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NATURAL COLORANTS FROM ZONGO VALLEY (BOLIVIA) AND THEIR TEXTILE APPLICATIONS

COLORANTES NATURALES DEL VALLE DE ZONGO (BOLIVIA) Y SUS APLICACIONES TEXTILES

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ABSTRACT

Three plants collected at the Zongo Valley were evaluated for textile dyeing applications. The species selected were extracted following acidic and basic procedures to obtain colored samples, that were submitted to direct and indirect (with mordents) dyeing techniques. In all experiments the need of a mordant was observed and the type of chemical mordant was identified. In each work, the strength of the fabric's dye with the natural colorant was evaluated using a series of washing, rinsing, drying, ironing and exposure to sun light protocols. It was found that the acidic extract of *Brachyotum microdon* (flowers) needs $Al_2(SO_4)_3$ to dye the fabric with a red cabernet tone, while the basic extract fixes the fabric with a brown color with $FeCl_3$ and green with $FeSO_4$. The acid extract of the stems of *Souroubea fragilis* dyed the fabric with a light orange color when $Al_2(SO_4)_3$ was used as a mordent, while the basic extract provided brown tones thanks to $CuSO_4$ or $FeSO_4$. Finally, the acidic extract of *Fuchsia boliviana* (flowers and fruits) gave a pale rose and a green tinting with $Al_2(SO_4)_3$ and $FeCl_3$, respectively. The basic extract of this plant provided a green dyeing with $CuSO_4$ and orange shading with $FeCl_3$. Different types of fabrics were evaluated, and the best dyeing results were observed with cotton. All the extracts studied presented antioxidant and photoprotector activities. The acidic extract of *Brachyotum microdon* (flowers) showed 82.5% of inhibition at 10µg/ml against DPPH and it absorbed the harmful UV B radiation. The acidic extract of *Fuchsia boliviana* (flowers and fruits) gave an 86.7% of inhibition at 10µg/ml against DPPH and it also absorbed the damaging UV B radiation. Finally, the acidic extract of *Souroubea fragilis* (stems) presented 92.1% of inhibition at 100µg/ml against DPPH and it absorbed both UV-B and UV-A radiations.

Keywords: Zongo Valley, *Souroubea fragilis*, *Brachyotum microdon*, *Fuchsia boliviana,* Dyes, Mordents, Antioxidant Activity, DPPH, Photo-protector Property, UV-A and/or UV-B Absorptions.

RESUMEN

Tres plantas colectadas en el Valle de Zongo fueron evaluadas para aplicaciones textiles. Las especies seleccionadas fueron extraídas siguiendo procedimientos ácidos y básicos para obtener muestras coloridas, que fueron sometidas a técnicas de tinción directa e indirecta (con mordientes). En todos los experimentos, la necesidad de mordiente fue observada y el tipo de mordiente químico fue identificado. En cada trabajo, el poder de la tinción del colorante natural en la tela fue evaluado usando una serie de protocolos de lavado, enjuague, secado, planchado y exposición a la luz solar. Se encontró que el extracto ácido de *Brachyotum microdon* (flores) necesita $Al_2(SO_4)_3$ para teñir la tela con un tono rojo cabernet, mientras que el extracto básico fija la tela de color café con $FeCl_3$ y verde con $FeSO_4$. El extracto ácido de los tallos de *Souroubea fragilis* tiñe la tela con un color naranja claro cuando se utiliza $Al_2(SO_4)_3$ como mordiente, mientras que el extracto básico proporciona un tono café gracias a $CuSO_4$ o $FeSO_4$. Finalmente, el extracto ácido de *Fuchsia boliviana* (flores y frutos) dio tinciones rosa pálido y verde con $Al_2(SO_4)_3$ and $FeCl_3$; respectivamente. El extracto básico de esta planta proporcionó un teñido verde con $CuSO_4$ y una tonalidad naranja con $FeCl_3$. Diferentes tipos de telas fueron evaluadas y los mejores resultados de teñido se observaron con algodón. Todos los extractos estudiados presentaron actividades antioxidantes y fotoprotectoras. El extracto ácido de *Brachyotum microdon* (flores) mostró 82.5% de inhibición a 10µg/ml contra DPPH y absorbió la dañina radiación UV B. El extracto acídico de *Fuchsia boliviana* (flores y frutos) dio una inhibición de 86.7% a 10µg/ml contra DPPH y también absorbió la nociva radiación UV B. Finalmente, el extracto ácido de *Souroubea fragilis* (tallos) presentó 92.1% de inhibición a 100µg/ml contra DPPH y absorbió ambas radiaciones UV-A y UV-B.

Palabras Clave: Valle de Zongo, *Souroubea fragilis*, *Brachyotum microdon*, *Fuchsia boliviana,* Tintes, Mordientes, Actividad Antioxidante, DPPH, Propiedad Fotoprotectora, Absorciones UV-A y/o UV-B.

1. INTRODUCTION

The use of natural dyes has again resurfaced in the industry due to the toxicity found in their synthetic derivatives [1], [2]. Worldwide, an important campaign has emerged to support nature as a fountain of colorants and dyes. The production sources vary from the vegetal, animal, and insect kingdoms to soils and microorganisms [3]. Several natural

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dyes have been studied and evaluated for their coloring properties but also for their possible antioxidant, photoprotector and/or nutraceutical applications [4], [5].

In Bolivia, a country rich in vegetal and animal biodiversity, only some species have been used as natural colorants following traditional extraction and dyeing techniques. Among these species, only a few have been scientifically reported or evaluated [6]. Among the species used to color fabrics, we highlight thola (*Baccharis dracunculifolia*), nogal (*Juglans regia*), achiote (*Bixa orellana*), mole (*Schinus molle*), eucalyptus (*Eucalyptus* sp.), chillca (*Baccharis* sp.) and kewiña (*Polylepis* sp.) [7]. There are different techniques to extract natural colorants from plants and the applications in dyeing of fabrics are also assorted. Aqueous, alcoholic, basic and acidic extractions as well as those with vegetal oils were reported. The selection of the extraction technique depends on the type of metabolite responsible for the dyeing property. To fix the dye in the fabric, there are the direct methods (where the dye is directly boiled with the fabric) and the indirect ones (where mordants are used to fix the color in the substrate) [8].

Bolivia has different ecosystems each of them characterized by a specific weather, altitude and soil type. One region in Bolivia that has many ecosystems is the Zongo valley, situated at the northeast of La Paz city. This valley starts at the high Andean prairie at 4800 m.a.s.l. and it extends to the humid tropical region called Yungas at 800 m.a.s.l. [9], [10]. It has been reported that 109 vegetal families and 158 species exist in the Zongo Valley [9].

