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Characterization of Bixin by UHPLC-UV-Vis and Application as Latent Fingermark Developer

Danielle Tapia Bueno [1,2], Amanda Fonseca Leitzke [1,3], Rosane Lopes Crizel [4], Cristina Jansen-Alves [1,4], Juliana Porciuncula da Silva [1], Gabrielly Quartieri Sejanes [1], Kristiane de Cássia Mariotti [5] and Claudio Martin Pereira de Pereira [1,2,3,*]

[1] Laboratory of Innovation and Solutions in Chemistry, Bioforensics Research Group, Federal University of Pelotas, Pelotas, RS, 96160-000, Brazil
[2] Postgraduate Program in Materials Science and Engineering, Federal University of Pelotas, Pelotas, RS, 96010 -610, Brazil
[3] Postgraduate Program in Biotechnology, Federal University of Pelotas, Pelotas, RS, 96160-000, Brazil
[4] Department of Food Science and Technology, Federal University of Pelotas, Pelotas 96010-610, Brazil
[5] Identification Group, Brazilian Federal Police, Porto Alegre, RS, 90610-093, Brazil
* Correspondence: claudiochemistry@gmail.com

Abstract: Fingerprints are crucial to human identification and represent a valuable form of physical evidence in criminal investigations. Consequently, research into the development of new materials for digital printing is constantly expanding. In this context, the use of materials derived from natural compounds has received increasing attention, mainly due to the toxicity associated with commercial powders. In this sense, interest in bixin arose, which is extracted from annatto seeds and has traditionally been used as a natural dye and has recognized medicinal applications. Still, its potential in the field of papilloscopy remains unexplored. In this study, composites were developed from extracted bixin. The extracted bixin was characterized by UV-visible spectroscopy, showing absorption peaks at 429, 453, and 481 nm. Meanwhile, standard bixin had peaks at 429, 457, and 487 nm. Thin-layer chromatography revealed bands with an R_f value of 0.4 for both extracted and standard bixin. Liquid chromatography confirmed identical retention times of 7.07 minutes for both bixins. The bixin/$ZnCO_3$ and bixin/kaolinite composites demonstrated effective development of natural and sebaceous latent fingerprints, allowing the visualization of sweat pores in some of the developed fingerprints. Thus, the formulated composites present an innovative and promising alternative for application in the area of papilloscopy.

Keywords: bixin; liquid chromatography; composites; zinc carbonate; kaolinite; fingermark; fingerprint revealers and papilloscopy

1. Introduction

Carotenoids are the most diverse and widespread pigments found in nature, exhibiting various biological properties. Comprising more than 700 compounds, carotenoids are lipophilic and typically exhibit yellow, orange, or red colors [1]. Compounds resulting from the cleavage of carotenoids are commonly referred to as apocarotenoids, representing carotenoids in which the carbon skeleton has been shortened by removing fragments from one end or both ends. Apocarotenoids are garnering attention due to their potential contributions to positive health-promoting actions [2].

Bixin is a liposoluble apocarotenoid and the main compound extracted from the annatto seed (*Bixa orellana L.*) of the *Bixaceae* family [3]. Bixin is a shrub native to tropical countries in America. However, its cultivation has now expanded to the Caribbean, Africa, and Asia regions. The pigment extracted from its seeds is commercially referred to as annatto. Annatto serves both as a seasoning and dye, providing a yellow-orange hue to a variety of food products [4]. It is approved as a colorant and additive in the food industry by the Food and Drug Administration (FDA) [5]. Bixin exhibits various beneficial health properties, including antioxidant effects [6], antimicrobial activity [7], anticancer potential [8], anti-inflammatory properties [9], and nephroprotective activity [10].

Bixin ($C_{25}H_{30}O_4$) constitutes 80% of the annatto seed extract and is comprised of an open chain of 25 carbons with a methyl ester group and a carboxylic acid present at the end [5]. Norbixin ($C_{25}H_{30}O_4$) is an apocarotenoid present in small quantities in annatto seeds. Despite its limited abundance, this carotenoid serves as a natural dye and boasts high antioxidant properties, attributed to its extensive system of conjugated double bonds [11]. To analyze annatto, specifically bixin and norbixin, various detection techniques are employed, including ultraviolet (UV), photodiode array (PDA), and mass spectrometry (MS), in conjunction with liquid chromatography (LC) [12]. The identification of carotenoids is commonly performed using UV-vis instruments as detectors. These detectors are user-friendly and can be employed to verify the type, location, and number of functional groups with ease [13].

