CHARACTERIZATION AND POTENTIAL APPLICATION OF DEXTRAN-BASED BIOPOLYMER POWDER OBTAINED FROM HYDRANGEA MACROPHYLLA LIQUID ANTHOCYANINS EXTRACT BY ULTRASONIC EXTRACTION

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Abstract: Biopolymers have numerous advantages, such as their biodegradable, non-toxic, non-inflammatory and biocompatible properties, and, therefore, have a potential for various applications. In this paper dextran-based biopolymer powder, obtained from *Hydrangea macrophylla* liquid anthocyanins extract by ultrasonic extraction and simple precipitating method, was synthetized and characterized in order to determine its properties and potential application. Attenuated total reflectance Fourier-transform infrared spectroscopy (ATR FT-IR) analyses showed interactions between the components of the system. Optical microscopy suggested that the prepared biopowder formulation was small and somewhat uniform in size, and also showed its easy binding to the fingerprint residues. Additionally, the prepared biopolymer powder was used to visualize latent fingerprints left on different non-porous and semi-porous surfaces, i.e. flat wood, glass, plastic and rubber. The results demonstrated the potential of the obtained dextran-based biopowder to complement routinely applied systems in developing latent fingerprints.

Keywords: (bio)polymers, dextran, *Hydrangea macrophylla*, latent fingerprints, forensics

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INTRODUCTION

Dactyloscopy represents the investigation of ridges of the inner surfaces of the human hands and feet. Over the past 100 years, it has been one of the most reliable methods for identifying individuals, since the characteristics of the hand and foot prints are unique features of every person (Champod, Lennard, Margot, & Stoilovic, 2004; Mitrović, 1998). In forensic practice, the fingerprints have been used for that purpose for years (Bumbrah, Sharma, & Jasuja, 2016). Fingerprints often remain on surfaces of different objects, when the fingertip comes in contact with the substrate. There are three specific types of fingerprints that could be found in forensic practice: patent (transferred together with blood, oil, dirt, etc.), plastic (three-dimensional impressions) and latent (invisible) (Champod, Lennard, Margot, & Stoilovic, 2004; Lennard, 2007), and the latest are of particular interest from the forensic point of view. These traces are imperceptible and (when freshly deposited) consist of lipid secretions and sweat (containing water, minerals, organic compounds and other residuals) (Färber, Seul, Weisser, & Bohnert, 2010).

In general, three types of methods are used for visualization of latent fingerprints: optical, chemical and physical methods (Mozayani & Noziglia, 2006). These methods have been routinely employed during the last decades. However, the last two have many disadvantages regarding their operational application and potential hazards (Champod, Lennard, Margot, & Stoilovic, 2004), and the biggest concern is related to their toxicity and detrimental effect to the human health. Therefore, the researchers are resorting to some novel systems (or even methods) that could overcome the aforementioned problem and, additionally, satisfy cost-benefit demands. In this regard, scientists employ various (bio) polymers, whose utilization is still insufficiently known to the scientific public, especially in visualizing latent fingerprints (Milašinović, 2016; Vučković, Dimitrijević, & Milašinović, 2020; Vučković, Glođović, Radovanović, Janaćković, & Milašinović, 2020). In our previous research, conjugates based on chitosan were used to develop and enhance latent fingerprints. The results demonstrated sufficient powder application efficiency, due to the small diameter of prepared micro particles and the specific mechanism of binding to the fingerprint residues. Additionally, these systems were non-toxic, easily applicable and the method itself was non-destructive (Vučković, Glođović, Radovanović, Janaćković, & Milašinović, 2020). A new approach described by Costa et al. (2020), based on the electrodeposition of bilayer systems based on conjugated and fluorescent polymers was used for the development of latent fingerprints on stainless steel. The first layer of Polypyrrole or PEDOY was electrodeposited onto the surface containing a latent fingerprint and the second layer of a fluorescent Poly(2,2′ :5′ ,2′ -terthiophene) was electrodeposited onto the first layer. Such bilayer systems showed fluorescent properties and could be applied for the development of latent fingerprints on stainless steel, due to the high definition of images in both visible and UV light. This enabled the recognition of the ridge patterns and minutiae points. Additionally, researchers attempt to develop polymer nanoparticles with the aim to visualize latent fingerprints. A novel Poly(*p*-phenylene vinylene) (PPV) nanoparticles in aqueous colloidal solution were used to immerse substrates (with latent fingerprints). The initial study on the adhesive tapes showed that the developing solution was very effective in fluorescence development on both fresh and aged visible fingerprints. Further study on latent fingerprints demonstrated that PPV nanoparticles in colloidal solution have high sensitivity in developing fingerprints to give very clearly fluorescent patterns (Chen, Ma, Chen, & Fan, 2017). However, all these systems should be further examined.

