and heartwood samples were taken at two meters from the second fork branch (one per tree). All sampled branches were over 50 cm in diameter (Figure 2). Were also collected botanical exsiccates and wood specimens for scientific identification by Federal University of Western Pará’s herbarium and xylarium experts, who identified them as *Dipteryx odorata* (Fabaceae), *Manilkara huberi* (Sapotaceae), *Hymenaea courbaril* (Fabaceae) and *Handroanthus serratifolius* (Bignoniaceae). These woods are also known in the international market as cumaru, ironwood, Brazilian cherry and yellow ipe, respectively.

The wood branches samples were mechanically transformed with an electric planer into sawdust (retained in a 75 µm mesh sieve) and later passed through a Willey mill resulting in a fraction called wood powder (600 µm mesh). In this study, we used a mixed wood powder sample of the five trees per specie for characterization.

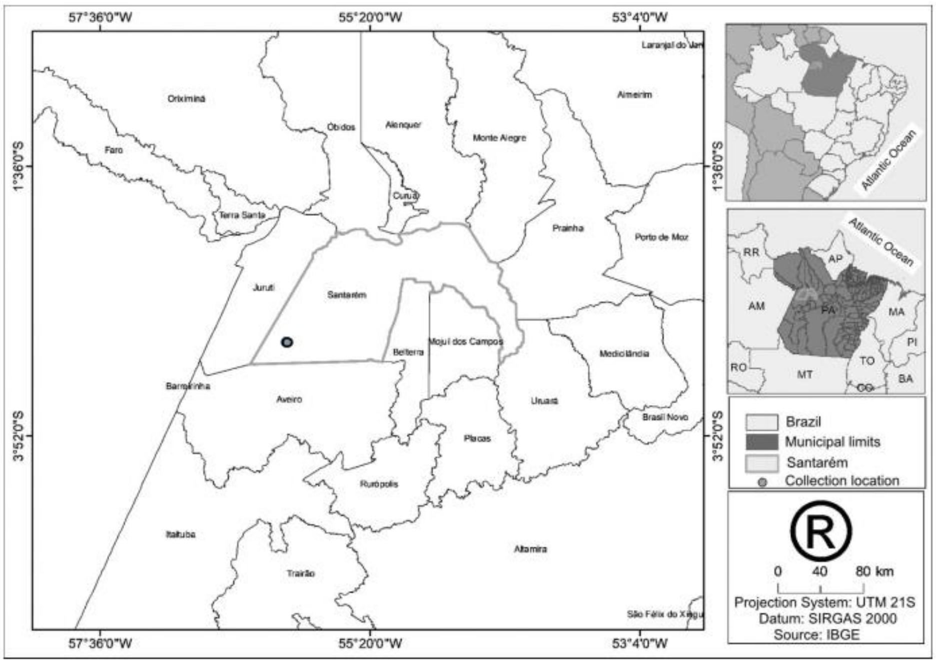

Figure 1. Location map of the region where wood residue was collected.

Figure 2. Collection of plant material. A and B – sawing the second fork branch of Manilkara huberi and Dipteryx odorata respectively. C and D – measuring the branch diameter of Manilkara huberi and Hymenaea courbaril respectively. E – Sampling Dipteryx odorata for laboratory analysis

Physical-chemical characterization

The determination of moisture content, ash content, granulometry and pH was carried out using the wood residue powders. All physical-chemical characterization tests were performed according to the methodologies recommended by the Brazilian Pharmacopoeia (ANVISA, 2010).

Natural dyes extraction

The natural dyes were extracted at high temperatures in a Soxhlet apparatus according to D'Auria et al. (2020). The alcoholic extract from each species was prepared by mixing 50 g of mixed powder wood residue with 700 mL of ethanol (absolute) for 8 hours. The mixture was concentrated under reduced pressure at 50°C in a rotary evaporator with an integrated cooling system. Each extraction was carried out in triplicate for all species (3 samples replicate x 4 species, totalizing 12 observations). At the end of this process, the yield of the extract was calculated with the equation: [extract mass (g) / dried material mass (g)] x 100.