In addition to its applications in the pharmaceutical, food, ornamental, and textile industries, bixin has the potential to be applied in several fields, including the area of criminalistics, mainly in the study of Papilloscopy. Papilloscopy is a quick and effective expert method that is low-cost and highly practical. In this method, the crystals on the ends of the fingers, palms of the hands, and soles of the feet are utilized to identify human beings, as the papillary impressions left are unique [14]. Fingerprints can be found in visible, latent, or molded forms, typically visualized using powder [15].

Using powder to develop fingerprints is the most common procedure. However, currently available powders still have some limitations, including toxicity, low surface contrast, and poor adhesion to latent fingerprints [16]. Recently, alternative materials to improve latent fingerprints have been developed. Passos et al. (2020) [17] evaluated the potential of algal biomass (*Chlorella sp.*, *Desmarestia anceps*, *Laurencia dendroidea*, *Lessonia searlesiana*, and *Spirulina sp.*) as developers of latent fingerprints. Similarly, Poletti et al. (2022) [18] evaluated the effectiveness of cinnamaldehyde-derived curcumin analogs as fingerprint powder.

Fingerprint powders consist of dye for contrast and a resinous polymer for adhesion. Kaolin and zinc carbonate ($ZnCO_3$) are commonly applied as adhesives [19]. Kaolinite is a polymeric nanocomposite with chemically modified layers, formed by silicate sheets linked to aluminum oxide/hydroxide layers through the arrangement of tetrahedral and octahedral sheets [20], $ZnCO_3$ is a natural crystal with high chemical stability in the air and is notable for its broad applications in optical, physical, and chemical fields [21]. $ZnCO_3$ has been widely used for fingerprint development, as it enables the development of latent fingerprints on a broad spectrum of moist, non-porous surfaces [22, 23, 24].

Considering the beneficial properties of bixin, the objective of this study was to extract and characterize bixin by UHPLC-UV-Vis. Later, develop bixin-based composites associated with materials such as kaolinite and $ZnCO_3$ for detecting latent fingerprints.

2. Materials and Methods

Bixin (9-cis-6,6′-Diapo-ψ,ψ-carotenedioic acid 6-methyl ester - purity ≥ 90.0% (HPLC)) and kaolinite® were purchased from Sigma-Aldrich (St. Louis, USA). Bixa orellana seeds were purchased at a local market in Pelotas, Rio Grande do Sul - Brazil. Methanol was purchased from J.K.Baker SOLUSORB® (San Pedro Xalostoc, Méx - HPLC ≥ 99,96%). Other chemicals were of analytical grade from commercial sources.

Extraction of bixin

The extraction methodology was adapted [25]. Bixin was obtained from a reaction at 60 °C with a seed: solvent ratio (methanol) of 1:15, under magnetic stirring for 30 min. The aqueous extract was filtered, and The solvent was evaporated under vacuum, leaving the powder that was dried in an oven at 50 °C.

Preparation of Bixin Composites

The bixin composites were formulated with zinc carbonate ($ZnCO_3$) and kaolinite in a ratio of 70:30, respectively. To obtain the formulations, the powders were macerated using a glass mortar and pistil, and a homogeneous powder was obtained.

Characterization

Ultraviolet-visible spectroscopy (UV-Vis)

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Ultraviolet-visible spectroscopy (UV-Vis) of standard bixin end bixin extracted was performed in an LGS53 instrument (Monza, Italy – BEL Engineering), with the samples diluted in methanol solvent.

Thin-layer chromatography analysis

Thin-layer chromatography (TLC) was performed on a silica gel (2.5 cm × 6.3 cm plate size, MACHEREY-NAGEL (MN)) by dissolving the compounds in methanol. An aliquot of each sample was spotted on the silica gel plate with a developing solvent system of methanol. The spots were checked under a UV detector at 254 nm (short range) and visible light.

High-performance liquid chromatography with UV-vis detector (UHPLC-UV-Vis)

The chromatographic analyses were performed using a Thermo Scientific Ultimate 3000 model (Waltham, USA) with a standard auto-injector and UV-Vis detector. The separation of analytes was carried out on a C18 column (Ascentis, Bellevue, USA) with a particle size of 5 μM and a pore size of 90 Å. The method employed was adapted [26], where the isocratic mode (A:B 5:95) was used with a mobile phase consisting of water: formic acid (98:2, v/v) (solvent A) and methanol:formic acid (98:2, v/v) (solvent B). The analysis was performed at a temperature of 29 °C, with an injection volume of 20 μL and a flow rate of 0.9 mL/min. The detection wavelength was 459 nm, and the samples were diluted in methanol.