This paper deals with dextran-based biopolymer powder, obtained from *Hydrangea macrophylla* liquid total anthocyanins extract by ultrasonic extraction and simple precipitating method, synthetized and characterized with the aim to determine its properties and potential application. Dextran is a

complex (yet cheap and non-toxic water soluble), branched and hydrophilic polysaccharide composed of anhydroglucose rings, obtained from bacteria (particularly from *Lactobacillus*, *Leuconostoc* and *Streptococcus* species), widely used in medicine and pharmacy, as a component of drug-delivery (nanoparticle) systems, material that reduces blood viscosity and prevents the formation of blood clots, etc. (Wang, Dijkstra, & Karperien, 2016; Wasiak, et al., 2016). Additionally, dextran-based biopolymer powder dyed with anthocyanin extract was used to visualize latent fingerprints left on different non-porous and semi-porous surfaces, i.e. flat wood, glass, plastic and rubber surface. The results demonstrated the capability of prepared dextran-based biopowder in visualizing latent fingerprints, as well as the potential to supplement some of the routinely applied physical methods.

MATERIALS AND METHODS

Materials

Dextran powder was purchased from Sigma-Aldrich (USA) and methanol from Centrohem (Serbia). Distilled water was used for the preparation of extraction medium. The medium was prepared by dissolving the sufficient amount of citric acid in distilled water, in order to obtain the solution with concentration of 0.0033M. The extraction medium was used for total anthocyanins extraction from *Hydrangea macrophylla* flowers and afterwards, the obtained total anthocyanins extract was used to dissolve dextran powder. Besides *Hydrangea macrophylla*, all materials were used without further treatment or purification.

Preparation of Dextran-based Biopowder

The extraction medium (pH ~ 3.84) was prepared by applying the experimental procedures described by Adjé et al. (2010). This medium was used for total anthocyanins extraction from flowers of *Hydrangea macrophylla*. Briefly, 3.5000 g of chopped *Hydrangea macrophylla* flowers were added to 350 ml of extraction medium in a round-bottom flask, then transferred to an ultrasonic bath (*VabSonic*, Serbia; 20 kHz operating frequency and with max. input power of 150 W) for one hour, and at room temperature. Afterwards, the suspension was filtered using a metal sieve and filter paper, respectively, and the filtrate (i.e. liquid total anthocyanins extract) was kept at 4 °C until further use. The obtained extract was used to achieve the different colours of the desired biopowder, as well for better enhancement through complexing with fingerprint sweat and lipid residues, since it was demonstrated that anthocyanins have indicator chemical properties (i.e. colour change in accordance with the change in pH value) (Chandrasekhar, Madhusudhan, & Raghavarao, 2012; Vučković, Dimitrijević, & Milašinović, 2020).

Furthermore, the dextran-based biopowder was prepared by simple precipitating method. Briefly, 1.0000 g of dextran powder was dissolved in 100 ml of prepared total anthocyanins extract. The mixture was stirred at low speed (~300 rpm) and at room temperature using a magnetic stirrer. After homogenization, methanol in 1:3 v/v ratio (mixture:methanol) was added, in order to precipitate polymer from the mixture, and the suspension was filtered using a filter paper. After air-drying at room temperature for ~24h, dry precipitate was kept in the drying oven at 37 °C for additional few hours. Finally, the obtained dry formulation was ground with pestle and mortar to fine powder and kept in desiccator until further application.

