

Review

# Towards a Sustainable Preservation of Medieval Colors through the Identification of the Binding Media, the Medieval Tempera

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**Abstract:** Medieval colors used in illuminated manuscripts from the 12th to 15th centuries can be at risk. Knowing the binding media used, the medieval tempera, is fundamental to developing new and greener methodologies to increase sustainability in Cultural Heritage. A closer look at the tempera used in medieval illuminated manuscripts kept in Portuguese collections, namely, the *Ajuda Songbook* (13th c.), the winter breviary (14th to 15th c.), the books of hours (15th c.), and a Renaissance Charter (1512), shows that most of the paints analyzed used a tempera similar to gum mesquite. Infrared spectra were the basis for the differentiation between the gums used in medieval times originating from *Prosopis* spp. and *Senegalia* spp., gum mesquite and gum arabic, respectively. The ethnobotanical uses of gum mesquite further engage the reader. This micro review represents a significant step forward in Heritage Conservation, offering new perspectives for innovative and greener treatments. Our research, focusing on the differentiation of gums used in medieval times and the identification of the binding media, has the potential to revolutionize our understanding and the preservation of illuminated manuscripts in Cultural Heritage.

**Keywords:** medieval illuminated manuscripts; gums; gum mesquite; gum arabic; *Prosopis* spp.; *Senegalia* spp.; infrared spectroscopy; *Ajuda Songbook*; books of hours; *Charter of Flower Town*



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## 1. Preamble

The medieval colors used in illuminated manuscripts from the 12th to 15th centuries were based on a luxurious and restricted palette dominated by a certain number of inorganic pigments and organic dyes, as described in Figures 1 and 2. The medieval codex, with the Bible as its foundation, started in the monastic scriptoria and evolved into lay workshops for producing books of hours. To sustainably preserve these precious colors for future generations, we conducted interdisciplinary research at the frontiers of the social and natural sciences, promoting public engagement [1].

These medieval colors are highly complex systems of intrinsically heterogeneous compositions in which the tempera plays a crucial role [1,2]. The medieval treatise “De Arte Illuminandi” perfectly describes the medieval tempera, the binding medium, as [3]: “II. Dei liquidi con cui si temperano i colori per fissarli sulla carta- I liquidi con cui si fissano i colori sono questi: cioè l’albumine e il tuorlo d’uovo di gallina, la gomma arabica e la gomma adragante disciolte in acqua limpida di sorgente. Talora a raddolcirli, è necessaria l’acqua di miele, o di zucchero o di candito, come esporrò minutamente, (...)”. (English version: “To prepare colors applied on supports, the following tempera are used: egg white (glair) and hen’s egg yolk, gum arabic and gum tragacanth dissolved in clear spring water. Sometimes, honey, sugar, or candied water are necessary to sweeten them, as I will explain in detail (...)”).



**Figure 1.** The colors used in Portuguese monastic production from the 12th to the 13th centuries. lapis lazuli was the primary blue; azurite and malachite were seldom used. A proteinaceous tempera was consistently applied in the monastic manuscripts. Design by Nuno Gonçalves.



**Figure 2.** The colors used in the books of hours in Portuguese collections. The tempera evolved to the use of gums and mixed tempera (proteins and gums). The most used gum is mesquite gum.

In this micro review, we will address the importance of the medieval tempera for the preservation of color in illuminated manuscripts.

This endeavor aligns with numbers 4, 8, and 11 of the United Nations' Sustainable Development Goals for 2030 by taking bold and transformative steps to shift the world

towards a more sustainable and resilient path. It will enhance scientific research and promote sustainable conservation.

### 1.1. Towards an In-Depth Understanding of Medieval Colors for Their Preservation for Future Generations

We have consistently studied medieval color formulations in illuminated manuscripts. These studies began within interdisciplinary teams that brought together art historians, conservators, codicologists, and conservation scientists. This research focused on the monastic production of the monasteries of Lorvão, Santa Cruz (Holly Cross), and Alcobaça during the 12th and first quarter of the 13th centuries (Romanesque period). A proteinaceous tempera was consistently applied in all illuminated manuscripts, even though each monastery had its specificities [4,5].