Fingermark deposition

For the revelation of fingerprints, the powder technique was employed using specific brushes (132LBW and CFB100, acquired from Sirchie®). Subsequently, the fingerprints that were revealed were photographed with a Canon EOS Rebel T6 18MP digital camera. For preliminary tests, glass slides were used to deposit fingerprints from four random donors. The method by Pacheco et al. (2021) [27], was used for the deposition of natural and sebaceous fingerprints. To simulate a natural fingerprint deposition, participants washed their hands using neutral soap and water, subsequently engaging in their routine activities for a duration of 30 minutes. For a sebaceous deposition, the donor rubbed their thumb on facial areas, including the forehead and nose, followed by fingertip rubbing to enhance the print with oily components. During the fingerprint placement, the pressure applied was subjectively firm (exerting medium pressure), and the contact time ranged between 3 and 5 seconds. To compare natural and sebaceous fingerprints with the commercial powder, the same fingerprint was deposited on two glass surfaces, with the left half of the fingerprint revealed using the composites and the right half revealed using the White® fingerprint powder acquired from Sirchie (Youngsville, USA).

3. Results

In Figure 1, the UV–Vis absorbance spectrum is shown for standard (a) and extracted (b) bixin, both dissolved in methanol. Absorption of extracted bixin (b) reached peaks at 429, 453, and 481 nm. Meanwhile, the standard bixin had maximum absorption peaks at 429, 457, and 487 nm (Figure 1a), which are very close to the extracted bixin. This indicates that the bixin has been extracted (Figure 1b).

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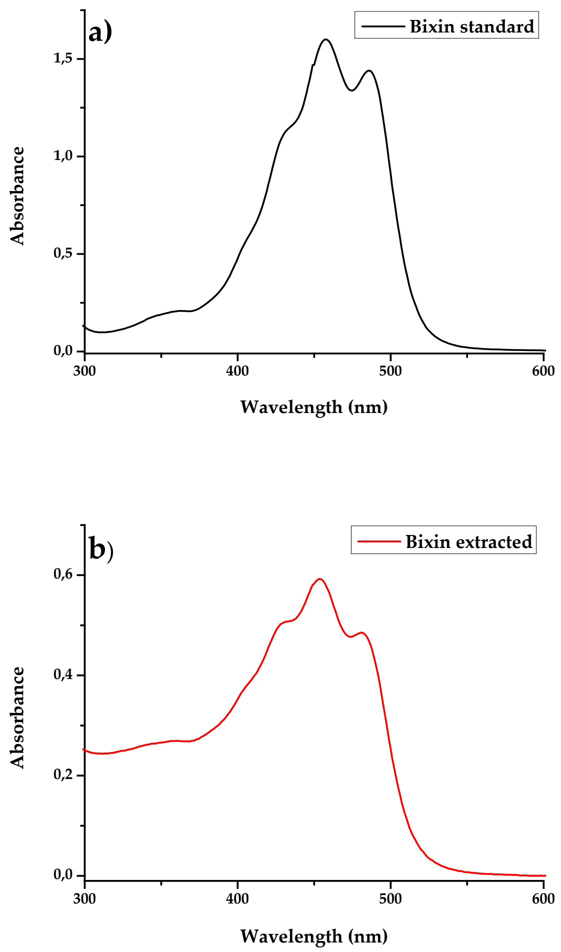

Figure 1. UV–vis absorption spectra of (a) bixin standard and (b) bixin extracted in MeOH.

The extracted bixin and the standard were subjected to thin-layer chromatography (TLC), and bands with the same of the retention factor (R_f) values (0.4 cm) and colors was observed for both, as shown in Figure 2.

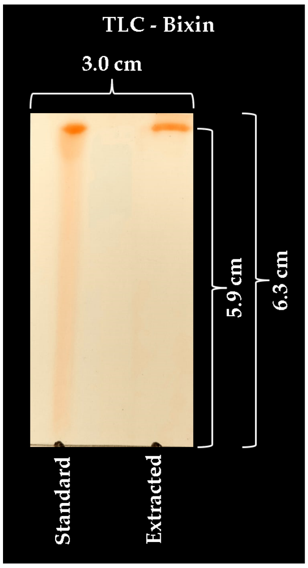

Figure 2. Thin-layer chromatography of standard and extracted bixin in methanol solvent.

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The UHPLC chromatograms of standard bixin (a) and extracted bixin (b) are represented in Figure 3. Peaks with retention times of 7.07 and 8.04 minutes, representing bixin and norbixin, were observed in both samples.

