# Characterization and dyeing potential of colorant-bearing plants of the Mayan area in Yucatan Peninsula, Mexico

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## Abstract

Natural dyes are receiving increasing attention from researchers and manufacturers, given its perceived eco-friendly nature. Yet, adjunct agents known as mordants that help bond the molecules of the fabric being dyed and the colorant used to dye it are often toxic. There is scant published information describing the dyeing potential and toxicity of colorant-bearing plants and the dye uptake with respect to the mordant treatments. A preliminary survey based on a range of sources of information showed that of the Yucatan peninsula harbors 23 plants showing dyeing properties. Four of them (*J. spicigera*, *B. orellana*, *B.glabra* and *R. discolor*) were selected to extract their natural dyes, which were chemical and toxically characterized. *B. orellana* was successfully employed to dye the fabric, viz. ordinary cotton cloth, manta.The dye baths without mordant only present few toxicity, the *J. spicigera* and *B. glabra* dyes being the most toxic. The *B. orellana* dye was less toxic, although its toxicity is increased when mordants are used. Regarding color performances of the dyed fabrics, mordanting influenced the depth of the shades, improving dyeing and color fastness. However the mordant had no significant effect on color values. All dyed fabrics had similar color, with hue angle values indicating yellowish, orange-yellowish color.

***Keywords***: Plant dyes; *Bixa orellana;* mordant; fabric materials; color characterization; Mayan handcraftsmanship.

## Highlights

‣23 plants from Yucatan peninsula (Mexico) were inventoried in an ethnobotanical study. ‣Potential toxicity of 4 of them was studied with *A. salina* and *L. sativa*. ‣*B. orellana* dye extract was employed in dyeing ordinary cotton cloth (manta). ‣ A measurement protocol for the color characterization of dyed fabrics was developed. ‣ Mordanting did improve dyeing and color fastness but not changed color values.

## 1. Introduction

Mesoamerican civilizations such as Olmecs, Mayas, Aztecs and Teotihuacans have been reported using natural substances for the dyeing production (Guirola, 2010). During the Pre-Hispanic time (2000 B.C. – 1524 A.D.), the application of dyes, extracted from plants and combined with several organic and inorganic elements, can be noticed in artistic pieces such mural paintings, decorated masks and stelas, and textile fragments (Cabezas, 2005). A wide variety of textile representations can be contemplated in the famous murals of Bonampak (Chiapas, Mexico) one of the few ancient Maya archaeological sites still preserved. On those representations, the social class division was represented throught the type of clothing weared by the characters. The Maya occupied the eastern third of Mesoamerica, and primarily the Yucatan peninsula (Mexico). It is one of the most emblematic civilizations of the world numbering millions of people that inhabited parts of the present-dayMexico, Guatemala, Belize, El Salvador and Honduras (**Figure 1**). The Maya used natural dyescomprising both biological-derived (mainly from plants)and inorganic dyes. In fact, the Maya Blue is perhaps the most studied pigment due to its remarkable chemical stability and resistance in the aggressive tropical environment of Central America. Maya Blue is a combination of both inorganic and organic elements: palygorskite or attapulgite clay (also called *yucatec sak tu'lum* in Maya) and a blue dye that is produced by the indigo plant (or *ch'oh* in Maya) (Chiari et al., 2003). On the contrary, the perishable nature of other dyes and colorants used by the Maya civilization made it difficult for archeologists to find evidence of their use among the ancient Mayan remains and the extent of their employment remains uncertain. Some investigators argue that the first evidence of the use of dyes in the area was found among human groups that used the pigments as body paintings, for ritual purpose and for environmental adaptation, probably using them as bug repellents (Ivic and Berger, 2008). Then, during the colonial period (from the Spaniards first settlement in 1524, to 1821) dyeing plants started to be traded as luxury goods for a very high price. Their value was only exceeded by the gold and silver that was also found in the American continent. The interest that the Spanish had in the dyeing techniques used by the indigenous people, is also documented by the detailed registries that they made of such techniques. Unfortunately, in some parts of Mesoamerica there is still a lack of information about this topic (Ivic and Berger, 2008; Guirola, 2010).

In Europe, during the XIX century, the textile industries acquired huge quantities of dyes that were easily available. This situation triggered the invention of synthetic dyes, offering a vast range of new colors at a lower-cost, and imparting better properties to the dyed materials (Garfield, 2000). In many Mayan regions the incursion of such dyes partially, and sometimes fully, replaced the use of local dyeing, although the original dying substances remained. Nowadays synthetic dyes are still the primary option chosen to dye textiles, in spite of the potential health and environmental risks, as some of them have been reported to have carcinogenic and other cytotoxic effects (Sewekow, 1988). Yet, in some region this discovery has prompted a resurgence of the use of dyes derived from natural to replace, at least partially, synthetic dyes. This renewed interest in plant dyes goes in line with a worldwide concern for the development of eco-friendly and sustainable productions (Padhy and Rathi, 1990; Garg et al., 1991; Eom et al., 2001), including in the textile field (Guinot et al., 2006; Bechtold et al., 2007; Islam et al., 2013). However, contrary to dominant public opinion, natural dyes are neither systematically safer nor more ecologically sound than synthetic dyes. A few natural dyes, such as logwood (*Haematoxylum campechianum*), which contains haematin and haematoxylin, are themselves significantly poisonous (Buchanan, 1987). Adjunct agents known as mordants, applied in conjunction with the natural dye to increase its affinity, substantivity and fastness properties, may be toxic at different levels (Haaret al., 2013). Most mordanting agents are metallic salts of chromium, tin, iron, copper, and aluminum; heavy metals significantly toxic and harmful to the environment and human health. Copper and chromium containing compounds (viz. copper sulfate and potassium dichromate) were widely used as mordants, but their usage has declined because of their toxicity (Cardon, 2007; Haaret al., 2013), and so as iron and tin mordants. The aluminum-mordanting agents despite its high toxicity are often considered in the application of natural dyes to fabric materials and they are probably the most commonly used in dyeing techniques.

There are very limited published studies that describe the dyeing potential of colorant-bearing plants and the dye uptake with respect to the mordant treatments. However, natural dyes are receiving increasing attention from researchers and manufacturers. Modern Maya artisans use both synthetic and plant-derived colorants to dye textiles and vegetable fibers to craft hats and decorative items. In the Campeche region (one of the states comprising the Yucatan peninsula), where this study was carried out, some of the rural craftsmen extract dyes from the leaves, roots, flowers or bark of some plant species mostly by boiling and by performing other procedures that they are reluctant to disclose. It is apparent that countless natural resources that have been used in Mesoamerica since the Pre-Hispanic epoch and some are still used thanks to the oral transmission of this knowledge. But there is insufficient information regarding the characterization of many plant species used for dyeing purposes.