This significant plant biodiversity has captured our attention to evaluate and validate their possible attributes as colorants, antioxidants and/or photo protectors [11], [12]. We have evaluated the antioxidant and photoprotector activities of 15 plants with possible dyeing properties. The vegetal species selected for the preliminary dyeing studies were chosen based on colored organs, biological properties (antioxidant and/or photoprotector activities), preliminary dyeing tests, and the amount of the collected plant. Among the species collected, *Fuchsia boliviana*, *Brachyotum microdon*, *Souroubea fragilis*, *Monnina bridgesii* and *Rubus floribundus* caught our attention due to their biological and coloring properties. In this publication we submit the preliminary dyeing studies on fabrics of *Fuchsia boliviana*, *Brachyotum microdon* and *Souroubea fragilis*. It has been reported that these three species present phenolic compounds, tannins and anthraquinones. In addition, *Brachyotum microdon* has anthocyanines; while *Fuchsia boliviana* has isoflavones. *Fuchsia boliviana* has an important photoprotector activity since their flowers and fruits absorb both UV radiations (UV-B and UV-A) at 50 ppm, while their leaves absorb the harmful UV B radiation at 100ppm. The leaves of *Souroubea fragilis* attract both UV-B and UV-A radiations at 50ppm, while their steams and fruits absorb the UV B radiation at 100ppm [11]. For the antioxidant activity, using the DPPH assay, it has been reported that *Fuchsia boliviana* is the most active specie. At 10µg/ml, the leaves of *Fuchsia boliviana* present a 93.2% of inhibition, while their flowers and fruits have 86.7% at the same concentration. *Brachyotum microdon* is another interesting antioxidant specie since their flowers reported a DPPH assay of 82.5% of inhibition at 10µg/ml. The control used in all the DPPH assays was ascorbic acid that shown 90% of inhibition at 10µg/ml [12].

2. EXPERIMENTAL WORK

2.1 General

Ultraviolet studies were done on UV/VIS spectrophotometer Biochrom, model Libra S12 and on Spectro quant Phano 300. To dry the aqueous extracts, a Biobase BK-FD 18P freeze dryer machine was used. All supports and reagents used in this work were obtained from Merck and Sigma.

2.2 Collection of plant species

Plant species were collected in the Zongo Valley on November 2017. The collection started near the Zongo Dam at altitude 4715 m.a.s.l. (68°05'02'' longitude and 16°15'02'' latitude) and ended near the Huaji Hydroelectric Power Station at 941 m.a.s.l. (67°55'04'' longitude and 16°00'05'' latitude). All species were identified, and their bouchers were deposited in the Bolivian National Herbarium, La Paz.

2.3 Extracts preparation

The species collected were air-dried at room temperature, in a dry place protected from solar radiation. The dried specimens were separated into their different organs, grinded, weighed and the important organ (based on previous works) was Soxhlet extracted following an acidic and a basic procedure. For the acidic extract a solution of ethanol-water (70%) with 0.6g of citric acid was used. For the basic extract 0.5N of NaOH was employed. The vegetal material submitted to the Soxhlet extraction was around 20g. After 5 hours of extraction, the colored extract was filtered and used to dye different types of fabrics ranging from cotton, crepe, velvet, chiffon and linen. For each dyeing process, approximately 30 ml of extract and 10 x 4.5 cm of clean fabric was used.

2.4 Direct dyeing using cotton fabric

For the direct dyeing, the fabric was boiled directly in the colored extract. The boiling process lasted 30 minutes and then, the fabric was soaked in the colorant for 24 hours. After the dyeing, the fabric was rinsed with water an submitted to the dyeing control procedures.

2.5 Indirect dyeing using cotton fabric

For the indirect dyeing, the fabric was first exposed to a mordent for 30 minutes, rinsed and then boiled in the colored extract following the protocol presented in the previous section. The chemical mordents used to fix the dye in the fabric were: $Al_2(SO_4)_3$, $FeCl_3$, $FeSO_4$ and $CuSO_4$. For each fabric, 150 ml of a 20% mordent solution was used.

2.6 Dyeing control procedures

For each tinted fabric (directly or indirectly done), a series of procedures were followed to verify the efficiency of the dyeing technique. The control procedures executed and the order pursued for the direct dyeing are submitted below. After each protocol, the fabric was dried at room temperature.

- the fabric is rinsed with water
- the fabric is washed and rubbed with water
- the fabric is washed with a detergent and then it is rinsed. In both cases, rubbing the fabric was avoided
- the fabric is washed with a detergent and then it is rinsed. In both cases the fabric was rubbed

The control procedures executed and the order pursued for the indirect dyeing is presented below. After each protocol, if indicated otherwise, the fabric is rinsed and dried at room temperature.

- the fabric is rinsed with water
- the fabric is washed with a detergent avoiding rubbing it
- the fabric is washed with a detergent avoiding rubbing it. Repeat this procedure twice
- the fabric is washed with a detergent while rubbing it. Repeat this procedure twice
- the fabric is rinsed with water, dried and ironed. Repeat the procedure for four days
- the fabric is washed with a detergent while rubbing it. After drying, the fabric is ironed. Repeat the procedure for four days
- the fabric is washed with a detergent while rubbing it. After drying, the fabric is ironed and exposed to sun light for four weeks. The fabric is examined weekly

2.7 Indirect dyeing using different fabrics

For the indirect dyeing using other fabrics than cotton, the followed protocol was that described in section 2.5 (Indirect dyeing using cotton fabric). The fabrics used in this study were crepe, velvet, chiffon and linen. The chemical mordents, used to fix the dye in the fabric selected, were chosen based on each samples' result in the indirect dyeing of cotton experiments and on its respective control assays. The concentration of each mordent used was 20% in water.

2.8 Spectroscopic studies

For each of the colored extracts, used in the dyeing techniques, a spectroscopic study was done to analyze its dyeing, antioxidant and/or photoprotector regions.

3. RESULTS AND DISCUSSION

3.1 Collection of plant species

Three vegetal species were collected in the Zongo valley on November 2017 and belong to 3 different families. Two of them, *Brachyotum microdon* (Melastomataceae) and *Fuchsia boliviana* (Onagraceae) were collected at the Yungas Mountain Brow; while, *Souroubea fragilis* (Marcgraviaceae) was collected in the Yungas region. Figure #1 presents the photographs of the collected species and Table #1 shows the collection's coordinates and the collection's codes. The mentioned species were collected based on their antioxidant and/or photoprotector activities and because of their preliminary dyeing properties evaluated on paper and cardboard.