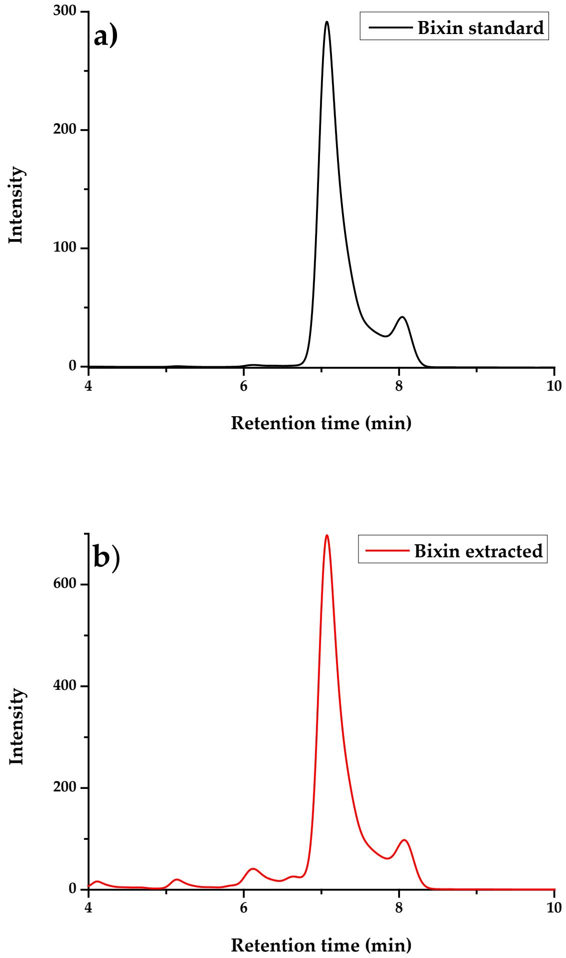

Figure 3. Chromatograms of standard bixin (a) and extracted bixin (b) were monitored at λ=459 *nm*. The bixin retention time (RT) is approximately 7.07 minutes.

The bixin/kaolinite and bixin/$ZnCO_3$ composites were then applied to develop fingerprints by the physical spray method on glass substrates. Figure 4 shows the results obtained for natural and sebaceous fingerprints from a donor. In Supplementary Fig. 1 and 2, it is possible to observe the development obtained for the natural and sebaceous fingerprints of the four donors.

Figure 4. Natural (N) and sebaceous (S) latent fingerprints deposited onto glass surfaces developed using bixin/kaolinite and bixin/$ZnCO_3$.

Furthermore, Figure 5 demonstrates the same fingerprint (natural and sebaceous) developed on the left with the composites and on the right with the commercial white powder. In Supplementary Figures 3 and 4, it is possible to observe the development obtained for the same natural and sebaceous fingerprints from the four donors, with the composites and the commercial powder.

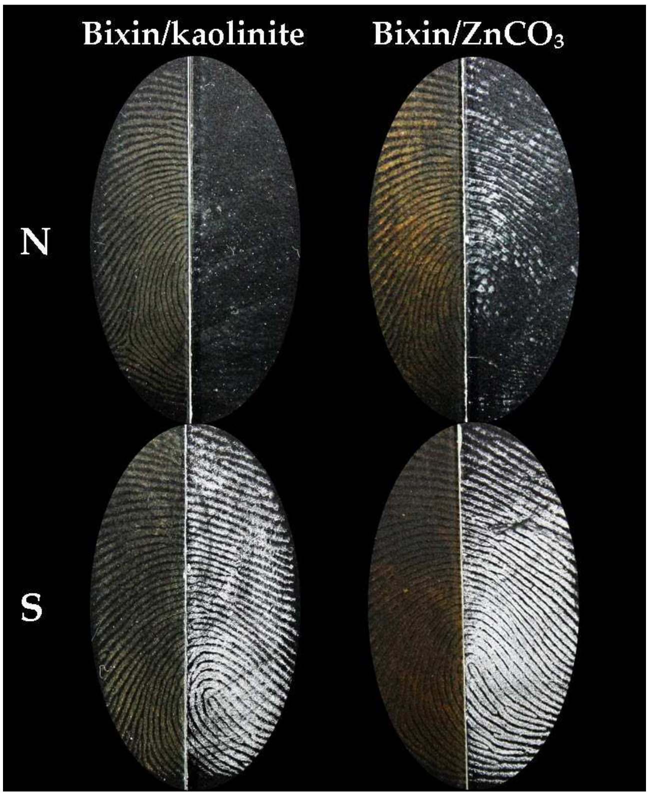

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Figure 5. Digital mark (Natural (N) and sebaceous (S)) sectioned and revealed the left half with composites and the right half with White® commercial powder purchased from Sirchie.