This study aims at characterizing some natural dyes derived from plants used in different areas of the Yucatan peninsula (Mexico) and will hopefully contribute to spread safe and sustainable ancestral techniques of hand-crafted productions essential to rural livelihood. Thus, the objectives of our study were to: (1) carry out a preliminary plant inventory and ethnobotanical study of the traditional dyeing resources from this region (Yucatan peninsula), (2) extract the natural dyes from the four most used plant species, (3) determine the main chemical constituents of these natural selected dye extracts, (4) assess the potential toxicity of these selected natural dye extracts through bioassays of *Artemia salina* and *Lactuca sativa*, and, lastly (5) to dye ordinary cotton cloth (manta) with *Bixa orellana* dye extract (one of the four selected), analyze the fabric dyed and to examine the color properties.

## 2. Materials and methods

### 2.1.*Plant inventory and ethnobotanical study*

An ethnobotanical inventory, in which we related the plant species and their utilization by the local people inhabiting in the region, was conducted in the Mayan area from Yucatan peninsula (Mexico). Three main sources of information were used to conduct the inventory: (i) analysis of regional herbaria data, (ii) bibliographic studies, and (iii) a number of interviews withcraftsmen and herbalists. The data collected also included the local names of the plants and the parts of the plant used to prepare the dye.

### *2.2. Dye extraction procedure*

The extraction of dyestuffs was carried out using four plant species of all native plants inventoried in the previous Section 2.1. These plants were selected, taking into account their common use by local rural handicraftsmen, and abundance in the region, as evidenced by the survey that was conducted. An additional criterion was that the local Ministry of Commerce and Industrial Development expressed its interest in performing research that may reach eventually application in local hand craftsmanship.

Plant samples were purchased in the local market. A botanist from the Autonomous University of Campeche (Mexico) confirmed their identity. The four plant species selected were *Justicia spicigera*, *Bixa orellana*,*Bougainvillea glabra* and *Rhoeo discolor*. The part of the plants employed for extraction included leaves, seeds, bracts and leaves, respectively. The material was dried at room temperature, stored in the dark, and extracted through two methods: a traditional (hot water extraction) and an alternative modern method (microwaves). Two solvents were employed for each method: water and ethanol diluted to 10% strength. All the experiments were carried out in duplicate.

### *2.2.1. Decoction or hot water extraction*

A known amount (20 g) of dry plant materialwas extracted with 400 ml of boiled distilled water, following the standard procedure in which the ratio of plant biomass to solvent volume is 1:20. Extraction was performed for 60 min at 100°C in an open beaker. Extracts were cooled at room temperature (26°C) and filtered using Whatman No.40 filter paper. Samples were frozen and freeze-dried (Labconco). The same extraction procedure was performed using 10% ethanol as extraction solvent.

### *2.2.2. Microwave-assisted extraction*

The process of microwave assisted extraction method was performed in a domestic microwave oven (Sharp model R-501CW) with the power of 800 W and the frequency set on 2450 MHz. A weighted amount (20 g) of dry plant material was subjected to extraction by adding 400 ml distilled water in a 1000 ml glass beaker. The glass beaker was placed in the center of the microwave oven. Samples were exposed to microwave irradiation during five cycles (one cycle equals 1 minute of duty cycle followed by 1 minute break to avoid overheating, i.e. 1 minute on / 1 minute off). Extracts were cooled at room temperature and filtered using Whatman filter paper No. 40. Samples were frozen and lyophilized to dryness and stored at room temperature (26°C).

### 2.3.Chemical characterizationof the extracted dyes

The water extracts of *Justicia spicigera*, *Bixa orellana*, *Bougainvillea glabra* and *Rhoeo discolor*, have been chemically characterized by spectroscopic and chromatographic techniques. The dye extracts were subjected to UV-VIS spectrophotometer (UNICO SQ-2800) after suitably diluting the extracted dye with water (1 mg/ml). The UV-VIS spectra of the natural dyes from the four plant species were obtained in visible region of 400-750 nm and the peak absorbance ($\lambda_{max}$) was measured and recorded. Additionally, all samples were examined by thin-layer chromatography (TLC) on silica gel 60 G F-254 plates (Merck). Chromatography was performed in following solvent systems: ethyl acetate-formic acid-water (33:7:10) and benzene-acetone (95:5). The chromatograms were observed first without chemical treatment, under UV 254 and UV 365 nm light, and subsequently using the spray reagents, namely, Dragendorff reagent for alkaloids, Bornträger reagent for antraquinones, Liebermann-Buchard reagent for triterpenes, and natural products and polyethylenglycol (NP/PEG) reagent for flavonoids.

### 2.4. Toxicity study of the extracted dyes

In order to assess the potential toxicity of the four extracted dyes, bioassays with the microcrustacean *Artemia salina* (brine shrimp) and the annual plant *Lactuca sativa* (lettuce) were carried out. The *Artemia salina* assay was performed according to the modified method of Solis et al. (1993). Larvae of *A. salina* (24 hours old) were exposed to different concentrations of the *Justicia spicigera*, *Bixa orellana*, *Bougainvillea glabra* and *Rhoeo discolor* residual dyeing baths at 25°C for a period of 24 hours. The dyeing baths were tested in a concentration series of 50%, 25%, 12.5% and 6.25%, in the 96-well microplates in triplicate. Negative controls were performed in parallel using an artificial marine salt solution (38 g/l). After an incubation of 24 hours, the number of dead larvae was counted and the percentage of dead nauplii calculated. The *Lactuca sativa* seeds (germination) assay was performed in Petri dishes, where filter paper disks (Whatman No. 5) were placed and wetted with 5 ml of the *Justicia spicigera*, *Bixa orellana*, *Bougainvillea glabra* and *Rhoeo discolor* residual dyeing baths. In this case, the dyeing baths were tested in a concentration series of 100% and 50%. Negative controls were performed in parallel using distilled water (5 ml). A total of twenty lettuce seeds were distributed in each dish. The dishes were incubated for germination at 24 ± 1°C under 12 h light / 12 h dark cycles. After an incubation of 96 hours, the germination percentage and radical length were recorded to calculate the germination index (GI).

The germination index (GI) was calculated according to the formula GI = $G/G_0 \times L/L_0 \times 100$, where $G_0$ and $L_0$ are respectively the germination percentage (number of seeds germinated) and root growth (radical length) of the 100%, distilled water control. The global germination index (GI) was the GI averages of the 100% and 50% extract treatments. This last bioassay was not used for the residual dyeing baths from the pre-mordanting method assays (see Section 2.5), because the simultaneous mordanting or 'single-bath method' (see Section 2.5) is used in most of studies on seeds germination of *Lactuca sativa*.

### *2.5. Dyeing fabric with* **B. orellana** *dye extract*

The ordinary cotton cloth (manta), which in the early twentieth century was the material used for clothing by two-thirds of the inhabitants of Mexico (Russell, 1914), was the fabric selected for the study. It was scoured in an aqueous solution containing 0.5 g/l sodium carbonate and 2 g/l non-ionic detergent at 50°C for 25 min. The scoured fabric was thoroughly washed with deionized water and soaked in clean water for 30 min. Subsequently, circular pieces of fabric material between 33.20 $cm^2$ (6.5 cm aprox. of diameter) and 38.50 $cm^2$ (7 cm aprox. of diameter) were prepared for dyeing and mordanting.