CHARACTERIZATION OF THE PREPARED BIOPOWDER FORMULATION

ATR FT-IR Analyses

The ATR-FTIR analyses were performed using Nicolet iS10 FTIR spectrometer (Thermo Scientific, USA), with a diamond attenuated total reflectance (ATR) smart accessory in the range of 4000-400 cm^{-1} at a resolution of 2 cm^{-1} and at 25 °C.

Optical microscopy

The obtained biopowder formulation and BVDA magnetic silver powder (control powder; BVDA, the Netherlands) were recorded with optical microscope Leica FS C comparison microscope, equipped with the Leica IM Matrox Meteor II Driver Software Module. Powders were recorded in dry state, with and without backlight. Prior to imaging under the microscope, latent fingerprints left on microscope glass slides were developed using prepared biopowder and the control powder.

Development of latent fingerprints

In order to determine the capability and performances of dextran-based biopowder, using only a thumb of the right hand, one male donor deposited sebaceous/oily fingerprints onto different non-porous and semi-porous surfaces, i.e. flat wood, glass, plastic and rubber surface. The prints were then left under laboratory (humid) conditions for a short period of time. That period allowed the traces to dry and reduce the residues, by the time the latent fingerprints were developed with synthetized biopowder and the control powder, using BVDA Squirrel hair brush (BVDA, The Netherlands) (International Fingerprint Research Group (IFRG), 2014).

Optical microscopy was used in order to approximate the size and uniformity of dextran-based biopowder, as well to estimate their performances in developing latent finger-marks on glass surface (on which the best results were obtained). Therefore, sebaceous fingerprints randomly deposited onto the glass microscopic slides (properly labelled), were left for a few minutes and then the prepared biopowder formulation and BVDA magnetic silver powder were used for their visualization (International Fingerprint Research Group (IFRG), 2014).

RESULTS AND DISCUSSION

The efficiency of obtained dextran-based biopowder to visualize latent fingerprints was tested on prints left onto different substrates: flat wood, glass, plastic and rubber surface and BVDA magnetic silver powder was then used for comparison (control powder). Both dextran-based biopowder and control powder were applied on multiple numbers of fingerprints on each substrate, to determine the capability and reproducibility of their application, i.e. to achieve the desired results. The best results were obtained with the sebaceous fingerprints deposited onto the glass surface and developed with

dextran-based biopowder. On the other hand, the development of fingerprints on rubber surface was poor, since that surface contains many bulges and indentations, thus disabling the binding of the prepared biopowder to the fingerprint residues. The results obtained on flat wood surface were somewhat satisfying, but non-uniform distribution of particles might have influenced the binding to the fingerprint residues, thus showing weak contrast. Finally, fingerprints enhanced on plastic surface showed better results than those on rubber and wood surface, due to the higher intensity and better quality of the obtained fingerprint images.

Figure 1 shows sebaceous fingerprints of one donor, developed with the prepared biopowder and the control powder on four different substrates, i.e. flat wood, glass, plastic and rubber surface. The prints were then photographed under visible light with a 12 MP camera (aperture $f/2.2$, pixel size 1.22 μm) using black background surface in order to achieve the satisfying contrast.

When comparing fingerprints developed with dextran-based biopowder and BVDA magnetic silver (control) powder on different surfaces, it is evident that the best results were obtained on glass surface, with visible and clear fingerprint image and some minutiae points as well (Figure 1d). Dextran-based biopowder showed poor results on flat wood and rubber surface, due to many bulges and non-uniform distribution of particles (Figure 1a, b). On the other hand, by comparing dextran-based biopowder with control powder in visualizing latent fingerprints, it is obvious that only the development of fingerprint with dextran-based biopowder on glass surface (Figure 1d)) was approximately good as those visualized with BVDA magnetic silver powder.