Other manuscripts were studied, which included the *Ajuda Songbook* (end of the 13th or beginning of the 14th century), books of hours (late 14th and 15th centuries), a winter breviary (14th century), and the first page of a Portuguese Renaissance Charter (1512). In the Charter of *Vila Flor* (Flower Town), we discovered for the first time a tempera based on gum mesquite [6]. Several binding media were identified in the other systematically studied manuscripts, including a proteinaceous tempera, mixtures of proteins and gums, and, again, a binding medium based on gum mesquite. Cases of using gum arabic are rare. This will be further expanded upon in Section 4.

### 1.2. Why Is It Essential to Know the Tempera Used In-Depth?

These binding media are the invisible components of a color. For our ancestors who produced them, all these mixtures that today we describe as “matte” colors had multiple meanings and different brightnesses [5]. This is why we need to know everything about these formulations and their state of degradation to choose the best way to propose future interventions that will respect the integrity of these colors that have survived to us without being restored [5,7–9]. They are precious and unique colors that must be protected and preserved for future generations.

### 1.3. A Medieval Color from the 12th Century to the 15th Century

Heritage materials produced in medieval times can be very resilient [1,5,7]. The main components of a medieval paint (the paint formulation), including colorants, binders, varnishes, and other additives, can be identified using the advanced analytical methods described in Section 1.4. All these constituents are essential for the applicability and durability of a medieval paint. Systematic investigations of historical reproductions have proven crucial for advancing paint analysis research [1,4,7].

Between the 12th and 15th centuries in Europe, the color palette expanded, increasing the options for greens and yellows. Vermilion (HgS) was the material of choice for the reds, and red lead (Pb<sub>3</sub>O<sub>4</sub>) was used for the oranges. In addition to these two pigments, red/brown iron oxides were also used to a lesser extent [10–21]. Within the yellows, orpiment (As<sub>2</sub>S<sub>3</sub>) was used until at least the 13th–14th centuries [15,22], after which it was substituted by other materials, such as mosaic gold (SnS<sub>2</sub>) [23], lead–tin yellow (Pb<sub>2</sub>SnO<sub>4</sub>) [14,22,24], and yellow ochres [14,21]. Massicot (PbO) and organic yellows were used to a lesser extent [13,25–28]. According to the analysis of several European manuscripts, the source of greens was mainly copper-based greens. Some authors have indicated the presence of verdigris-based colors, other basic copper acetates, and basic copper sulfates [8,10,29–31]. Bottle green is the characteristic color in Portuguese Romanesque manuscripts; it is a synthetic copper-based pigment within a proteinaceous matrix [8,9,32–34]. Malachite was seldom used in monastic Portuguese manuscripts and was identified more frequently in manuscripts produced during and after the 14th century [34]. Three pigments were applied for the blues: the precious lapis lazuli, indigo, and azurite. Carbon-based blacks and lead white (2PbCO<sub>3</sub>·Pb(OH)<sub>2</sub>) were the preferred materials for the blacks and whites, respectively. Finally, the pink and carmine colors were mainly derived

from dyes and were used as lake pigments. In Portuguese monastic manuscripts, lac dye is a characteristic material in the color palette. It produces pink to carmine colors, either by itself or in a mixture with other pigments and additives [4,35,36]. The pinks were based on a brazilwood lake pigment in the winter breviary, the *Ajuda Songbook*, and the books of hours [6,36–39]. This colorant has been consistently identified in books of hours of French and Flemish production [38–40]. Within the organic materials, orchil-based purples have also been used and identified in the *Book of Kells* (8th c.) and the 14th-century *Lineage Book* (now part of the *Ajuda Songbook*) [23,37,41,42].

#### 1.4. Advanced Analytical Methods to Disclose a Medieval Color

Several advanced analytical methods are now available to investigate paints for in-depth characterization. The multi-analytical approach combines a high spatial resolution (microRaman) and the highest sensitivity (microspectrofluorimetry). UV–VIS spectra in absorbance or reflectance are also very useful for preliminary dye investigations, while Fourier Transform Infrared microspectroscopy (microFTIR) is needed to discover more details regarding paint formulations.