Dyes from *J. spicigera, B. glabra,* and *R. discolor* without mordant demonstrated to be adjectives, since they had poor fixation to the fabric, whereas the *B. orellana* dye adhered to the fabric without losing much dye during the rinse. Based on these results, the extracted dye solution from *Bixa orellana* (Rajendran, 1990; Sinha et al., 2013; Tamil Selvi et al., 2013) was employed to dye cotton cloth (manta). The fabric was dyed with the *B. orellana* extract colorant without the use of mordants (**Figure 2**) in open beakers with manual agitation of the material. The dye (2.5% w/v) was dissolved in water with increasing temperature up to 50°C. The fabric samples were submerged in the solution, which was further heated to 80°C. The temperature was maintained with continuous agitation for 1 hour. After dyeing, the dye surplus was removed from the dyed materials by rinsing three times with water at 50°C. Furthermore, mordants such as tannic acid (0.2% w/v), sodium citrate (2.9% w/v), aluminum potassium sulfate (0.625% w/v), and ferrous sulfate (0.1% w/v) were used to mordant the fabric (**Figure 3**). Two mordanting processes were used: pre-mordanting, a process in which the sample is first mordanted and after dyed, and simultaneous mordanting or comordanting, where the sample is treated simultaneously with dye and mordant. For the pre-mordanting method, the fabrics were first immersed in the aqueous solutions of each one of the aforementioned mordants at 50°C for 30 minutes.The samples were then removed and without drained were immersed in a water extract of *B. orellana* (2.5%w/v) at 80°C for 1 hour with constant stirring. The samples were washed (two baths of 1 min) in distilled water at 50°C. Subsequently the pre-mordanted fabrics were washed twice (1 min each) at 50°C with distilled water. For the simultaneous mordanting, (i.e. dyeing in the presence of mordants), the fabrics were immersed in a bath containing a mordant and the dye extract of *B. orellana* at room temperature (26°C). Undyed fabric samples were used as controls. Experiments were performed by triplicate.

### *2.6. Colorimetric analysis of fabric dyed with* **B.orellana** *dye extract*

Dyed fabrics processed in this study (see Section 2.5) were subjected to the reflectance color measurements using a Konica Minolta colorimeter with a CR–300 measuring head (8-mm-diameter viewing area). The following measurement conditions were selected: illuminant D65, which represents a phase of daylight, with a CCT of approximately 6500 K, including the ultraviolet region spectrum, and 2° observer (CIE 1931). The measurements were made by spectral reflectance, using the diffuse illumination geometry with an integration sphere, covered with a white material, so that the light is uniformly diffuse in all directions illuminating the sample, and is observed with the specular component included in 8° in relation to normal (d/8°). The color measurements were analyzed by considering the CIE 1976 (L*a*b*) or CIELAB color system, which is widely accepted by both the scientific community and industry, since is the most *perceptually uniform* of the color spaces (Berns, 2000; Völz, 2001). *Perceptually uniform* means that a change of the same amount in a color value should produce a change of about the same visual importance. The CIELAB color system is organized with three axes in a spherical form: L*, a* and b*. The L* axis is associated with the lightness of the color and moves from top (value: 100, white) to bottom (value: 0, black), whereas the a* and b* axes are associated with changes in redness-greenness (positive a* is red and negative a* is green) and in yellowness-blueness (positive b* is yellow and negative b* is blue); both move in the two axes that form a plane orthogonal to L*, and do not have specific numerical limits. Furthermore, the color parameters most closely related to the psychophysical characteristics of color, i.e. more related to color perception, and which correspond to the angular coordinates $C^*_{ab}$ and $h_{ab}$ were also calculated. $C^*_{ab}$ is the chroma or saturation of color ($C^*_{ab}=[a^{*2}+b^{*2}]^{1/2}$) measured as the length of the line from the neutral point to the sample point in the a*b* plane, and $h_{ab}$ is the hue angle or tone ($h_{ab}$= arctan (b*/a*)) refers to the dominant wavelength, as is the CIELAB color parameter that represents the major color perception attribute, indicating redness, yellowness, greenness, or blueness in a circular scale that starts at 0° and increases counter clockwise to 360° (Wyszecki and Stiles, 1982; Boonsong et al., 2012). Likewise, the partial (ΔL*, Δa* and Δb*) and the total ($\Delta E^*_{ab}= [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$) color differences between the textile samples were calculated. The samples compared were: (i) the dyed with the undyed samples, (ii) the dyed samples with and without the use of mordant, and (iii) the pre-mordanting and simultaneous mordanting dyeing samples. Likewise, both sides of the same textile sample were compared using $\Delta E^*_{ab}$. With the purpose of establishing the minimum number of measurements required to quantify the color of the dyed textile material under study, ten measurements at random positions onto circular areas of the textile material have been previously carried out. From them, the minimum number of measurements required was analyzed by taking into account the cumulative averages of the CIELAB color system coordinates: L*, a*, and b* (Prieto et al., 2010a).

## 3. Results and discussion

With ever-increasing demand for eco-friendly, non-toxic colorants, dyes derived from natural sources have emerged as a potential alternative to relatively toxic synthetic dyes. In this context, the list of dyeing plant species inventoried in the ethnobotanical study is arranged in alphabetical order using the botanical family as a reference (**Table 1**). The most prevalent families are Fabaceae followed by Rubiaceae. A total of twenty-three different plants were identified as common sources of dyes; although other plants appeared to be also a source of dyes, they were not used as much and therefore they are not included in this study. Leaves are the most prevalent type of biomass material that is used to obtain dyes, followed by bark. Most of the plants were readily identified by their Mayan names by local handicraftsmen working with them. Taking into account the criteria explained previously (see Section 2.1) four of these plants were selected for the extraction of dyes, namely *Justicia spicigera*, *Bixa orellana*, *Bougainvillea glabra* and *Rhoeo discolor*.

A comparative study of the effects of processing parameters of extraction on the yield of *Justicia spicigera*, *Bixa orellana*, *Bougainvillea glabra* and *Rhoeo discolor* dyes revealed that in all cases the yield of extract material was better in the traditional heating method (decoction) than that from the microwave method. In contrast, the study on pomegranate (*Punica granatum L.*) rind by Sinha et al. (2012) reported that the application of microwave irradiation method improved the yield of dye. This diversity within the natural dyes probably results from differences in the main chemical constituents and morphology. Moreover for the dye extraction Sinha et al. (2012) used statistical analyses (response surface optimization and artificial neural network modeling), which predict the optimal experimental conditions for maximum extraction of dye. More recently, the same authors (Sinha et al., 2013) published a study in which used the same two statistical analyses for simulation and optimization of the dye microwave-assisted extraction process from *Bixa orellana*, one of the species selected by this study. Unfortunately decoction extraction was not included in their study. Besides, a binary solvent extraction system (water and ethanol) was superior to a monosolvent system (water) in most cases. The four plant species increase the percentage of extracted matter (from 20 g of plant sample) in the order of *J spicigera* > *B. glabra* > *B. orellana* and *R. discolor* (**Table 2**).