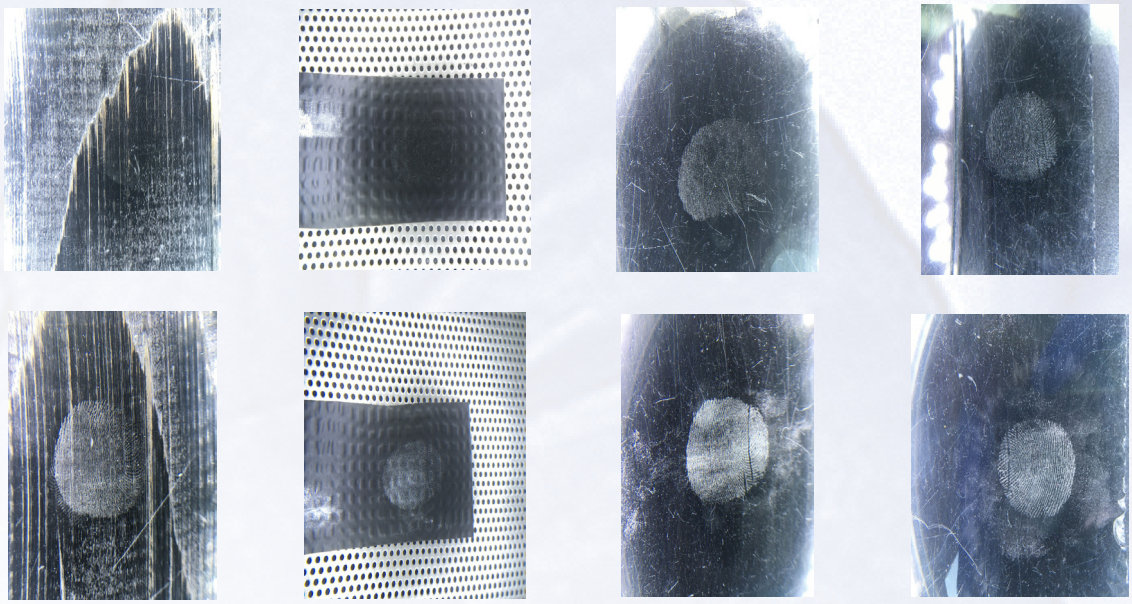

Figure 1. Sebaceous fingerprints developed on: a) flat wood, b) rubber, c) plastic and d) glass surface, using the following powders: 1) dextran-based powder and 2) BVDA Magnetic silver powder, recorded under visible light using black background surface for appropriate contrast.

ATR FT-IR Analyses

ATR FT-IR analyses were performed in order to evaluate interactions between the components of the prepared system. Figure 2 shows the spectra of pure dextran, the initial dextran solution, the total anthocyanins extract and the prepared biopowder formulation. All spectra in Figure 2 contain some characteristic bands: 3700-3000 cm^{-1} due to the O–H stretching and 2360 cm^{-1} due to the stretching of C–H (Carp, et al., 2010; Mehta, Rucha, Bhatt, & Upadhyay, 2006; Mitić, Cakić, & Nikolić, 2010). The band at 1154 cm^{-1} at spectra of pure dextran, initial dextran solution and dextran-based biopowder (Figure 2a, c, d) can be assigned to the stretching vibrations of the C–O–C bond and glycosides bridge, while the band at 1017 cm^{-1} can be associated with the stretching of C–O–H (Chiu, Hsiue, & Chen, 2004; Mehta, Rucha, Bhatt, & Upadhyay, 2006; Mitić, Cakić, & Nikolić, 2010). The weak band at 1110 cm^{-1} can be ascribed to the vibration of the C–O bond at the C4 position of the glucopyranose units (Mitić, Cakić, & Nikolić, 2010). The peaks at 905, 841, and 758 cm^{-1} can be assigned to the α-glucopyranose ring deformation modes (Cakić, Nikolić, Ilić, & Stanković, 2005; Carp, et al., 2010). Nevertheless, small shoulder peak at 1077 cm^{-1} may be due to the complex vibrations involving the stretching of the C6–O6 bond with the participation of deformational vibrations of the C4–C5 bond (Guerrero, Kerry, & de la Caba, 2014; Nikolić, Cakić, Mitić, & Ilić, 2008). However, according to Mitić et al. (2010), the peaks at 1041 and 1017 cm^{-1} present in spectra a, c and d at Figure 2 are related to the crystalline and amorphous phases respectively, and can be responsible for more and less ordered structures of dextran chains.