3.2 Extracts preparation

The flowers of *Brachyotum microdon* (batches of 20 g) were Soxhlet extracted following the acid and basic protocols. The resulting acidic extract had a red cabernet tone while the basic one had a dark brown color. The acidic and basic extraction of the flowers of *Fuchsia boliviana* (10 g for each extraction) gave a pale rose color and a dark brown tint;

respectively. Finally, the steams of *Souroubea fragilis* (lots of 20 g) provided a light orange acidic extract and a burgundy wine like tone following the basic treatment. All the extracts obtained were submitted to freeze dry to obtain their yields. Table 2 presents the amount of the collected species, the amount processed in the acidic extraction and the yield obtained after freeze-drying. Table 3 presents the same parameters shown in Table 2 but referred to the basic extraction.

Brachyotum microdon *Fuchsia boliviana* *Souroubea fragilis*

Figure 1: Photographs of the collected vegetal species in the Zongo Valle.

TABLE 1 - COLLECTION'S COORDINATES AND COLLECTION'S CODES OF THE STUDIED PLANTS FROM THE ZONGO VALLEY

FAMILY	SPECIE	COLLECTION CODE	COLLECTION COORDINATES	COLLECTION REGION
Melastomataceae	*Brachyotum microdon*	MZ 4094	16°10'24.1'' (Latitude S) 68°08'04.7'' (Longitude W) 3397 m.a.s.l (Altitude)	Yungas Mountain Brow
Onagraceae	*Fuchsia boliviana*	MZ 4096	16°07'24.0'' (Latitude S) 68°04'99.3'' (Longitude W) 2303 m.a.s.l (Altitude)	Yungas Mountain Brow
Marcgraviaceae	*Souroubea fragilis*	MZ 4097	16°03'63.1'' (Latitude S) 68°01'04.3'' (Longitude W) 1486 m.a.s.l (Altitude)	Yungas

TABLE 2 - AMOUNT OF COLLECTED SPECIES, ACIDIC SOXHLET EXTRACTIONS AND FREEZE-DRIED RESULTS

SPECIE/ ORGAN	COLLECTED AMOUNT [gr]	AMOUNT USED IN THE ACIDIC EXTRACTION [gr]	YIELD OF ACIDIC EXTRACT [%]
Brachyotum microdon/ Flowers	178	20	4.51*
Fuchsia boliviana/ Flowers and Fruits	27.2	10	5.44
Souroubea fragilis/ Steams	346	20	NA**

* Some fractions of the extract had a fatty appearance. ** Not available since the extract was an aqueous- oily liquid.

TABLE 3 - AMOUNT OF COLLECTED SPECIES, BASIC SOXHLET EXTRACTIONS AND FREEZE-DRIED RESULTS

SPECIE/ ORGAN	COLLECTED AMOUNT [gr]	AMOUNT USED IN THE BASIC EXTRACTION [gr]	YIELD OF BASIC EXTRACT
Brachyotum microdon/ Flowers	178	20	NA*
Fuchsia boliviana/ Flowers and Fruits	27.2	10	11.14
Souroubea fragilis/ Steams	346	20	NA*

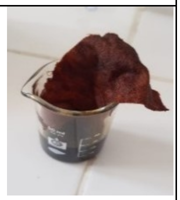
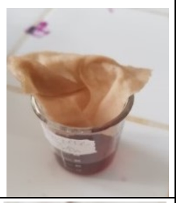
* Not available since the extract was an aqueous- oily liquid.

3.3 Direct dyeing

For the preliminary direct dyeing assays, cotton was selected as the substrate because it has been reported as the fabric that better absorbs natural colorants. All the natural extracts obtained perfectly tinted this fabric as it can be seen in Table 4; however, none of them went through the dyeing control procedures. Table 4 presents a summary of the direct dyeing technique of the acidic and basic extracts of the three studied species.

Figure 2 shows the results of the dyeing control process done on the cotton fabric tinted with the acidic and basic extracts of *Brachyotum microdon* (flowers). In this figure we can see that the natural color transferred to the fabric was vanished in all protocols. In addition, it is important to remark that the acidic extract of *Brachyotum microdon* (flowers) presented an interesting color palette depending on the pH worked. This palette goes from a red cabernet tone from pH 4 to 7 to a turquoise and green color from pH 9 to 14 as it can be seen in Figure 3.

TABLE 4 - SUMMARY OF THE ACIDIC AND BASIC EXTRACTS OF THE PLANTS STUDIED AND THE RESULTS OF THEIR DIRECT DYEING ON COTTON

VEGETAL SPECIE		EXTRACTION	EXTRACT'S COLOR	COLOR OF TINTED FABRIC	
Brachyotum microdon/ Flowers		Acidic	Red cabernet (deep red)	red cabernet (deep red)	
		Basic	Dark brown	Dark brown	
Fuchsia boliviana/ Flowers		Acidic	Pale rose	Pale rose	
		Basic	Dark brown	Dark brown	
Souroubea fragilis/ Steams		Acidic	Light orange	Light orange	
		Basic	Burgundy wine (brownish red)	Burgundy wine (brownish red)	