Thin-layer chromatography (TLC) profiles of natural dyes from the four plant species were similar (data not shown), which is consistent with the results obtained in the absorption spectra (**Figure 4**). In all extracts obtained from *J. spicigera*, *B. glabra*, and *R. discolor* were detected flavonoids as metabolites responsible of coloration. It has been previously reported that both *J. spicigera* as *R. discolor* contain flavonoids, particularly anthocyanins, which may be responsible for the signals observed in the absorption spectra (Asen et al., 1972). In *B. glabra*, signals of betalains were not detected, although from previous studies the occurrence of flavonoids in the bracts of this plant is known (Kumar et al., 2013). We expected the presence of bixin but they were not actually detected in *B. orellana* extracts. This is probably due to the extraction procedure itself, as these molecules were probably eliminated when the extracted plant material was filtered to obtain a water soluble dye. The absence of norbixin can be explained because of its insoluble nature in polar solvents (**Figure 5**).

In order to assess the toxicological response of the residuary dyeing baths on living organisms, *A. salina* and *L. sativa* were used as toxicity level bioindicators due to their fast response to concentrated and diluted by-product solutions. The dye residual baths without mordant did not present toxic activity against nauplii of *A. salina*, but exhibited certain phytotoxic activity on *L. sativa* seeds to concentrations of 100% and 50%, the *J. spicigera* and *B. glabra* being the most toxic dyes (**Table 3**). The *B. orellana* dye was less toxic in the test of *L. sativa* seed germination, though its toxicity increased when the colorant was mixed with the corresponding mordant used in the dyeing process **(Table 4)**. This result was also observed with *A. salina* in some combinations. The combination of *B. orellana* dye and aluminum potassium sulfate was the most toxic for the microcrustacean *A. salina.* The combination of *B. orellana* dye and sodium citrate was the most toxic for *L. sativa* seeds (**Table 4**).

In previous studies with leather fabrics, *B. orellana* extract appeared to be a viable option to natural dyed process (Tamil Selvi et al., 2013) and the same result is achieved for the ordinary cotton cloth (manta). Importantly, cotton fibre has very low affinity for most of the natural dyes (Shahid et al., 2013). Pieces of ordinary cotton cloth (manta) colored using *B. orellana* extract have been subjected to reflectance measurement. The color measurement parameters L*, a*, b*, $C^*_{ab}$ and $h_{ab}$ were recorded. The minimum number of measurements required to characterize these parameters in each sample of dyed textile material was defined from the steady section of the inverted exponential decay graph, obtained by plotting the cumulative averages of Cartesian CIELAB coordinates (L*, a* and b*) against number of measurements (Prieto et al., 2010a, 2010b, 2011). Thus, it was 8 measurements to characterizean area between 33.20 and 38.50 $cm^2$ approximately, with an 8-mm viewing aperture diameter. Once the measuring protocol was defined, the color of each dyed textile sample was characterized by carrying out eight measurements at random positions on three replicates of each type of sample, following the conditions described in Section 2.6. The values of the CIELAB color coordinates: L*, a*, b*, $C^*_{ab}$ and $h_{ab}$ for the dyed fabrics processed in this study are shown in **Table 5**. The L* parameter (color lightness) varied between 74.17 and 62.32 CIELAB units for dyed samples, 80.60 CIELAB units was the L* value of the control (undyed fabric, U), indicating a clearly perceptible darkening of the fabric due to dye (Hardeberg, 1999; Giacomucci et al., 2012). The value of a* (associated with greenness (-) to redness (+) changes) varied from -0.11 (U) to 3.22 (DSF) and 24.63 (DSC) range of color data, conferring the fabric a reddish color. The changes in b* (associated with blueness (-) to yellowness (+) changes) and $C^*_{ab}$ (color chroma) were very similar except for DSC; in both cases they increased by at least 11 CIELAB units, which means more yellow and chromatic colors. The hue angle, $h_{ab}$, for dyed fabrics with and without mordant, fell within the interval 83.87º (DSA) and 60.90º (DSC), so they were located in the yellow, yellow-orange hue area. All the mordants influenced the depth of the shades, which suggests that the mordants allow the fixation of *B. orellana* dye to the natural fibers of cotton cloth (manta) by the formation of a chemical bridge between the dye and the fiber, improving the dyeing and color fastness. This result is similar to that achieved by Meksi et al. (2012) in wool fabrics colored using olive mill wastewater in which mordanting gave higher dyeability and enhanced fastness properties. The fabrics dyed without mordant (DWM) showed lighter shades, i.e. higher values of L*, except those dyed with aluminum mordant (DPA and DSA). However values of $h_{ab}$ in DWM moved away control (undyed fabric, U) almost as much (DSC and DPC is further away) or even more that the cotton fabrics with mordant (**Table 5**). The value of $h_{ab}$ represents the major color perception attribute therefore these results led us to conclude that mordant had no significant effect on color values (Boonsong et al., 2012). Simultaneous mordanting with sodium citrate (DSC) showed a deeper maximum color than the rest of fabrics dyed did, with a maximum value of a* (associated with redness increase), b* (associated with yellowness increase) and $C^*_{ab}$ (color chroma), and a minimum value of L* (color lightness) and $h_{ab}$ (hue angle). It was only surpassed in L* parameter by the simultaneous mordanting with ferrous sulfate (DSF), which is related with darkening of the color. In all cases the partial and total color differences between the dyed fabrics (**Table 6**), with respect to the control (undyed fabric), surpassed the visual threshold of 3 CIELAB, perceptible to the human eye (Wyszecki and Stiles, 1982;Völz, 2001). The greatest difference was observed in the simultaneous sodium citrate-mordanted fabrics (DSC). The mordants in the order of DPC<DST<DPA<DPT<DSA<DPF<DSF<DSC increased the color change of the dyed fabrics. From the results, it was also confirmed that the pre-mordanting method versus the simultaneous mordanting gave rise to fabrics colored with a perceptibly different color, with the exception of tannic acid-mordanted (DPT and DST) fabrics. Finally to

analyze color variations between both sides of the dyed fabric, the total color differences ($\Delta E^*_{ab}$) between both sides for each dyed textile sample were calculated and their average values were plotted in **Figure 6**. The greatest differences were observed with the simultaneous mordanting with aluminum potassium sulfate (DSA) and the simultaneous mordanting with ferrous sulfate (DSF), which reached values of 1.34 and 1.82 CIELAB units, respectively. However these values do not exceeded 2 CIELAB units, considered imperceptible to the human eye (Wyszecki and Stiles, 1982; Völz, 2001; Giacomucci et al., 2012). In all other cases, $\Delta E^*_{ab}$ was less than 1 CIELAB unit, defined as the visual color difference threshold or just noticeable difference (jnd) which constitutes the lowest limit of perception for an individual with normal color vision (Wyszecki and Stiles, 1982; Völz, 2001; Prieto et al., 2010a, 2011).