Finally, a detailed evaluation of the fingerprint natural developed with the bixin/kaolinite composite is shown in Figure 6, showing the predominance of a Left Loop fingerprint pattern and some characteristic regions of pores, ridge ending, eye, lake, dot, bifurcation and double bifurcation.

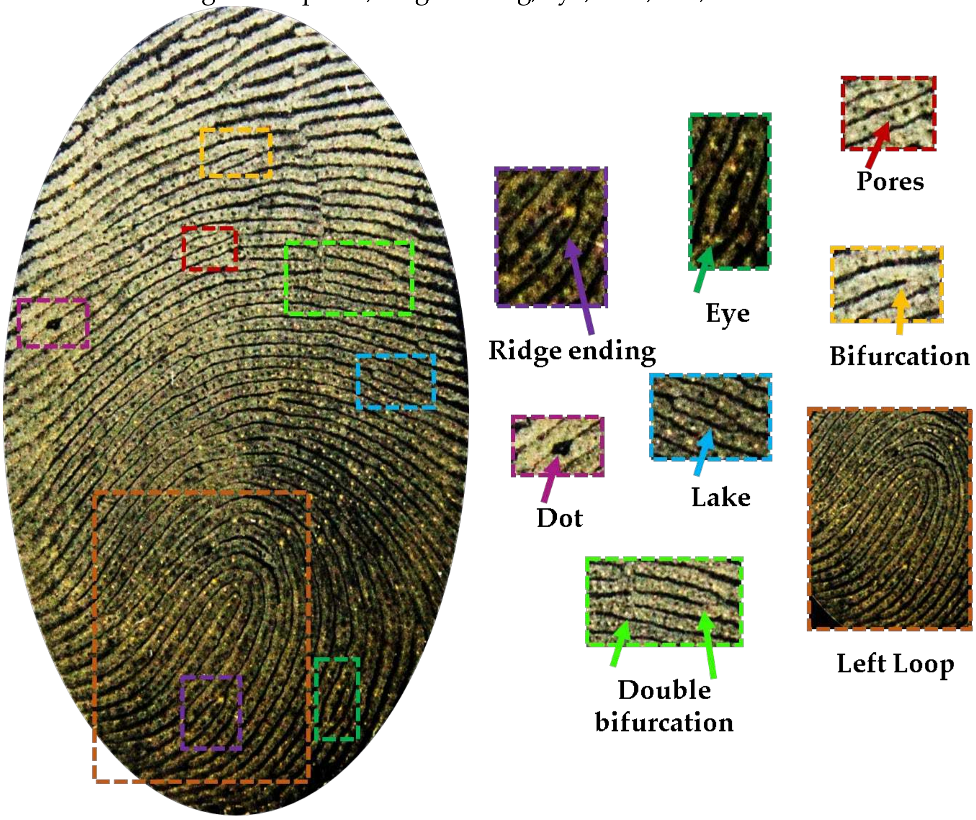

Figure 6. In detail, the fingermark developed with composite bixin/kaolinite and its minutiae identification.

4. Discussion

UV-Vis Spectroscopy analysis showed the bands characteristics of bixin already mentioned in the literature, were observed in the spectra [28]. UV-Vis spectrum analysis is a cheap and easy-to-use technique. It can be observed that there is an alternation between single and double bonds in the structure of carotenoids (Figure 7), which helps in the absorption of light in the visible range of the spectrum [13]. However, for the complete characterization and confirmation of chemical compounds, more refined techniques must be used together [29]. Different authors recommended the use of visible absorption (λ) spectra solubilized in different solvents for the identification of carotenoids and with the use of chromatography through different detection systems, preferably thin layer chromatography and liquid chromatography high efficiency. Furthermore, it is necessary to know the molecular mass of the compounds using a mass spectrum [13].

TCL is a subdivision of liquid chromatography known as planar chromatography. In this technique, the sample is deposited on a surface formed by a thin layer of porous sorbent on an inert surface, called the stationary phase. The mobile phase, which is a liquid solvent, migrates through the stationary phase by capillary action [30]. TLC has already been used in the analysis of biomedical products [31] and vitamins [30]. A similar study has already used TLC for qualitative assessment of bixin [25], with results similar to this study. In this context, the TLC method could be used as a rapid test for the identification of bixin and can also be useful in quality assurance and detection of dye adulteration.