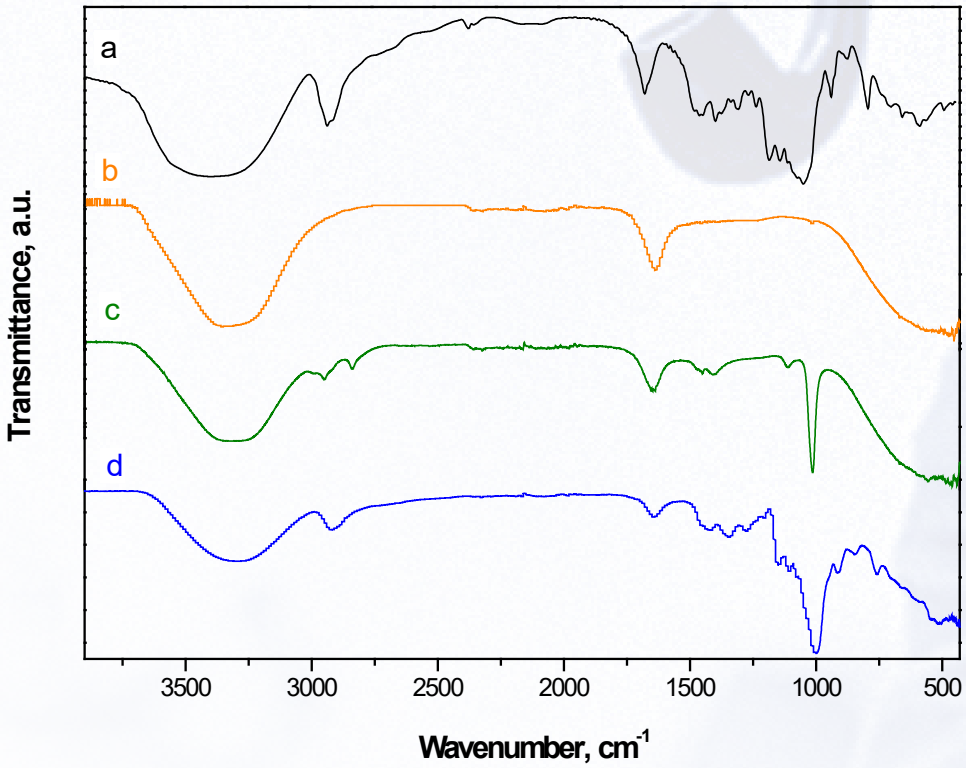

Figure 2. FT-IR spectra: a) pure dextran; b) total anthocyanins extract, c) initial dextran solution and d) dextran-based biopowder.

On the other hand, a decrease in intensity of the peak at 1041 cm^{-1} at the spectra of prepared biopowder (Figure 2d), when compared to the spectra of pure dextran (Figure 2a), can be associated with complexing with the compounds (polyphenols, anthocyanins, etc.) present in the total anthocyanins extract. Additionally, a slight peak shifting (and increase in peak intensity) from 1017 to 1000 cm^{-1} may be related to the interactions between dextran chains and the compounds present in the medium.

Optical microscopy

Figure 3 shows the images of used powders taken by Leica FS C Comparison Macroscope, equipped with the Leica IM Matrox Meteor II Driver Software Module, using magnification ×75 and dark-field contrast technique (backlighting). Since the best results were obtained on non-porous (glass) surface, the same surface was used for further analyses. Powders were deposited onto microscopic glass slides in the form of a fine (thinner) and amassed (thicker) layer, in order to compare the uniformity and size of the particles.