## 4. Conclusion

During the last two decades, natural dyes have become an interesting subject of study due to their better properties in comparison to their synthetic counterparts. They are considered as healthier for humans and their environment, some of them with antibacterial properties, and therefore are sometimes preferred in textile industry. In this study, the preliminary survey of Yucatecan flora based on a range of sources of information showed its resourceful potential to obtain natural dyes. From the 23 plants included in the inventory and ethnobotanical study, four were selected to yield natural dyes. Dyes from *J. spicigera*, *B. glabra*, and *R. discolor* without mordant demonstrated to be adjectives, contrary to fabrics dyed *with B. orellana* dyes without mordanting, which showed an adherence to the fabric material. In all cases the yield of extracted material was better with the traditional heating method (decoction) than with from the microwave method. Moreover, 10% ethanol replacing distilled water proved to be more efficient. The dye baths without mordant only present few toxicity, the *J. spicigera* and *B. glabra* dyes being the most toxic. The *B. orellana* dye was less toxic, although its toxicity is increased when mordants are used. Consequently, caution should be taken when stating that natural-derived dyes are safe to the environment and to the manufacturer or end user, as many of them require the use of mordants that shows a level of toxicity. Regarding the color performances of the dyed fabrics, mordanting influenced the depth of the shades, improving dyeing and color fastness, although it had no significant effect on color values. All dyed fabrics had similar color, with hue angle values indicating yellowish, orange-yellowish color. A perceptibly different color was obtained between the pre-mordanting and simultaneous mordanting dye processes, except in tannic acid-mordanted fabrics.

## Acknowledgments

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## References

Asen,S., Stewart, R.N., Norris, K.H., 1972. Co-pigmentation of anthocyanins in plant tissues and its effect on color. Phytochemistry 11, 1139-1144.

Bechtold, T., Mahmud-Ali, A., Mussak, R., 2007. Natural dyes for textile dyeing: A comparison of methods to assess the quality of Canadian golden rod plant material. Journal of Cleaner Production 75, 287-293.

Berns RS. 2000. Billmeyer and Saltzman’s principles of color technology. 3rd ed. New York (USA): John Wiley & Sons.

Boonsong, P.,Laohakunjit, N.,Kerdchoechuen. O., 2012. Natural pigments from six species of Thai plants extracted by water for hair dyeing product application. Journal of Cleaner Production 37, 93‑106.

Buchanan, R., 1987. A Weaver's Garden: Growing Plants for Natural Dyes and Fibers. Interweave Press Inc. Loveland Colorado.

Cabezas, H., 2005. Unidad geográfica-cultural, Flora Mesoamericana; Mesoamérica. Universidad Mesoamericana. Guatemala.

Cardon, D., 2007. Natural dyes: Sources, tradition, technology and science. London, England: Archetype Publications.

Chiari, G., Guistetto, R., Ricchiardi, G., 2003. Crystal structure refinements of palygorskite and Maya Blue from molecular modeling and powder synchrotron diffraction. European Journal of Mineralogy 15, 21-33.

CIE Publication 15-2. Colorimetry. 1986. CIE Central Bureau, Vienna.

Eom, S., Shin, D., Yoon, K. 2001. Improving the dyeability of natural colourants on cotton by cationization. Indian Journal of Fibre and Textile Research 26, 425-431.

Garfield, S., 2000. Mauve: How One Man Invented a Color That Changed the World. Faber and Faber.

Garg, A., Shenda, S., Gupta, K.C., 1991. Effect of mordants on color of natural dye extracted from tissue flowers (*Butea mongsperma*). Colourage 38, 50-53.

Giacomucci, L., Toja, F., Sanmartín, P., Toniolo, L., Prieto, B., Villa, F., Cappitelli, F., 2012. Degradation of nitrocellulose-based paint by *Desulfovibrio desulfuricans* ATCC 13541.Biodegradation 23, 705-716.

Guinot, P., Rogé, A., Gargadennec, A., Garcia, M., Dupont, D., Lecoeur, E., Candelier, L., Andary, C., 2006. Dyeing plants screening: an approach to combine past heritage and present development. Color Technology 122, 93-101.

Guirola, C., 2010. Natural dyes used in Mesoamerica since prehispanic age. Cajas, A. ed. FLAAR Mesoamérica. Guatemala.

Haar, S., Schrader, E., Gatewood, B.M., 2013.Comparison of Aluminum Mordants on the Colorfastness of Natural Dyes on Cotton. Clothing and Textiles Research Journal 31(2), 97-108.

Hardeberg,A.Y., 1999. Acquisition and reproduction of color images: colorimetric and multispectral approaches. Ph.D. thesis (Ecole Nationale Superieure des Telecommunications).

Islam, S.,Shahid, M., Mohammad,F., 2013.Perspective for natural product based agents derived from industrial plants in textile applications – a review. Journal of Cleaner Production 57, 2–18.

Ivic de Monterroso, M., Berger de White, M., 2008.El arte sale de las casas: los tintes naturales en San Juan la Laguna, Sololá. En: *Ciencia y Técnica Maya*. Ivic de Monterroso, M., Azurdiaq Bravo, I. eds. Fundación Solar. Guatemala.

Kumar, S.N.A, Ritesh, S.K., Sharmila,, G., Muthukumaran C., 2013. Extraction optimization and characterization of water soluble red purple pigment from floral bracts of *Bougainvillea glabra*. Arabian Journal of Chemistry (*In press*).

Meksi, N., Haddar, W., Hammami, S., Mhenni M.F., 2012. Olive mill wastewater: A potential source of natural dyes for textile dyeing. Industrial Crops and Products 40, 103– 109.

Padhy, R.N., Rathi, D., 1990. Effect of mordants on dyeing of cotton with vegetable dyes. Textile Dyer and Printer 23, 27-28.

Prieto, B., Sanmartín, P., Silva, B., Martínez-Verdú, F., 2010a. Measuring the color of granite rocks: a proposed procedure. Color Research and Application 35, 368–375.

Prieto, B., Sanmartín, P., Aira, N., Silva, B., 2010b. Color of cyanobacteria: some methodological aspects. Applied Optics 49, 2022–2029.

Prieto, B.,Ferrer, P., Sanmartín, P., Cárdenes, V., Silva, B., 2011. Color characterization of roofing slates from the Iberian Peninsula for restoration purposes. Journal of Cultural Heritage 12, 420–430.

Rajendran, R., 1990.Achiote: Bixa orellana L. A Natural Food, Colour and Dye, AES, College of Agricultural Science, Mangilao, Guam, 1st edition.

Russell, T.H., 1914. Mexico in peace and war. Published by Reilly & Britton Syndicate, Chicago.

Sewekow, U., 1998. Natural dyes: an alternative to synthetic dyes? Melli and Textilberichte 69, 145-148.

Shahid, M., Islam, S., Mohammad, F., 2013. Recent advancements in natural dye applications: a review. Journal of Cleaner Production 53, 310‑331.

Sinha, K., Saha, P.D., Datta, S., 2012. Response surface optimization and artificial neural network modeling of microwave assisted natural dye extraction from pomegranate rind. Industrial Crops and Products 37, 408–414.