Thin–layer chromatography (TLC)

The phytochemical profile of phenolic compounds in the wood residues extracts was obtained by Thin–Layer Chromatography (TLC), according to the Brazilian Pharmacopoeia Standards (ANVISA, 2010). For the analysis, aluminum chromatoplates (10 x 10 cm) and 60 mesh silica gel stationary phase with a 0.20 mm thickness layer were used. A 10 µl aliquot of each extract with a concentration of 10 mg mL–1 in ethanol was applied in duplicate to the chromatographic plate. Quercetin was used as a reference standard for flavonoids and gallic acid, for hydrolysable tannins. The mobile phase used in the chromatographic analysis was a quaternary mixture with composition (v/v) of ethyl acetate: formic acid: acetic acid: water (100:11:11:27). The specific developer used for the visualization and detection of phenolic compounds was an AlCl3 ethanolic solution at 5%. The plates were read using an Entela UVGL 58 ® UV–vis light chamber under UV365 nm light (Américo et al., 2020).

Total flavonoid content

After obtaining the phenolic compounds' phytochemical profile by TLC, total flavonoid content was determined by the spectrophotometric method, using rutin and quercetin standards (Sigma–Aldrich®), according to Nunes et al. (2011), with modifications. Two external standardization calibration curves were obtained from the rutin and quercetin standard solutions, with methanol as a solvent. Six dilutions in triplicates were acquired from the standard solutions in the concentration of 1:1 (m/v). The flavonoid content absorbances were obtained in a spectrophotometer (LGI SCIENTIFIC model LGI–VS– 721 N), at a wavelength of 350 nm. The flavonoids quantification in wood residue extracts was calculated by linear regression equations obtained from the rutin and quercetin calibration curves. The total flavonoids content was calculated in rutin and quercetin equivalents and expressed in µg mL-1 (Nunes et al., 2011).

Facial biocosmetics development and organoleptic analysis

For all biocosmetic samples, organoleptic analyzes (appearance, color and odor) were performed according to official compendia (Isaac et al., 2008). Two types of facial biocosmetics formulations were developed, powder and cream foundation (Table 1). Powder foundations were obtained by mixing white clay and starch in proportions described in Table 1, and then between 10 to 25% of the wood residues, extracted dyes were incorporated. For the cream foundations, an O/A emulsion was obtained by the phase inversion method, the oil (A) and aqueous (B) phases were subjected to heating in a thermostatic bath at a temperature between 40–45°C and then phase B was poured over phase A.

Table 1. Components of facial biocosmetics formulations.

COMPONENTS	% (W/W)
FACE POWDER FOUNDATION	
WHITE CLAY	22.9
STARCH	45.7
LAVANDER ESSENCIAL OIL	9.8
DYE	21.0
FACE CREAM FOUNDATION	
CARNAÚBA WAX	1.6
CUPUAÇU BUTTER	1.6
GRAPE SEED OIL	32.1
ZINC OXIDE	16.0
DYE	3.6
GLYCERYL STEARATE	4.8
DISTILLED WATER (Q.S.P)	100.0

Statistical analysis

We assessed the data using descriptive statistics and analysis of variance ($p < .05$) with four treatments (species) and three repetitions per treatment. We compared the average values with the Tukey test at the level significance of 5%. Statistical analysis was done in Minitab software, version 19.2020.1 (Minitab Inc.; NY, USA).

Results

We observed that *Hymenaea courbaril* presented the highest gravimetric yield, differing from the others, but also had a higher ash content. On the other hand, *Dipteryx odorata* had low yield and ash values (Table 2). The granulometry of *Handroanthus serratifolius* was classified as semi-fine and the others had a moderately thicker powder.

Table 2. Physical-chemical characteristics of Amazonian species wood residues.

Wood species	*Desiccation loss*	*Gravimetric Yield (%)*	*Ash content (%)*	*pH*	*Powder granulometry*
Dipteryx odorata	9.03(±0,08) b	6.5 (±0,6)c	0.30(±0,01)b	4.95	MT*
Manilkara huberi	10.3(±1,16) a	7.4 (±1,1)bc	0.28(±0,02)b	5.05	MT*
Hymenaea courbaril	8.9(±1,14) b	16.3 (±0,4)a	0.50(±0,01)a	5.44	MT*
Handroanthus serratifolius	8.12(±1,17) c	8.8 (±1,0)b	0.29(±0,02)b	5.56	SF**

* Moderately Thick; ** Semi–fine; Means that do not share a letter, in column, are significantly different. Standard deviation in parenthesis.