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As is known, annatto harbors two main carotenoids, bixin, and norbixin (Figure 7a and 7b, respectively), both identified in the sample bixin sigma standard [32]. The identification of these carotenoids revealed a predominance of 90% bixin, in accordance with the expected purity of the sample, highlighted by the highest intensity peak with a retention time of 7.07 minutes. Furthermore, a second peak, with a retention time of 8.04 minutes, was identified and attributed to the norbixin molecule (Figure 3a). The chromatogram of the extracted bixin (Figure 3b) corroborated with the results obtained in the standard sample, presenting the same chromatographic profile and thus confirming the effectiveness of the bixin extraction process from annatto seeds.

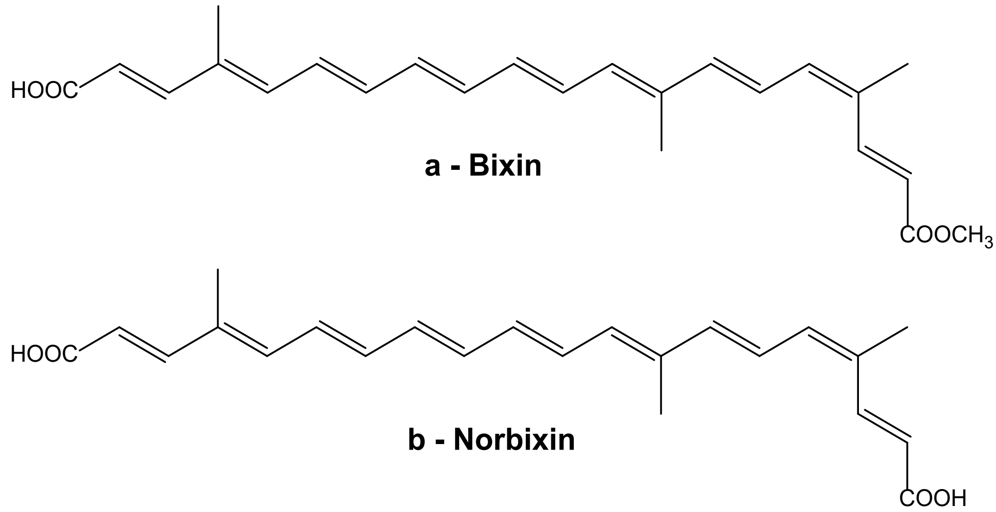

Figure 7. Chemical structures of bixin (a) and norbixin (b).

Papilloscopy is the science that studies human identification through fingerprints, which serve as a valuable source of evidence at crime scenes, given their uniqueness that varies not only between fingers but also among individuals. The use of fingermarks in human identification dates back over a century [33]. In the field of forensic sciences, fingerprints are based on Locard's exchange principle, which posits that every contact between two objects results in a transfer of traces between them. Thus, the contact of a fingerprint facilitates the transfer of materials from the papillary ridges of the fingers to the surface during this interaction [34].

In criminal investigations, fingerprints take the form of physical evidence as the residue left by the fingermark can preserve exogenous compounds such as drugs, explosives, and chemicals. Additionally, the physical properties of fingerprints are utilized in identifying the perpetrator of a crime, given that the details of the ridges generate a unique pattern [35].

Frequently, the fingerprints found are latent, meaning they are invisible to the naked eye, necessitating the use of specific methods for their visualization [36]. Various methods for the revelation of latent fingerprints (LFP) are employed, including optical methods (ultraviolet laser), chemical methods (fuming with cyanoacrylate), and physical methods (spraying and powder deposition) [37]. The technique for revealing latent fingerprints using either conventional or unconventional powders is contingent upon the physicochemical properties of their components and the particle size that constitutes them. The $ZnCO_3$ is known for presenting small particles, having good adhesion, and the ability to reveal clear fingerprints on dark-colored articles [33].

Rohatgi et al. (2015) [38] developed a formulation containing a suspension of $ZnCO_3$, crystal violet dye, and a commercial liquid detergent, and the results obtained were satisfactory. The formulation was able to reveal clear, crisp, and detailed fingerprints on non-porous items. The formulation can be applied to wet and dry surfaces. Similarly, formulations containing $ZnCO_3$ ($ZnCO_3$ + commercial liquid detergent + water + eosin Y) produced positive results under destructive conditions, where successful development of latent prints was observed on smooth surfaces exposed to high temperatures of 200 °C and water [39]. Thus, even in destructive crime scene conditions, zinc carbonate is a suitable reagent for the development of latent marks.

Typical latent fingerprint development powders are comprised of a resinous polymer for adhesion and a colorant for contrast. Commonly used adhesives include kaolin, rosin, starch, and silica gel. The colorants may vary and can be either organic or inorganic derivatives [40]. Inorganic-based powders are commonly applied for fingerprint development, including formulations containing titanium dioxide and kaolin, ferric oxide and rosin, manganese dioxide, and kaolin [41]. However, inorganic salts of manganese, lead, cadmium, mercury, and titanium represent an operational risk due to their toxicity. Thus, organic-based formulations have become more popular.