Sinha, K., Chowdhury, S., Das Saha P., Datta S., 2013. Modeling of microwave-assisted extraction of natural dye from seeds of *Bixa orellana* (Annatto) using response surface methodology (RSM) and artificial neural network (ANN). Industrial Crops and Products 41, 165– 171.

Solis, P.N., Wright, C.W., Anderson, M.M., Gupta, M.P., Phillipson, J.D., 1993. A microwell cytotoxicity using *Artemia salina*. Plant Medica 59, 250-252.

Tamil Selvi, A., Aravindhan, R., Madhan, B., Raghava Rao, J., 2013. Studies on the application of natural dye extract from *Bixa orellana* seeds for dyeing and finishing of leather. Industrial Crops and Products 43, 84–86.

Völz, H.G., 2001. Industrial color testing, Wiley–VCH. Weinheim.

Wyszecki, G., Stiles W.S., 1982. Color Science. Concepts and Methods. Quantitative Data and Formulae, Wiley, New York.

## Figure captions

**Figure 1**. Map of the Maya area within the Mesoamerican region (from http://www.latinamericanstudies.org/mayas.htm).

**Figure 2**. Picture of the ordinary cotton cloth (manta) undyed (left) and dyed using *B. orellana* extract colorant without the use of mordants (right).

**Figure 3**. Several pictures of the dyed fabrics with mordanting. A: pre-mordanting with tannic acid, B: simultaneous mordanting with tannic acid, C: pre-mordanting with sodium citrate, D: simultaneous mordanting with sodium citrate, E: pre-mordanting with aluminum potassium sulfate, F: simultaneous mordanting with aluminum potassium sulfate, G: pre-mordanting with ferrous sulfate, H: simultaneous mordanting with ferrous sulfate. Controls appear on the left in the pictures.

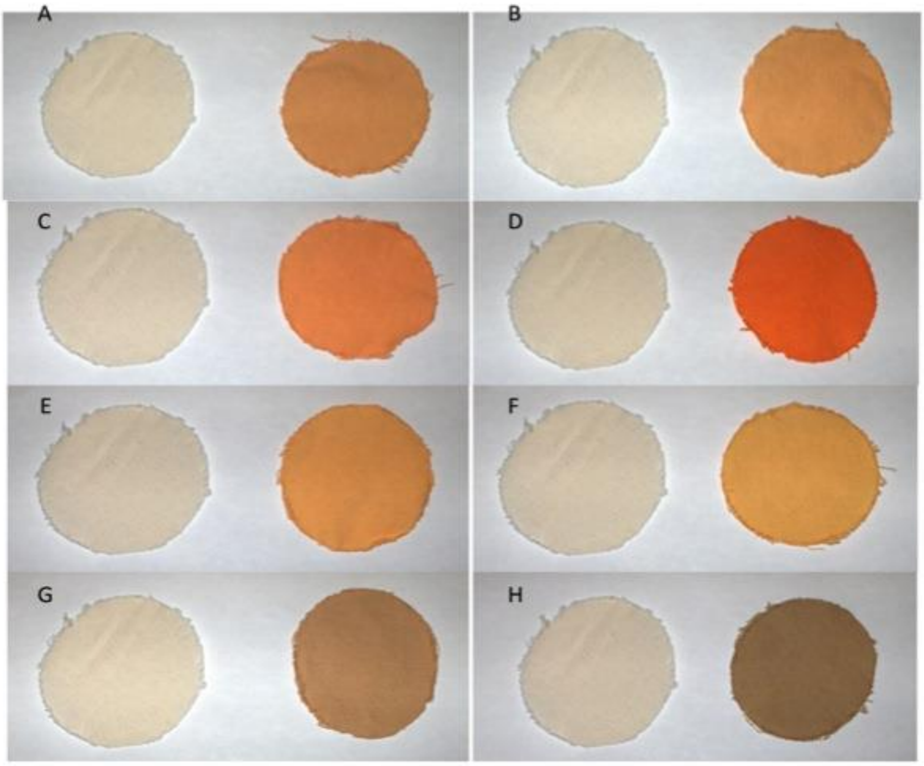


**Figure 4**. Absorption spectra for extracts of (a) *J. spicigera*, (b) *B. orellana*, (c) *B. glabra*, and (d) *R. discolor*.