The extractive method has a direct influence on the total yield of the extract (Dhanani et al., 2017), therefore, the choice of the extraction at high temperature in a Soxhlet apparatus was based on the optimization of the dye's extraction yield from the wood residues.

TLC analysis revealed a Retention Factor (Rf) of 0.87 for the quercetin standard and 0.85 for the gallic acid standard. The dyes extracts presented an Rf ranging from 0.81 to 0.87, whereas cumaru presented an Rf of 0.85 for quercetin and 0.87 for gallic acid, which was the closest to the standard Rf. The extract's colorings in the TLC, when compared to the reference standards, showed the characteristic coloring of phenolic compounds.

For the total flavonoids content determination, the rutin and quercetin standards calibration curves were plotted (Figure 3). The spectrophotometric method showed linearity at 350 nm for the analyzed concentrations (2.0–40.0 µg mL-1), the linear equation (leastsquares method) for the rutin calibration curve is y = 0.0179x + 0.017, with R2 = 0.996, and for the quercetin calibration curve is y = 0.0412x + 0.0243, with R2 = 0.997, where y is the absorbance (nm) and x is concentration (μg mL-1). We observe a higher concentration of flavonoids in

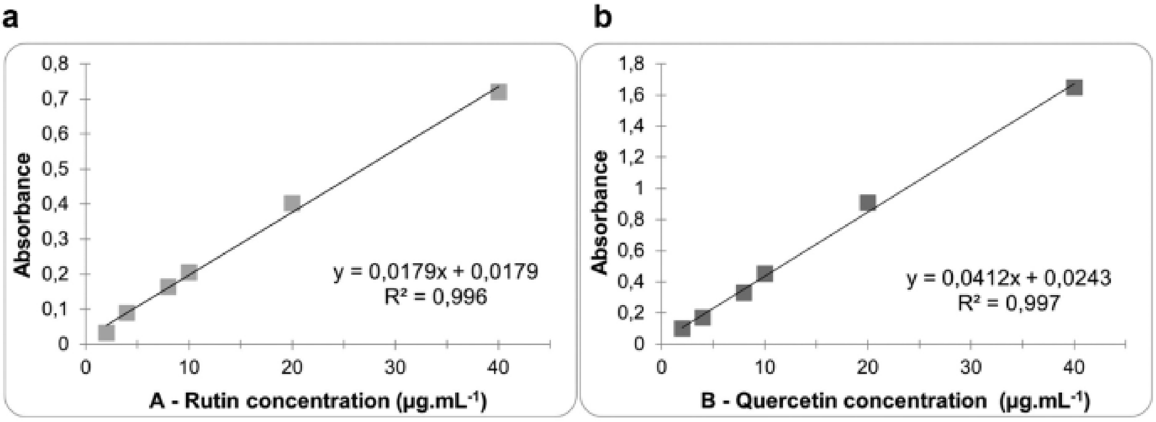

Figure 3. Calibration curve constructed with standards rutin (A) and quercetin (B). The points indicate the concentrations of the standards used for the construction of the curve and obtaining the equation for quantification of total flavonoids in the samples.