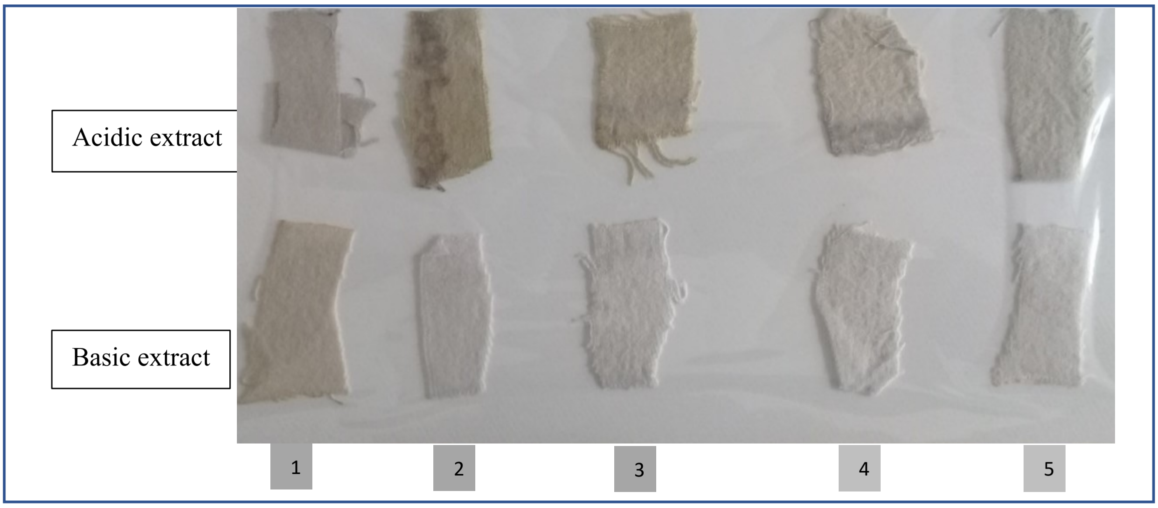


Figure 2: Results of the dyeing control process done on the cotton fabric tinted with the acidic and basic extracts of *Brachyotum microdon* (flowers). 1 Fabric rinsed with water; 2 Fabric washed and rubbed with water; 3 Fabric washed with a detergent and then rinsed (without rubbing); 4 Fabric washed with a basic detergent and rinsed (with rubbing); 5 Fabric washed with an acidic detergent and rinsed (with rubbing).

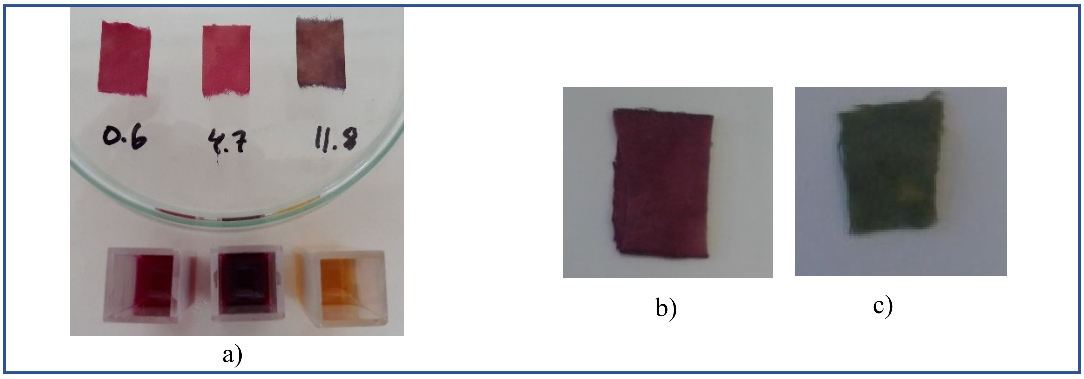


Figure 3: Color palette of the fabric tinted with the acidic extract of *Brachyotum microdon* (flowers) and exposed to different pH. a) colored extract exposed to pH 0.6, 4.7 and 11.8; b) tinted fabric exposed to pH 4 to 7; c) tinted fabric exposed to pH 9.

3.4 Indirect dyeing

For the preliminary indirect dyeing assays, cotton was also selected as the substrate to better absorb the natural colorants. In most of the indirect dyeing, the natural colorant was nicely transferred to the fabric previously treated with the mordent. Tables 5 to 7 present the summary of the indirect dyeing techniques of the acidic and basic extracts of each specie studied. In Table 5, it can be seen that the original acidic extract color of *Brachyotum microdon* (red cabernet or deep red) is perfectly obtained with aluminum sulfate while the dark brown tint of its basic extract can be successfully attained when ferric chloride is used as a mordent. For *Souroubea fragilis*, shown in Table 6, the light orange color of its acidic extract can be nicely accomplished with aluminum sulfate; unfortunately, its burgundy wine tone given by the basic extract, was not obtained with neither of the mordents used. Finally, in Table 7, the results of the indirect dyeing of *Fuchsia boliviana* can be observed. The pale pink tone reached with the acidic extract was well achieved with aluminum sulfate; however, the dark brown color of its basic extract could not be reproduced with the mordents used. It is important to highlight that only 3 mordents were used with Fuchsia's extracts because we did not have enough vegetal sample for the essays.

Each of the tinted fabrics obtained was submitted to resistance control assays to study the dyeing strength and durability. Figures 4 through 8 show the results of these controls. As it can be seen in Figures # 4 and # 5 for *Brachyotum microdon*, the mordents that best dye the fabric which remains colored after the control tests are $Al_2(SO_4)_3$ and $FeSO_4$ for the acidic and basic extracts, respectively. For *Souroubea fragilis*, the best mordents for the acidic extract are $CuSO_4$ and $Al_2(SO_4)_3$, while for the basic extract all mordents worked nicely except for $Al_2(SO_4)_3$. Finally, for *Fuchsia boliviana* none of the mordents used reproduced the original extract colors after the dyeing controls; however, nice tones (dark blue, dark green and orange) were obtained and maintained with $CuSO_4$ and $FeCl_3$.

TABLE 5 - SUMMARY OF THE ACIDIC AND BASIC EXTRACTS OF *BRACHYOTUM MICRODON* (FLOWERS) AND THE RESULTS OF THEIR INDIRECT DYEING ON COTTON

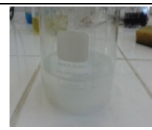
VEGETAL SPECIE		EXTRACTION	EXTRACT'S COLOR	CHEMICAL MORDENT		COLOR OF TINTED FABRIC	
Brachyotum microdon/ Flowers		Acidic	Red cabernet (deep red) 	$CuSO_4$ (Copper sulfate)		Dark red	
				$FeCl_3$ (Ferric chloride)		Dark red	
				$Al_2(SO_4)_3$ (Aluminum sulfate)		Red cabernet (deep red)	
				$FeSO_4$ (Ferrous sulfate)		Dark red	
		Basic	Dark brown 	$CuSO_4$ (Copper sulfate)		Green/brown	
				$FeCl_3$ (Ferric chloride)		Dark brown	
				$Al_2(SO_4)_3$ (Aluminum sulfate)		Light brown	
				$FeSO_4$ (Ferrous sulfate)		Dark green/brown	

TABLE 6 - SUMMARY OF THE ACIDIC AND BASIC EXTRACTS OF *SOUROUBEA FRAGILIS* (STEAMS) AND THE RESULTS OF THEIR INDIRECT DYEING ON COTTON