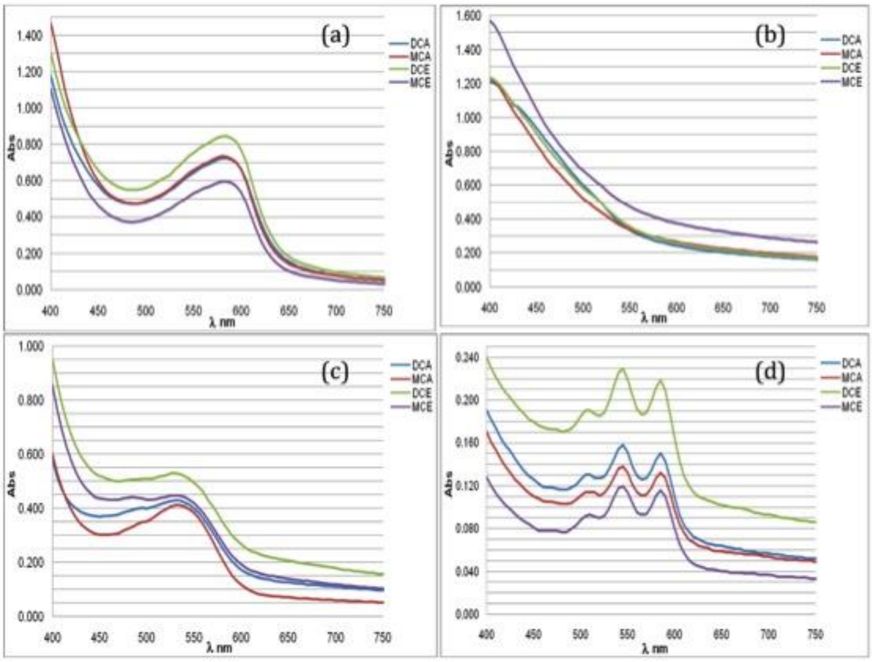


**Figure 5**. Chemical structure of the flavonoid pigments, main colorants present in the extracted plant materials.

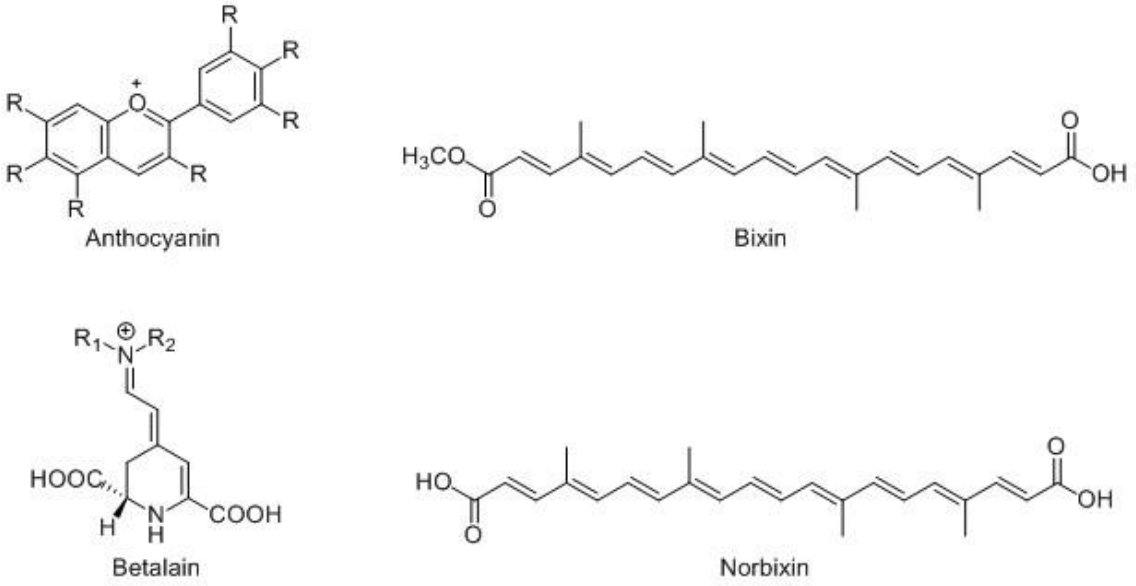
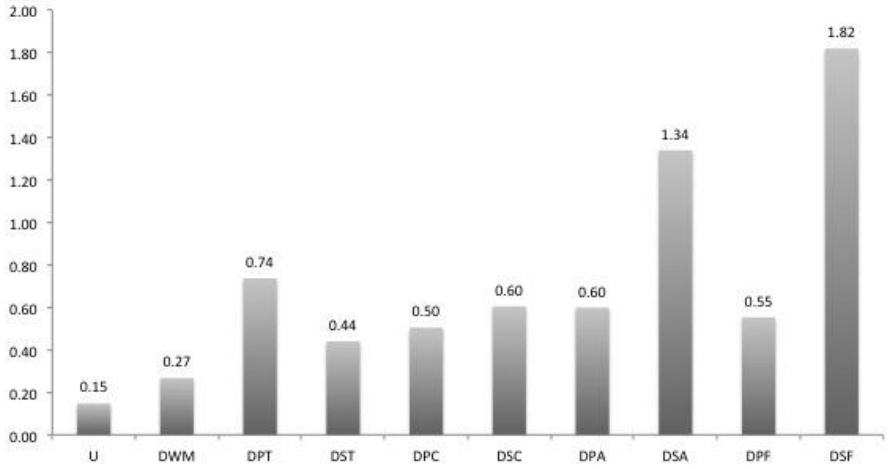


**Figure 6**. Total color difference ($\Delta E^*_{ab}$) between both sides for the dyed fabrics processed in this study.

Undyed (U), Dyed using *B. orellana* extract colorant without the use of mordants (DWM), Pre-mordanting with tannic acid (DPT), Simultaneous mordanting with tannic acid (DST), Pre-mordanting with sodium citrate (DPC), Simultaneous mordanting with sodium citrate (DSC), Pre-mordanting with aluminum potassium sulfate (DPA), Simultaneous mordanting with aluminum potassium sulfate (DSA), Pre-mordanting with ferrous sulfate (DPF), Simultaneous mordanting with ferrous sulfate (DSF).



## Table captions

### Table 1

Plants identified with dyeing potential that are of common use in the Yucatan Peninsula. The four plant species selected for this study are indicated in bold.

| Scientific name | Family | Local name (Mayan and Spanish languages) | Parts used for dyeing |
|---|---|---|---|
| ***Justicia spicigera*** | Acanthaceae | Chak xiu, muitle | Leaves, stem |
| ***Bixa orellana*** | Bixaceae | K´uxub, achiote | Seeds |
| *Hylocereus undatus* | Cactaceae | Chakam, pitaya | Fruit peel |
| ***Rhoeo discolor*** | Commelinaceae | Maguey morado | |

| | | | |
|---|---|---|---|
| | | | Leaves |
| *Euphorbia prostrata* | Euphorbiaceae | Xana mukuy | Leaves |
| *Acacia farnesiana* | Fabaceae | Subin, cascalote | Bark, fruit |
| *Caesalpinia vesicaria* | Fabaceae | Yaáx k´lin che´, mareña | Bark |
| *Gliricidia sepium* | Fabaceae | Kuytunuk, cacahuanano | Fruit |
| *Hematoxylum campechianum* | Fabaceae | Tiinta che´, palo de Campeche, palo de tinte | Bark |
| *Indigofera suffruticosa* | Fabaceae | Ch´oj xiiw, añil platanillo | Leaves |
| *Brysonima crassifolia* | Malpighiaceae | Nance | Bark, fruit |
| *Maclura tinctoria* | Moraceae | Chak oox, palo mora | Bark |
| ***Bougainvillea glabra*** | Nyctaginaceae | Bugambilia | Bracts |
| *Neea choriophylla* | Nyctaginaceae | Ta´tsi´, Ramón negro | Fruit |
| *Ouratea lucens* | Ochnaceae | Laurel del monte | Flowers |
| *Phytolacca icosandra* | Phytolaccaceae | X tel kox, | Fruit |
| *Karwinskia calderonii* | Rhamnaceae | Luum che, anonillo | Seeds |

| | | | |
|---|---|---|---|
| *Rhizophora mangle* | Rhizophoraceae | Ta’ab che’, Mangle rojo | Bark |
| *Cosmocalyx spectabilis* | Rubiaceae | Chakte´kook | Bark |
| *Hamelia patens* | Rubiaceae | Chak took´, coloradillo | Leaves |
| *Randia aculeate* | Rubiaceae | Tinta che´, cruz k´iix | Fruit |
| *Sickingia salvadorensis* | Rubiaceae | Sabakche´, palo de rosa | Bark |
| *Manilkara zapota* | Sapotaceae | Chak ya’, zapote, chico zapote | Bark, fruit peel |

**Table 2**

Comparison of the extraction yields (percentage of extracted matter from plant sample (20 g) for the decoction and microwave methods.

| Dyeing plant | DCA | DCE | MCA | MCE |
|---|---|---|---|---|
| *Justicia spicigera* | 31.3 | 34.6 | 32.1 | 32.0 |
| *Bixa Orellana* | 22.3 | 21.1 | 14.2 | 13.3 |
| *Bougainvillea glabra* | 28.9 | 26.3 | 26.7 | 26.6 |
| *Rhoeo discolor* | 18.1 | 17.7 | 17.8 | 16.8 |

DCA: decoction with distilled water; DCE: decoction with 10% ethanol; MCA: microwave with distilled water; MCE: microwave with 10% ethanol.