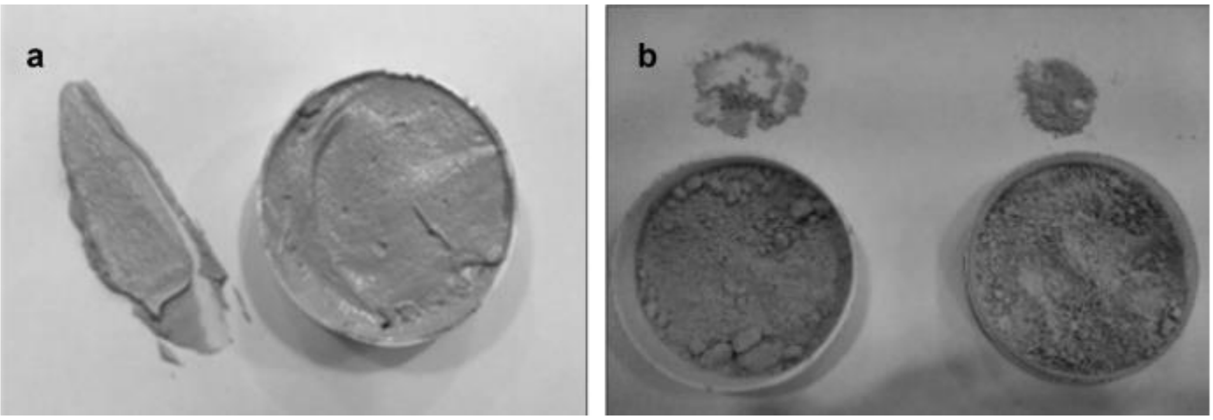

Figure 4. Facial biocosmetics: (A) creamy foundation with dyes extracted from Hymenaea courbaril wood residue (B) powder foundation with dyes extracted from Handroanthus serratifolius (left) and Manilkara huberi (right) wood residue.

In the biocosmetics organoleptic analysis, the powder foundations presented a dry powder aspect for the species *Manilkara huberi* (ironwood), *Handroanthus serratifolius* (yellow ipe) and *Hymenaea courbaril* (Brazilian cherry), with good skin coverage and easy spreading, and a granular aspect for the species *Dipteryx odorata* (cumaru), which impaired spreading. All powders presented a characteristic woody odor. The cream foundations presented a homogeneous appearance with a creamy texture, good skin coverage and easy spreading and a characteristic butter and vegetable oil odor. All cream foundations presented a light color palette, with shades of pink for ironwood and Brazilian cherry and beige for yellow ipe and cumaru (Figure 4).

Discussion

The wood industries in the Amazon region produce significant volumes of wood residues in their primary and secondary processing, without rational or economic use (Lima et al., 2020; Luz et al., 2021). Once accumulated, these residues have caused environmental damage through fires, leachate formation and gas emissions with toxic metals generation. Even with the National Solid Waste Policy (Government of Brazil, 2010) the country still cannot implement its goals of waste generation reduction, redirection, and reuse.

The performance evaluation of the extraction process is a conditioning factor that determines the technical viability of extracting natural dyes for biocosmetic production (Franzen et al., 2018). The chosen extraction method allowed the achievement of adequate extracts yield for coloring purposes and a desirable color palette for facial cosmetics was obtained. The natural dyes percentage used in the foundations ranged from 2.0 to 30%. The study by Cavalcanti et al. (2018) describes the development of a mineral cosmetic formulation containing inorganic dyes, such as red iron oxide and yellow iron oxide, with percentages ranging from 5.0 to 23%.

The particle size is decisive in the performed extraction process because the smaller the particle size, the greater the liquid extractor's contact surface with the plant material, which increases the extraction yield (Cho & Sohn, 2016). However, we observed that *Hymenaea courbaril*, which had a moderately thicker powder, had a higher yield than *Handroanthus serratifolius*, which had a semi-fine powder granulometry. This difference is probably related to the solvent chosen in the extractive method, since species differ in the solubility of their secondary metabolites, resulting in different yields.

The moisture content, determined by the desiccation loss test, is an important indicator of product stability and quality and varied between 8 to 10%, which corroborates with the values specified in official compendia for powders made with plant products (ANVISA, 2010). It is important to highlight that moisture content, when it comes to wood residues, can be related to the time of sawdust storage and it diverges from the values of materials collected in loco (Souza et al., 2012).

In the ash content test, wood residues presented percentages within the limits specified in the official compendia for plant products (0.2% to 0.5%). According to Couto et al. (2009), the ashes represent the inorganic matter, which could be a constituent or contaminant of vegetal products, therefore it is a parameter for quality control. In studies carried out with natural dyes, the authors found ash contents of 2.04% and 3.4% for *Bixa orellana* extract and *Hylocereus undatus* pulp, respectively (Silva, 2017). The barks of the Amazonian wood species *Parkia* *panurensis* and *Dicorynia paraensis* presented ash contents of 2.93 and 9.66%, respectively (Freire & Vianez, 2014).