For example, a medieval color based on a brazilwood lake pigment can be revealed by combining its infrared spectra with its molecular fluorescence spectra [1]. The former quantifies the binders and additives and their conservation condition. Microspectrofluorimetry allows the acquisition of emission and excitation spectra in the same micro-spot (in situ, without any contact with the sample or work of art being analyzed). Depending on the acquisition conditions, the excitation spectrum may be identical in shape to the absorption spectrum. These molecular data provide essential information on the specificities of the recipe, which can be determined by using a database of historically accurate reproductions, preferably in combination with statistical approaches, such as chemometrics [1,36,43].

## 2. Tempera Based on Gums in Medieval Manuscripts from Portuguese Collections

### 2.1. Winter Breviary

The collection of manuscripts from the monastery of Alcobaça is one of the most important in the Cistercian world. The scriptorium was active from the end of the 12th to the 16th centuries, and we have studied the production of its 12–13th-century illuminated manuscripts and of manuscript Alc. 54, known as the winter breviary [5,8,9,34,44]. The breviary is the book used to celebrate the liturgy of the hours, and as such contains chants such as responsories, versicles and antiphons, as well as prayers, hymns, and readings of the *Liturgy of the Hours*. The Alcobaça winter breviary, Alc. 54, measures 157 × 114 mm and contains a total of 340 folios organized into 31 quires; most are composed of 6 bifolios, and the first 27 quires belong to the original corpus of the manuscript (fol. 1r–fol. 309v), dating to the 14th century (Figure S1, Supplementary Material) [44]. The remaining four quires were added in the 15th century. To learn more about the palette employed by the scriptorium of the Alcobaça monastery in the 12th and 13th centuries and the changes observed in the earlier 14th century, see [9,34,44].

The tempera used in the 12–13th centuries was protein-based [4,5,9]. Concerning the winter breviary, it is noteworthy that we found a protein-based binding medium in both the main body of the manuscript, dated to the first half of the 14th century, and the two additions from the second half of the 15th century [44]. We also identified a mix of gum and protein that will be discussed in depth in this work in Section 4.

### 2.2. Ajuda Songbook

Standing as a monument to the Galician–Portuguese lyric, the *Ajuda Songbook* is an exceptional manuscript, replete with valuable illuminations (Figure S2, Supplementary Material) [45]. It was found in the 19th century at the Colégio Real (Royal College) in Lisbon and, in 1842, a quire and loose folios were discovered in the Biblioteca Pública de Évora (Public Library in Évora), pointing to its possible passage through Évora, sometime in its history [46]. It was left

unfinished, a fact denoted by the lack of musical annotation and the unfinished clothes of the troubadours and minstrels represented in the illuminations [23].

Why it was left unfinished is one of the many questions related to the history of the *Ajuda Songbook*. Recent material studies have reinforced the previous proposal that the Songbook was produced at the end of the 13th century, beginning of the 14th century; however, its place of production remains a mystery [23]. The previous proposal that it had possibly been produced in the scriptorium of Alfonso X, the Learned, due to iconographic similarities to the Songs of Holy Mary, has been refuted with the material study of its scriptorium [47,48].

### 2.3. Books of Hours

In the late Middle Ages, books of hours became the most popular and luxurious books among the royal aristocrats and the middle class [38,40]. They were frequently commissioned to celebrate a marriage and handed down from one family generation to another. Lavishly decorated and treasured as precious objects, enabling the private recitation of religious offices (*devotio moderna*), books of hours reflected the rise of religious individualism, which characterized men and women of this period [49,50]. Flemish, French, and particularly Parisian workshops were important production centers during the 15th century, where some of the finest books of hours were created, such as the famous “Bedford Hours” from the Bedford Master (London, British Library, Add. 18850) and “The Belles of the Duke of Berry” from the Limbourg Brothers (New York, The Metropolitan Museum of Art, Cloisters Collection, ms. 54.1.1). We studied the color palette of the books of hours preserved in Portuguese collections, with some examples shown in Figures S3–S6, (Supplementary Material); however, for more information see [40]. Contrary to the consistency of the tempera applied in Portuguese monastic production, here we observed significant variation in the use of tempera for the same color in the books of hours. Tempera can be protein-based, polysaccharide, or mixed tempera (protein + gum) [38,40]. In this paper, we discuss the tempera used in depth.