VEGETAL SPECIE		EXTRACTION	EXTRACT'S COLOR	CHEMICAL MORDENT		COLOR OF TINTED FABRIC	
Souroubea fragilis/ Steams		Acidic	Light orange 	$CuSO_4$ (Copper sulfate)		Light beige	
				$FeCl_3$ (Ferric chloride)		Light beige	
				$Al_2(SO_4)_3$ Aluminum sulfate		Light orange	
				$FeSO_4$ (Ferrous sulfate)		Light beige	
		Basic	Burgundy wine (brownish red) 	$CuSO_4$ (Copper sulfate)		Dark brown	
				$FeCl_3$ (Ferric chloride)		Brown with green tones	
				$Al_2(SO_4)_3$ Aluminum sulfate		Yellow	
				$FeSO_4$ (Ferrous sulfate)		Brown	

TABLE 7 - SUMMARY OF THE ACIDIC AND BASIC EXTRACTS OF *FUCHSIA BOLIVIANA* (FLOWERS) AND THE RESULTS OF THEIR INDIRECT DYEING ON COTTON

VEGETAL SPECIE		EXTRACTION	EXTRACT'S COLOR	CHEMICAL MORDENT		COLOR OF TINTED FABRIC	
Fuchsia boliviana/ Flowers		Acidic	Pale rose 	$CuSO_4$ (Copper sulfate)		Light Pink with dark green tones	
				$FeCl_3$ (Ferric chloride)		Dark green	
				$Al_2(SO_4)_3$ (Aluminum sulfate)		Pale rose	
		Basic	Dark brown 	$CuSO_4$ (Copper sulfate)		Green	
				$FeCl_3$ (Ferric chloride)		Light brown	
				$Al_2(SO_4)_3$ Aluminum sulfate		Light beige	

Figure 4: Summary of the resistance control assays of cotton fabrics tinted with the acidic extract of *Brachyotum microdon* (Flowers). a) Original dyeing, b) Fabric rinsed with water c) Fabric washed with detergent, d) Fabric washed with detergent, rubbed and dried (twice), e) Fabric washed with detergent, without rubbing and dried (twice), f) Fabric rinsed, dried and ironed (for 4 days).

Figure 5: Summary of the resistance control assays of cotton fabrics tinted with the basic extract of *Brachyotum microdon* (Flowers). a) Original dyeing, b) Fabric rinsed with water c) Fabric washed with detergent, d) Fabric washed with detergent, rubbed and dried (twice), e) Fabric washed with detergent, without rubbing and dried (twice), f) Fabric rinsed, dried and ironed (for 4 times), g) Fabric washed with detergent, rubbed, dried and ironed 4 times (resting one day between washes), h) Fabric washed with detergent, rubbed, dried, ironed and sun exposure for one month.

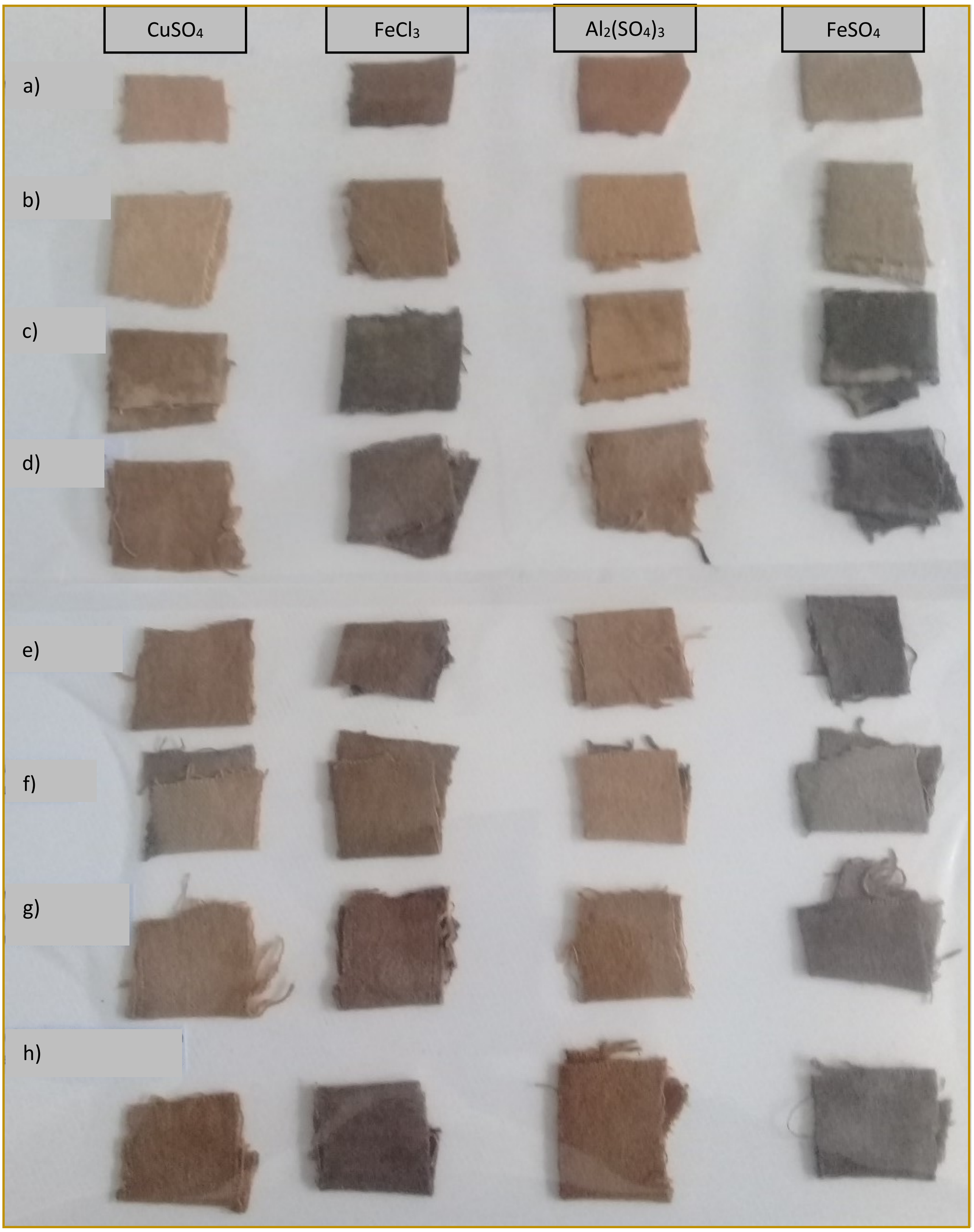
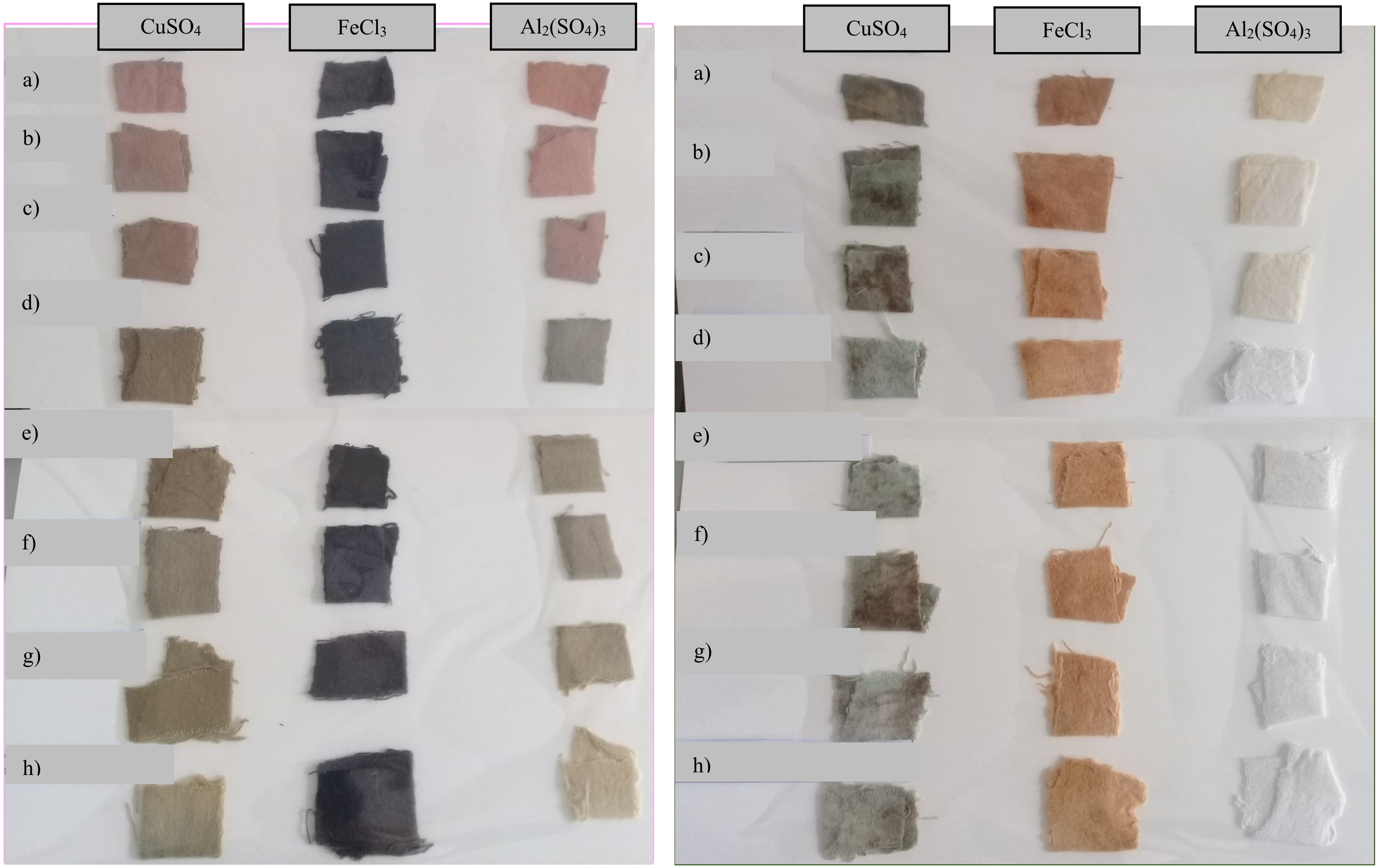