The use of natural sources, such as powders, for fingerprint enhancement still needs to be improved. Examples of successful applications include the use of turmeric powder on various surfaces in criminal investigations [42], Fuller's earth (*Multani Mitti*) powder, recognized for its affordability, simplicity, and easy availability in deciphering latent fingerprints on diverse substrates [43], and the use of marine biomasses (*Chlorella* sp., *Desmarestia anceps*, *Laurencia dendroidea*, *Lessonia searlesiana*, and *Spirulina sp.*) yielding satisfactory results in the development of natural and sebaceous latent fingerprints [17]. Thus, the assessment and exploration of novel sources for post-fingerprint enhancement are crucial to identifying materials characterized by minimal or no toxicity, excellent surface contrast, and enhanced adhesion to latent fingerprints. Bixin, proposed in this study for application in latent fingerprint development, has a high color range from orange-red to yellow tones in the visible region. During its isolation, it is chemically unstable to high temperature, light, pH, and oxygen conditions and then converts into trans-bixin (β-bixin). Bixin is soluble in fats, alcohols, and organic solvents but insoluble in water and is known as a hydrophobic compound [3].

Kaolinite is a promising clay mineral to be used as an adhesion polymer due to its intrinsic properties, such as chemical and thermal stability, easy processing, abundant resources, and low cost. Kaolinite has plate-like structures, and its cohesive energies between the layers differ significantly. However, it does not have intercalated charge-balancing cations due to little or no isomorphic substitution in the tetrahedral and octahedral sheets. Thus, adjacent layers are linked by hydrogen bonds, which makes direct intercalation of Kaolinite with inorganic and/or organic molecules much more difficult than other clays, such as swelling clays. Furthermore, it is also difficult to homogenize kaolinite surfaces, leading to poor dispersion in polymer matrices [44].

In this context, bixin/kaolinite and bixin/$ZnCO_3$ composites were tested as latent fingerprint developers on glass surfaces. The choice of this surface is crucial for the analysis, as various factors such as dryness, adhesive property, size, shape, smoothness, type of material, cleanliness, and porosity are significant [45]. This surface was selected as it is considered ideal for conducting these tests, providing favorable conditions for effectively evaluating fingerprint developers.

To assess the influence that the donor can have on the development of latent fingerprints, Supplementary Figures 1 and 2 display images of natural and sebaceous fingerprints from four different donors, revealed using bixin/kaolinite and bixin/$ZnCO_3$ composites 24 hours after deposition. The donor's impact may vary depending on factors such as sweat rate, quantity and composition of secretions, age, health status, mental stress, biological sex, and ethnicity, among others [45].

Moreover, residues from fingerprints constitute a complex mixture of different water-soluble and lipid-soluble compounds. Natural and sebaceous fingerprints were developed to assess the interaction of the composites with aqueous or lipidic residues from fingerprints. It is worth noting that since hands do not have sebaceous glands, the presence of these compounds in fingerprints occurs due to contact with other areas of the body, such as the nose and forehead [27].

As a result, it was possible to observe that both composites were able to provide general images of natural and sebaceous fingerprint marks for all donors (Supplementary Figures 1 and 2). The contrast difference between the composites is noticeable, with the bixin/$ZnCO_3$ composite standing out. Both powders used to formulate the composites effectively enhanced the adhesion of bixin, a key characteristic to be improved. This is particularly significant as bixin already exhibits intense coloration, i.e., high contrast, making it well-suited for application in the field of papilloscopy.

In general, when comparing natural and sebaceous fingerprints, a more significant enhancement in the development of natural fingerprints is observed with the use of composites. However, the results do not differ substantially for sebaceous fingerprints. For the bixin/kaolinite composite (Supplementary Figure 1), no significant differences were identified in natural fingerprints among the four donors. Regarding sebaceous fingerprints, clearer visualizations were noted in donors 1, 3, and 4, with donor 3 exhibiting slightly lower contrast, but this did not compromise the visibility of characteristic points. Similar patterns were observed in the results of sebaceous fingerprints developed with the bixin/$ZnCO_3$ composite (Supplementary Figure 2), highlighting a better development in the fingerprints of donors 1, 3, and 4. On the other hand, natural fingerprints showed a more noticeable improvement in donors 1, 2, and 3, with some fewer sharp areas in the case of donor 4.