### 2.4. Charter of Vila Flor

The *Charter of Vila Flor* was granted in 1512 by D. Manuel (King of Portugal, 1495–1521). These charters were documents granting rights and privileges to towns to encourage settlement when the Portuguese kingdom was created [6]. It is a bound book with the following dimensions: 275 × 190 × 200 mm. For more details, please see [6]. The colors of the front page of this Renaissance Charter were studied, Figure S7 in Supplementary Material. Except for the gold preparation in the initial capital letter in which a proteinaceous tempera was found, in all analyzed samples, the binding medium was a polysaccharide, such as gum mesquite, an arabinogalactan proteoglycan of Type II [6], the same type as gum arabic.

## 3. Materials and Methods

All reagents were of analytical grade, except for gum arabic in grains from *Senegalia senegal* (L.) Britton, which was acquired from Kremer, as well as gum mesquite (*Prosopis* spp.), which was provided by the Laboratory of Biopolymers—Centro de Investigación en Alimentación y Desarrollo, A.C (C.I.A.D., A.C.), México. Spectroscopic or equivalent grade solvents and Millipore water were used for all the spectroscopic studies.

### 3.1. Artworks

The manuscripts studied have been kept in Lisbon, the National Library (Biblioteca Nacional de Portugal, BNP), the Palace of Ajuda Library (Palácio Nacional da Ajuda, PNA), the Mafra National Palace (Palácio Nacional de Mafra, PNM), and the National Archives (Arquivo Nacional da Torre do Tombo, IAN-TT).

Fifteen manuscripts dating from the 13th to the 16th centuries were studied. The *Ajuda Songbook* (PNA, 13th–14th c.), the winter breviary Alc. 54 (BNP, 14th–15th c.) and twelve

books of hours, Ms. 22 (PNM, 1400–20), Ms. 23 (PNM, 1410–1430/1460–1470), Ms. 24 (PNM, 1420/1470), Ms.29 (PNM, second half of the 15th century), Ms.31 (PNM, ca. 1440/ca. 1460), IL 12 (BNP, 1476–1500), IL 15 (BNP, ca. 1450), IL 18 (BNP,1476–1500), IL 19 (BNP, 1420–30), IL 21 (BNP, 1460–70), IL 36 (BNP, 1476–1500), IL 42 (BNP, ca. 1470), and the *Charter of Vila Flor* (Foral de Vila Flor) (IAN-TT, 1512).

### 3.2. Micro-Sampling

The micro-samples are collected under the microscope using the following micro-tools from Ted Pella<sup>®</sup>, Redding, CA, USA: micro chisel n°13603 attached to a micro graver oval n°13611. The microscope is a Leica KL 1500 (7.1× to 115× objective) coupled to a Leica Digilux digital camera, with external illumination via optical fibers. The dimensions of the micro-samples range from 20 to 50 µm and, as such, are invisible to the naked eye. As we have not yet obtained their weight, although microscales were used, we can use their detection limit to conclude that they weigh less than 0.1 µg. Micro-samples were stored on microscope slides with a single cavity and covered with a microscope glass slide. They were closed with tape (3 M magic tape) and used as sample holders. In situ spectra are collected directly from the sample by opening the cover. These sample holders are then stored in a microscope slide tray cabinet, in a dust-free enclosure. The cabinet's outer shell is white polypropylene, and the tray rails are polystyrene.

Micro-sample collection under a microscope ensures the selective sampling of the dye paint; that is, the micro-sample will not include parchment support, ground layers (mainly applied to metallic colors), or any other external layers. Therefore, we can certify that the fillers and other additives present in the medieval colors are part of the paint formulation.