Figure 6: Summary of the resistance control assays of cotton fabrics tinted with the acidic extract of *Souroubea fragilis* (Steams). a) Original dyeing, b) Fabric rinsed with water c) Fabric washed with detergent, d) Fabric washed with detergent, rubbed and dried (twice), e) Fabric washed with detergent, without rubbing and dried (twice), f) Fabric rinsed, dried and ironed (for 4 times), g) Fabric washed with detergent, rubbed, dried and ironed 4 times (resting one day between washes), h) Fabric washed with detergent, rubbed, dried, ironed and sun exposure for one month.

Figure 7: Summary of the resistance control assays of cotton fabrics tinted with the basic extract of *Souroubea fragilis* (Steams). a) Original dyeing, b) Fabric rinsed with water c) Fabric washed with detergent, d) Fabric washed with detergent, rubbed and dried (twice), e) Fabric washed with detergent, without rubbing and dried (twice), f) Fabric rinsed, dried and ironed (for 4 times), g) Fabric washed with detergent, rubbed, dried and ironed 4 times (resting one day between washes), h) Fabric washed with detergent, rubbed, dried, ironed and sun exposure for one month.



1) **2)**

Figure 8: Summary of the resistance control assays of cotton fabrics tinted with *Fuchsia boliviana* (*Flowers*). 1) Acidic extract. 2) Basic extract.
a) Original dyeing, b) Fabric rinsed with water c) Fabric washed with detergent, d) Fabric washed with detergent, rubbed and dried (twice), e) Fabric washed with detergent, without rubbing and dried (twice), f) Fabric rinsed, dried and ironed (for 4 times), g) Fabric washed with detergent, rubbed, dried and ironed 4 times (resting one day between washes), h) Fabric washed with detergent, rubbed, dried, ironed and sun exposure for one month.

3.5 Indirect dyeing using different fabrics

Based on the results of the indirect dyeing experiments and their control assays, it was concluded that for the acidic extract of *Brachyotum microdon*, $Al_2(SO_4)_3$ should be used while the selection for the basic extract was $FeSO_4$. For *Souroubea fragilis*, the mordents used were $Al_2(SO_4)_3$ for the acidic solution while $CuSO_4$ for the basic one. Finally, for *Fuchsia boliviana* only the basic extract, with $FeCl_3$, was evaluated because the acidic extract was consumed in the previous tests. Figure # 9 presents the dyeing results of the acidic and basic extracts of *Brachyotum microdon*, Figure # 10 shows those for *Souroubea fragilis* and the results for the basic extract of *Fuchsia boliviana* are displayed in Figure # 11. In all colored fabrics the tones obtained are not as strong as those obtained with the cotton textile. Among the tested fabrics, linen, crepe and velvet presented the best color resolutions. All the fabrics exposed to the acid extract of *Brachyotum microdon* presented a light purple coloration, while the fabrics exposed to the other vegetal extracts presented copper colored tones.