In this way, the donor's influence in the fingerprint development process became evident, considering that the same deposition method was uniformly applied to all. Despite individual variations, consistent and satisfactory results were observed, enabling the identification of all analyzed fingerprints. In summary, the evaluated composites exhibit promising potential to enhance fingerprint revelation techniques in forensic investigations, emphasizing the importance of considering individual factors to optimize the effectiveness of these methods.

Furthermore, Supplementary Figures 3 and 4 demonstrated that the prepared composites were able to develop natural and sebaceous fingerprints similarly or even better than what is currently found in terms of image quality. As evidenced in Supplementary Figure 3, a notable improvement in the development of natural fingerprints was observed when using the bixin/kaolinite composite for donors 2 and 3. Donors 1 and 4, on the other hand, exhibited a development comparable to that obtained with the commercial powder. Regarding sebaceous fingerprints, a similar development pattern was observed for all four donors. Similar results were observed in natural fingerprints revealed by the bixin/$ZnCO_3$ composite (Supplementary Figure 4), indicating a significant improvement for donors 2 and 3. In contrast, donors 1 and 4 showed a development comparable to that obtained with the commercial powder. Concerning sebaceous fingerprints, it was noted that the bixin/$ZnCO_3$ composite provided a more pronounced contrast for donors 1 and 4 while showing a lower contrast for donors 2 and 3, compared to the employed commercial powder.

Finally, a thorough assessment of a natural fingerprint revealed using the bixin/kaolinite composite was conducted (Figure 6). In this regard, biometric fingerprint recognition involves three levels of evaluation. The first level focuses on the macro details of the fingerprint, encompassing the four fundamental types: arch, left loop, right loop, and whorl. The second level involves minutiae, such as bifurcations and terminations. Lastly, the third level incorporates all dimensional attributes of ridges, including width, shape, contour, sweat pores, scars, folds, and other permanent details. Pores, positioned in unique ways with varying shapes, quantities, and dimensions from person to person, are particularly crucial at this level and play a vital role in automatic fingerprint recognition methods [46].

In this context, it is worth highlighting the exceptional development showcased by the composites, allowing the observation of pores in fingerprints from multiple donors. Beyond various minutiae and the fundamental types of fingerprints, this underscores the potential and advancement in research demonstrated by the composites developed in this study, particularly in the realm of forensic fingerprint analysis.

5. Conclusions

This article proposes a simple and sensitive chromatography method with UHPLC-UV-Vis for the analysis of bixin molecules. Furthermore, this study described a simple methodology for obtaining composites from a natural and unexplored compound in the field of papilloscopy, bixin, was described. The choice of bixin was made due to its remarkable contrast, skillfully combined with compounds such as kaolinite and zinc carbonate. This synergy provided bixin with essential adherence, making it a highly effective revealing powder. That way, the bixin/kaolinite and bixin/$ZnCO_3$ composites stood out for their remarkable contrast and adherence to dermal ridges. These characteristics contributed significantly to the sharpness and identifiability of fingerprint marks, even allowing the visualization of sweat pores in some of the samples analyzed. Thus, the composites developed in this study emerge as a promising alternative with vast potential for application in latent fingerprint development, standing out for their simplicity, sensitivity, and effectiveness in revealing crucial details. This advancement provides new perspectives and opportunities in the field of papilloscopy, promoting an innovative and efficient approach to the identification and analysis of fingerprints.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: Natural (N) and sebaceous (S) latent fingerprints deposited onto glass surfaces developed using bixin/kaolinite. Figure S2: Natural (N) and sebaceous (S) latent fingerprints deposited onto glass surfaces developed using bixin/$ZnCO_3$. Figure S3: Digital mark (Natural (N) and sebaceous (S)) sectioned and revealed left half with composite bixin/kaolinite and right half with White® commercial powder purchased from Sirchie. Figure S4: Digital mark (Natural (N) and sebaceous (S)) sectioned and revealed left half with composite bixin/$ZnCO_3$ and right half with White® commercial powder purchased from Sirchie.

Author Contributions: D.T.P: Conceptualization, Investigation, Writing – original draft. A.F.L: Investigation, Data curation, Writing – review & editing. R.L.C: Investigation, data curation, Writing – review and editing. C.A.J: Investigation, data curation, Writing – review and editing. J.P.S: Methodology, Investigation. G.Q.S: Methodology, Investigation. K.C.M: Validation, Funding acquisition. C.M.P.P: Resources, Project administration, Funding acquisition. All authors read and approved the final manuscript.

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Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created or where data is unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in the section "MDPI Research Data Policies" at https://www.mdpi.com/ethics.

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Conflicts of Interest: The authors declare no conflicts of interest.

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