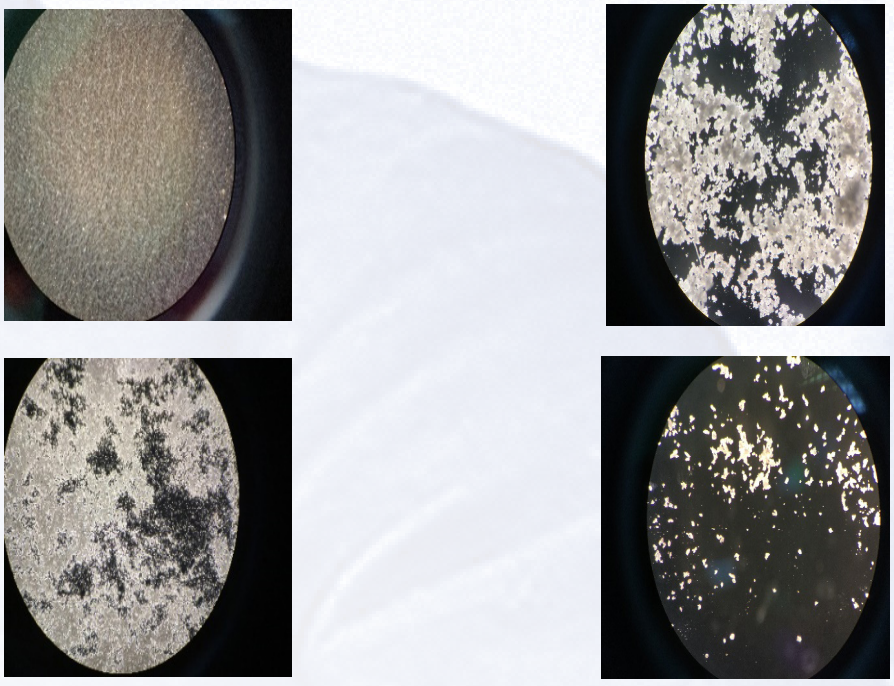

Figure 3. Microscopic images of used powders deposited onto microscopic slides and recorded with optical microscope (magnification ×75, using dark-field contrast technique): a) BVDA magnetic silver powder and b) dextran-based biopowder. Numbers 1 and 2 denote images with powders in form of amassed (thicker) and fine (thinner) layer, respectively.

When comparing both thick and thin layers, it is evident that BVDA magnetic silver powder (Figure 3a), 1) and 2) contain more uniform and smaller particles than prepared biopowder formulation. Prepared dextran-based biopowder (Figure 3, 1b) and 2b)) possessed many irregular and non-uniform particles when compared to control powder, which can be related to weak binding and contrast when dextran-based biopowder is applied. When observing thinner layers, these characteristics are even more obvious (Figure 3, 2).

Subsequently, in order to confirm the previous presumptions, dextran-based biopowder and BVDA magnetic silver powder were used to develop latent fingerprint on glass surface. Therefore, sebaceous fingerprints randomly deposited onto labelled glass microscopic slides using technical scale were left for a few minutes and then the prepared biopowder formulation and the control powder were used for their visualization, using BVDA Squirrel hair brush. Afterwards, the samples of the developed fingerprints were recorded under the optical microscope (magnification ×7.5), using dark-field (Figure 4, 1) and bright-field (Figure 4, 2) contrast techniques.