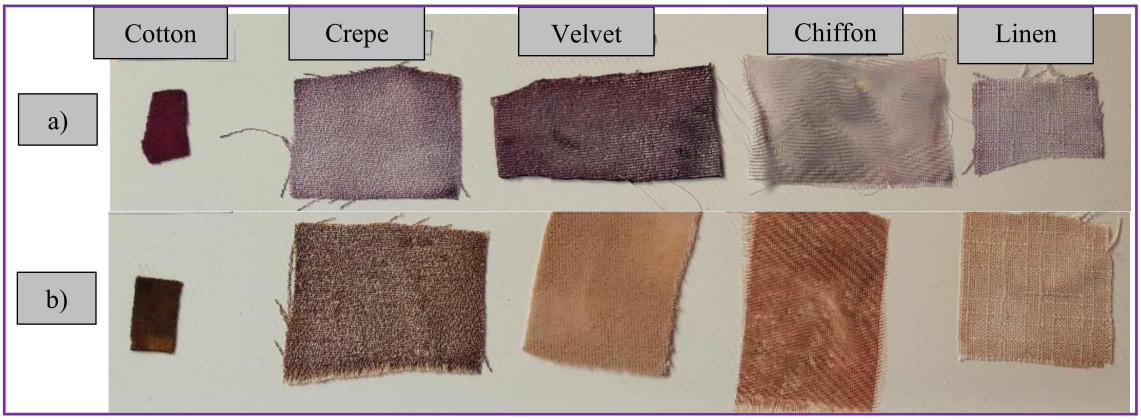


Figure 9: Summary of indirect dyeing of *Brachyotum microdon* (Flowers) on different fabrics. a) Acidic extract exposed to a fabric treated with $Al_2(SO_4)_3$, b) Basic extract exposed to a fabric treated with $FeSO_4$.

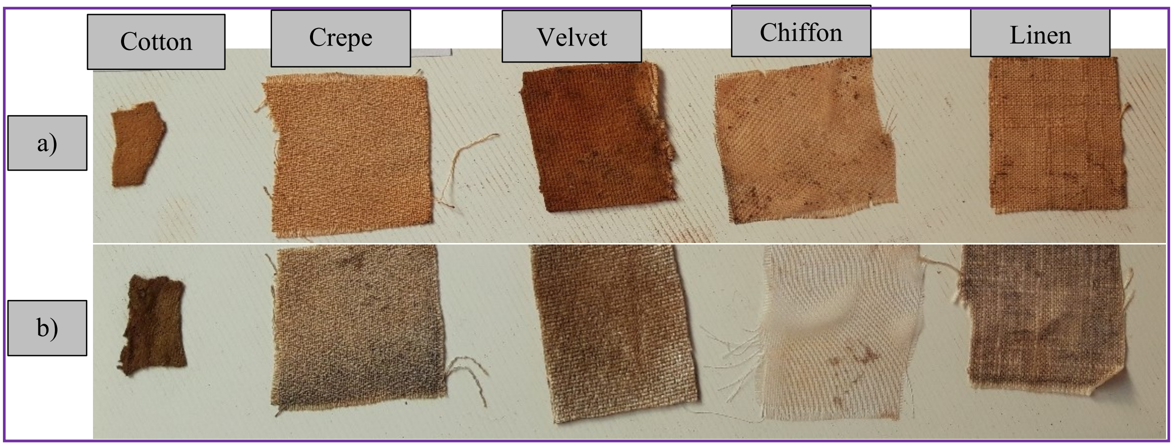


Figure 10: Summary of indirect dyeing of *Souroubea fragilis* (Steam) on different fabrics. a) Acidic extract exposed to a fabric treated with $Al_2(SO_4)_3$, b) Basic extract exposed to a fabric treated with $CuSO_4$.

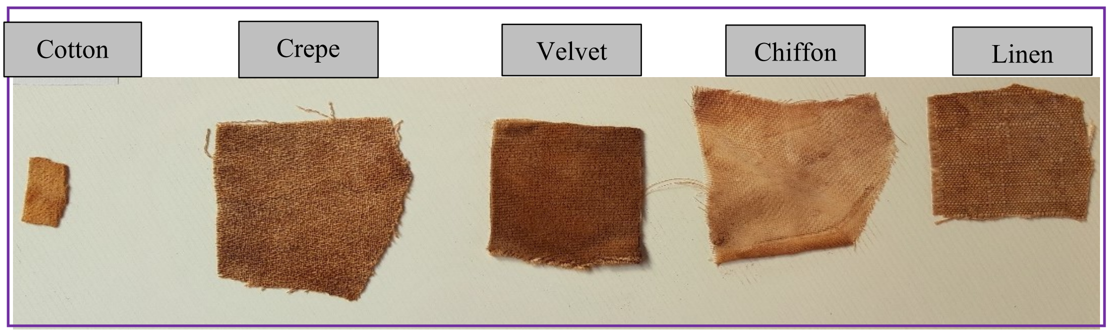


Figure 11: Summary of indirect dyeing of *Fuchsia boliviana* (Flowers) on different fabrics. Basic extract exposed to fabrics treated with $FeCl_3$.

3.6 Spectroscopic studies

Each of the colored extracts, used in the dyeing techniques, was submitted to a spectroscopic study to analyze its color, antioxidant and/or photoprotector regions. Figure12 presents the spectra of the acidic colored samples.