The phytochemical profile by TLC showed that the wood residues dyes from the analyzed species presented phenolic classes in their chemical composition, such as flavonoids and hydrolyzable tannins. These metabolic classes are widely described in the literature for their biological activities, most importantly their antioxidant and anti-inflammatory properties (Macedo et al., 2017), which could provide necessary functionality to serve several segments within the cosmetics sector, such as dermo-cosmetics and cosmeceuticals (Cherubim et al., 2019; Moreira et al., 2017).

The determination coefficients (R2) for rutin and quercetin standards calibration curves were greater than 0.99 as established in resolution (ANVISA, 2017), proving the method adequacy to the evaluated range and ensuring the reliability of the obtained data to determine the total flavonoid content (Table 3). Among the species with the highest total flavonoids content, yellow ipe stands out with 7.5 and 3.2 µg mL–1, expressed in rutin and quercetin, respectively.

Some studies emphasize the secondary metabolism of plants mentioned in this study. In *H. courbaril* the predominant class is flavonoids and its sap extract has already demonstrated the potential for inducing the scaring of cutaneous wounds (Da Costa et al., 2021; Costa et al., 2014). In Brazilian cherry, some chemical components, such as tannins and flavonoids, are probably associated with biological activities (Fernandes et al., 2005). In *Handroanthus serratifolius* there is the presence of reducer sugars, organic acids, alkaloids, depsides and depsidones, saponins, phenols and tannins (Duarte et al., 2015). In cumaru wood, there is the presence of isoflavone, retusin and its derivatives odoratin and dipterixin (Lorenzi & Matos, 2002). The chemical compounds diversity in ironwood (*Manilkara zapota*), such as phenolic compounds, has also been reported (Carvalho Filho et al., 2012).

Table 3. Total flavonoids content in extracts from Amazonian wood residues, expressed in rutin and quercetin.

SAMPLE	RUTIN µG ML–1	QUERCETIN µG ML–1
DIPTERYX ODORATA	4.586	1.837
MANILKARA HUBERI	0.106	0.109
HYMENAEA COURBARIL	2.743	1.03
HANDROANTHUS SERRATIFOLIUS	7.5	3.12

Our findings corroborate the hypothesis that processed residues from selected wood species are a potential natural dye source and have a promising chemical composition for their application in cosmetics. The presence of phenolic compounds, such as flavonoids, in addition to promoting beneficial effects to the skin with antioxidant action, can also function as a reducer agent for oxidation reactions, which occur in cosmetic formulations that contain an oil phase (Cherubim et al., 2019). This synergism between the action and functionality of wood residues and natural dyes may prevent organoleptic changes and enhance their applicability in cosmetics.

All the tested biocosmetic formulations presented compatibility between their excipients and incorporation of wood residues dyes with organoleptic properties suitable for facial cosmetics use (Daudt et al., 2015).

From this perspective, and in line with one of the 2030 Agenda for Sustainable Development (UN, 2015) goals, this work provides a rationale and sustainable alternative for managing forest products through the reuse of wood residues, converting them into natural dyes for the cosmetics industry. As a result, income is generated through new production chains that move the local bioeconomy, aiming the social and economic development in the Amazon.

Conclusions

The results demonstrated the technical viability of obtaining natural dyes from Amazonian species' wood residues. The dyes presented phenolic compounds in their chemical composition, which are of great interest to the cosmetic industry, due to their antioxidant activity. In addition, the blend of natural dyes showed appropriate physicochemical properties for facial biocosmetics, with a pink and beige color palette. Therefore, the work presented an innovative and sustainable technological strategy for wood residue reuse from the circular bioeconomy perspective, adding value to products from the Amazonian biodiversity.

Author contributions

BCN, MECS, CVPC, Jefferson M. Feitosa, Sara F. de Souza, conducted all the experimental essays: dyes extraction, physical-chemical and chemical characterization, biocosmetic formulation development. KCFC contributed by analyzing chemical and chromatography data and discussion. FWCA contributed to the revision of the manuscript and statistical analysis. VHPM and KMN contributed to the writing of the manuscript and coordinated the work. All authors read and approved the final version of the manuscript.

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