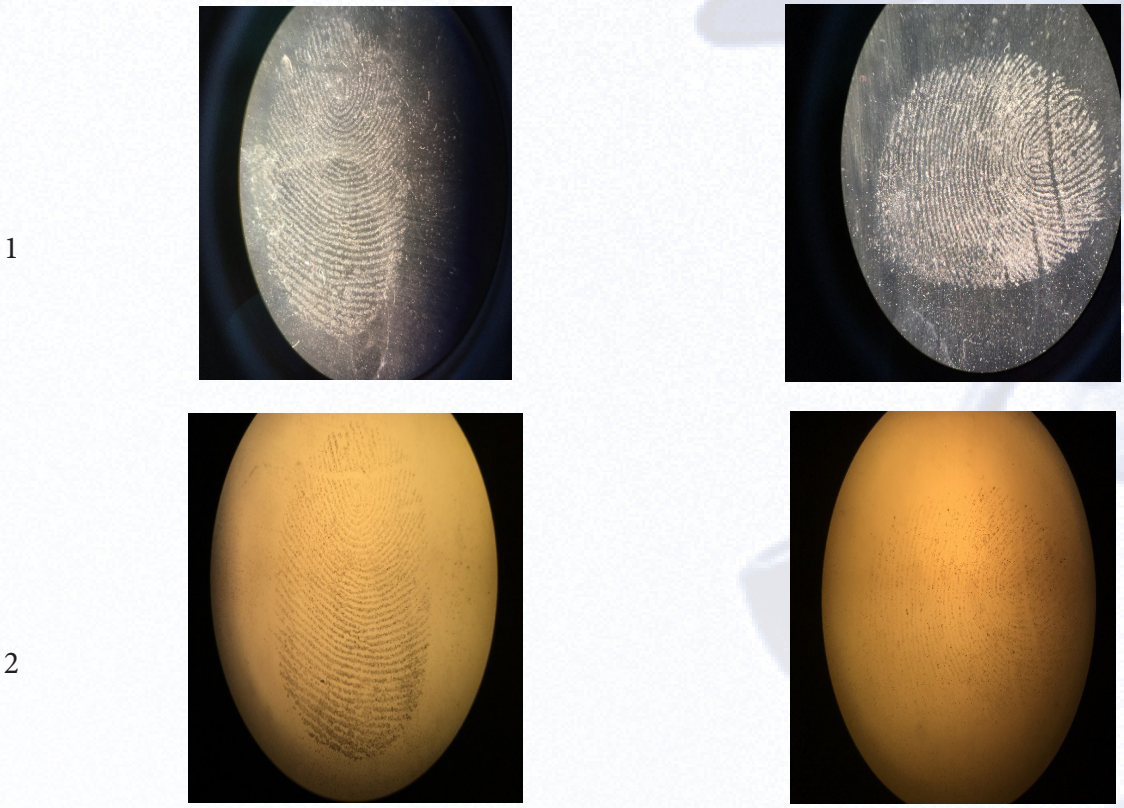

Figure 4. Sebaceous fingerprints deposited onto glass microscopic slides, left for a few minutes and developed using: a) BVDA magnetic silver powder and b) dextran-based biopowder, recorded with optical microscope (magnification ×7.5), using: 1) dark-field and 2) bright-field contrast techniques.

When compared to the BVDA magnetic silver powder, dextran-based biopowder showed even better results in terms of visualizing latent fingerprints, by developing the papillary lines with their continuous flow and making perceptible some minutiae as well. When observing the fingerprint developed with control powder, the obtained fingerprint pattern was somewhat blurred, but with visible papillary lines and some minutiae points. Additionally, when applied with a brush, the prepared biopowder formulation bound with fingerprint residues and did not remain in the interpapillary space. Based on the results, very promising visualization of sebaceous fingerprints was achieved using glass surface as substrate, and with cheap and non-toxic dextran-based biopowder system.

CONCLUSIONS

In this paper, dextran-based biopolymer powder obtained by ultrasonic extraction and simple precipitating method was obtained and characterized in order to determine its potential application in the development of latent fingerprints. Dextran-based biopowder dyed with total anthocyanins extract was used due to its availability and low price, water solubility and non-toxic properties. Based on the obtained results, the prepared biopowder formulation showed optimal properties, with satisfying binding to the fingerprint residues and their clear visualization on glass surface, and the system is less harmful and satisfies the cost-benefit requirements. However, the obtained results did not meet the requirements regarding colour appearance, since the colour of the applied biopowder was not appropriate to potentially visualize fingerprints on bright/white surfaces. On the other hand, as many commercial dusting formulations, this biopowder is easily handled and applicable, requiring no prior knowledge and the method itself is non-destructive, avoiding irreversible loss of traces. Finally, additional researches could comprise other (bio)polymers or addition of bio-based dyes and indicators, in order to expand the application of these systems on other surfaces and potentially complement some of the routinely applied physical methods in developing latent fingerprints.

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