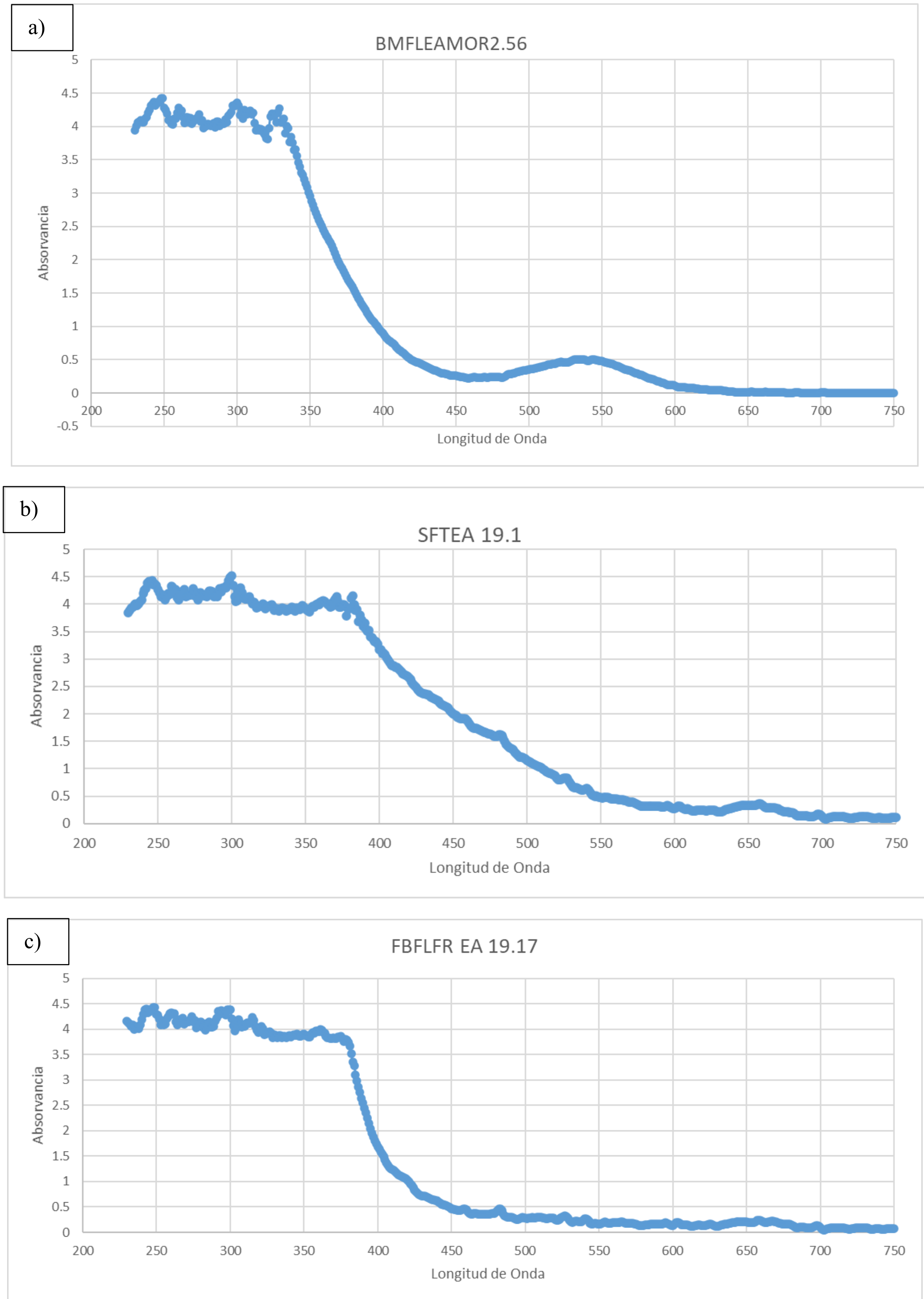


Figure 12: UV-VIS spectra of colored acidic extracts. a) *Brachyotum microdon* (Flowers) at 2.56 mg/ml, b) *Souroubea fragilis* (Steam) at 19.1 mg/ml, c) *Fuchsia boliviana* (Flowers) at 19.17 mg/ml.

In all spectra, the presence of the color absorption regions is evident. The maximum absorption for *Brachyotum microdon* is found between 470 -560 nm and for both *Souroubea fragilis* and *Fuchsia boliviana* it is between 460- 480 nm. These absorptions are consistent with the natural color of the extracted organ and the tone of the organic extract. For *Brachyotum microdon*, the purple/violet color of its flowers has an absorption range between 500- 550 nm and the red cabernet (deep red) color of its acidic extract is between 470- 500 nm. Both absorptions are present in the spectra and provide the colors observed and explained above. For *Souroubea fragilis* and *Fuchsia boliviana*, the absorbed wavelengths revealed a range of colors starting in orange and ending in red. This color range is consistent with the orange color of *Souroubea fragilis* 's steams and its acidic extract. The same analogy can be found between the red flowers of *Fuchsia boliviana* and its pale pink acidic extract.

In the studied spectra the absorptions of some active metabolites are also visible. The main absorption feature of phenolic and tannin compounds is found at 280 nm and depending on the type of phenolic compound other absorptions are characteristic. For instance, for the galloylated flavanols an additional absorption at 350 nm is observed, for anthocyanins the band at 520nm is characteristic for red color substances, hydroxycinnamic acids have an additional absorption at 320 nm and flavonols present another band around 360 nm. The UV-Vis spectra of anthraquinones show 4 bands in the wavelength range of 220-250 nm and one absorption band close to 400 nm. In all the spectra studied, the stronger bands for phenolic compounds, tannins and anthraquinones are observed. In addition, it is important to highlighting the presence of flavonols in *Souroubea fragilis* and *Fuchsia boliviana* with an additional band at 360 nm and anthocyanins in *Brachyotum microdon* with a complementary absorption band at 520 nm.

4. CONCLUSION

Brachyotum microdon, *Fuchsia boliviana* and *Souroubea fragilis* were collected in the Zongo Valley to study their dyeing properties. The acid and basic extracts of the analyzed species required the use of mordents to fix the dye into the cotton fabric. To obtain the dark red color of the acidic extract of *Brachyotum microdon*, $Al_2(SO_4)_3$ was the recommended mordent; while for the basic extract, with a dark brown tone, $FeSO_4$ gave the best dyeing results. $Al_2(SO_4)_3$ was also the chosen mordent to dye the fabric with the light orange tone of the acidic extract of *Souroubea fragilis*; while, $CuSO_4$ reproduced the dark brown tone of its basic extract. Finally, $FeCl_3$ provided the best finishing and colors for the dyeing techniques with *Fuchsia boliviana*. The same mordents were used to dye other fabrics with the natural colorants and among the fabrics tested, linen, crepe and velvet presented the best color resolutions. All the fabrics exposed to the acid extract of *Brachyotum microdon* presented a light purple coloration, while the fabrics exposed to the other vegetal extracts presented copper like colored tones. The spectroscopic studies on the acidic extracts presented absorptions consistent with their natural organs (flowers, fruits or steams) and extracts colors. In addition, the spectra presented absorptions for anthraquinones and phenolic compounds, some of them responsible for the species' antioxidant and photoprotector activities. To our knowledge, this is the first time that dyeing studies on *Brachyotum microdon*, *Fuchsia boliviana* and *Souroubea fragilis* are reported.

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