**Table 3**

Toxicity of plant dyes assessed in this study.

| Plant dye | Target organism | | | | | |
|---|---|---|---|---|---|---|
| | *Artemia salina* | | | | *Lactuca sativa* | |
| | Tested concentrations | | | | | |
| | 6.25% | 12.5% | 25% | 50% | 50% | 100% |
| *J. spicigera* dye | 0[a] | 0 | 0 | 0 | 52.9[b] | 28.0 |
| *B. glabra* dye | 0 | 0 | 0 | 0 | 58.6 | 29.9 |
| *R. discolor* dye | 0 | 0 | 0 | 0 | 69.5 | 59.8 |
| *B. orellana* dye | 0 | 0 | 0 | 0 | 82.9 | 63.1 |
| Artificial sea water | --- | --- | --- | 0 | --- | --- |
| Distilled water | --- | --- | --- | --- | --- | 100 |

[a] Dead nauplii percentage, [b] germination index percentage.

**Table 4**

Toxicity of different combinations of *B. orellana* dye and mordating agents.

| Treatment | Target organism | | | | | |
|---|---|---|---|---|---|---|
| | *Artemia salina* | | | | *Lactuca sativa* | |
| | Tested concentrations | | | | | |
| | 6.25% | 12.5% | 25% | 50% | 50% | 100% |
| *B. orellana* dye + tannic acid | 0[a] | 10.2 | 70.4 | 100 | 19.5[b] | 3.2 |
| *B. orellana* dye + sodium citrate | 0 | 0 | 0 | 0 | 0 | 0 |
| *B. orellana* dye + aluminum potassium sulfate | 100 | 100 | 100 | 100 | 0.9 | 0 |
| *B. orellana* dye + ferrous sulfate | 0 | 5.4 | 66.7 | 100 | 6.1 | 2.96 |
| Artificial sea water | --- | --- | --- | 0 | --- | --- |
| Distilled water | --- | --- | --- | --- | --- | 100 |

[a] Dead nauplii percentage, [b] germination index percentage.

**Table 5**

Colorimetric data, values of CIELAB color coordinates (CIELAB units) of the ordinary cotton cloth (manta) colored using *B. orellana* extract.

| CIELAB color coordinates | U | DWM | DPT | DST | DPC | DSC | DPA | DSA | DPF | DSF |
|---|---|---|---|---|---|---|---|---|---|---|
| L* | 80.63<br>(0.12) | 72.25<br>(0.26) | 70.10<br>(0.50) | 71.89<br>(0.23) | 71.30<br>(0.07) | 63.89<br>(0.14) | 74.08<br>(0.20) | 74.17<br>(0.32) | 67.66<br>(0.19) | 62.32<br>(0.48) |
| a* | −0.11<br>(0.03) | 10.69<br>(0.26) | 8.38<br>(0.15) | 6.68<br>(0.25) | 13.39<br>(0.23) | 24.63<br>(0.36) | 6.50<br>(0.24) | 3.36<br>(0.26) | 4.57<br>(0.17) | 3.22<br>(0.21) |
| b* | 6.28<br>(0.07) | 28.95<br>(0.24) | 25.58<br>(0.29) | 26.95<br>(0.23) | 30.08<br>(0.39) | 44.42<br>(0.40) | 29.16<br>(0.38) | 31.26<br>(0.73) | 17.84<br>(0.35) | 18.95<br>(0.93) |
| $C^*_{ab}$ | 6.28<br>(0.05) | 30.86<br>(0.54) | 26.92<br>(0.32) | 27.77<br>(0.27) | 32.93<br>(0.41) | 50.79<br>(0.47) | 29.88<br>(0.39) | 31.44<br>(0.81) | 18.42<br>(0.37) | 19.22<br>(1.02) |
| $h_{ab}$ | 91.00º<br>(0.15º) | 69.73º<br>(0.35º) | 71.86º<br>(0.46º) | 76.08º<br>(0.35º) | 66.00º<br>(0.10º) | 60.90º<br>(0.12º) | 77.43º<br>(0.22º) | 83.87º<br>(0.40º) | 75.63º<br>(0.23º) | 80.36º<br>(0.56º) |

Data are the mean of 8 measurements, done at random points on three replicates (standard deviation between brackets).

Undyed (U), Dyed using B. orellana extract colorant without the use of mordants (DWM), Pre-mordanting with tannic acid (DPT), Simultaneous mordanting with tannic acid (DST), Pre-mordanting with sodium citrate (DPC), Simultaneous mordanting with sodium citrate (DSC), Pre-mordanting with aluminum potassium sulfate (DPA), Simultaneous mordanting with aluminum potassium sulfate (DSA), Pre-mordanting with ferrous sulfate (DPF), Simultaneous mordanting with ferrous sulfate (DSF).

**Table 6**

Partial color differences (ΔL*, Δa* and Δb*) and total color difference ($\Delta E^*_{ab}$) between the dyed fabrics.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| | DWM - U | DPT - U | DST - U | DPC - U | DSC - U | DPA - U | DSA - U | DPF - U | DSF - U |
| ΔL* | 8.38 | 10.53 | 8.74 | 9.33 | 16.74 | 6.55 | 6.46 | 12.97 | 18.31 |
| Δa* | 10.80 | 8.49 | 6.79 | 13.50 | 24.74 | 6.61 | 3.47 | 4.68 | 3.33 |
| Δb* | 22.67 | 19.30 | 20.67 | 23.80 | 38.14 | 22.88 | 24.98 | 11.56 | 12.67 |
| $\Delta E^*_{ab}$ | 26.47 | 23.57 | 23.45 | 28.91 | 48.45 | 24.70 | 26.03 | 17.99 | 22.51 |
| | | | | | | | | | |
| | | DPT - DWM | DST - DWM | DPC - DWM | DSC - DWM | DPA - DWM | DSA - DWM | DPF - DWM | DSF - DWM |
| ΔL* | | 2.15 | 0.36 | 0.95 | 8.36 | 1.83 | 1.92 | 4.59 | 9.93 |
| Δa* | | 2.31 | 4.01 | 2.70 | 13.94 | 4.19 | 7.33 | 6.12 | 7.47 |
| Δb* | | 3.37 | 2.00 | 1.13 | 15.47 | 0.21 | 2.31 | 11.11 | 10.00 |
| $\Delta E^*_{ab}$ | | 4.62 | 4.50 | 3.08 | 22.44 | 4.58 | 7.92 | 13.49 | 15.95 |
| | | | | | | | | | |
| | | DPT - DST | | DPC - DSC | | DPA – DSA | | DPF - DSF | |
| ΔL* | | 1.79 | | 7.41 | | 0.09 | | 5.34 | |
| Δa* | | 1.70 | | 11.24 | | 3.14 | | 1.35 | |
| Δb* | | 1.37 | | 14.34 | | 2.10 | | 1.11 | |
| $\Delta E^*_{ab}$ | | 2.82 | | 19.67 | | 3.78 | | 5.62 | |

Undyed (U), Dyed using B. orellana extract colorant without the use of mordants (DWM), Pre-mordanting with tannic acid (DPT), Simultaneous mordanting with tannic acid (DST), Pre-mordanting with sodium citrate (DPC), Simultaneous mordanting with sodium citrate (DSC), Pre-mordanting with aluminum potassium sulfate (DPA), Simultaneous mordanting with aluminum potassium sulfate (DSA), Pre-mordanting with ferrous sulfate (DPF), Simultaneous mordanting with ferrous sulfate (DSF).