### 3.3. Fourier Transform Infrared Microspectroscopy (microFTIR)

Infrared analyses were performed using a Nicolet Nexus spectrophotometer coupled to a Continuum microscope (15× objective) with an MCT-A detector cooled by liquid nitrogen. Spectra were collected in transmission mode, using a Thermo diamond anvil compression cell, on 50 µm<sup>2</sup> areas, at a 4 or 8 cm<sup>-1</sup> resolution, and as a result of 128 scans. CO<sub>2</sub> absorption ca. 2400–2300 cm<sup>-1</sup> was removed from the acquired spectra. More than one spectrum was acquired from different sample spots to improve the robustness of the results.

## 4. Spectroscopic Information on the Gums of the Manuscripts

This section presents the tempera identified in the manuscripts for the following colors: black, orange, red, pink, purple, and blue. Reference spectra and the other colors can be found in the SI (Figures S8–S28, Supplementary Material). In the first observation, the books of hours are the manuscripts that present the most significant variation in terms of the materials identified.

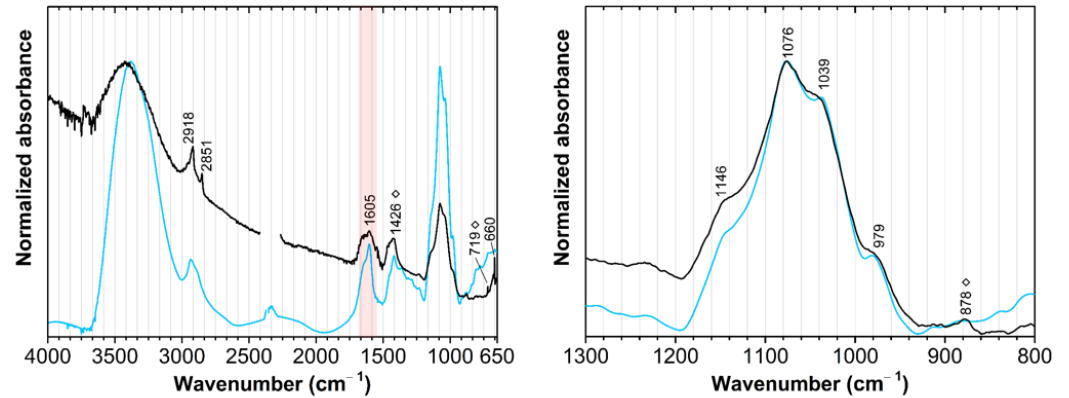
### 4.1. Black Paints

In the black paints, Figure 3, it was possible to verify the presence of a binder with a signal similar to gum mesquite by the bands at 1148–1152 cm<sup>-1</sup> ( $\nu(\text{COC})_{\text{glycosidic bond}}$ ) and 1112–1114 cm<sup>-1</sup> ( $\nu(\text{CC})(\text{CO})$ ) and the shoulder at approximately 1071 cm<sup>-1</sup> ( $\nu(\text{CO}) + \delta(\text{OH})$ ), 902–904 cm<sup>-1</sup>, and 833–838 cm<sup>-1</sup> ( $\gamma(\text{C-OH})_{\text{ring}}$ ) [51–55]. In all the books of hours, it was possible to identify the presence of protein, which was visible by the amide I at 1648 cm<sup>-1</sup> ( $\nu\text{C=O}$ ) in the IL 19 [56]. Proteins might have been mixed with the gum, which is an advised process in medieval treatises such as the *Liber diversum arcium*, or as a varnish [57]. Among the examples shown in Figure 3, only the IL 19 sample did not contain calcium carbonate, as detected in the other paints by the bands at 1418–1425 cm<sup>-1</sup> ( $\nu\text{CO}_3^{2-}$ ) and 875 cm<sup>-1</sup> ( $\delta_{\text{as}}\text{CO}_3^{2-}$ ) [58]. This material was possibly used as an additive to the paint; it could also be used to prepare the parchment support.



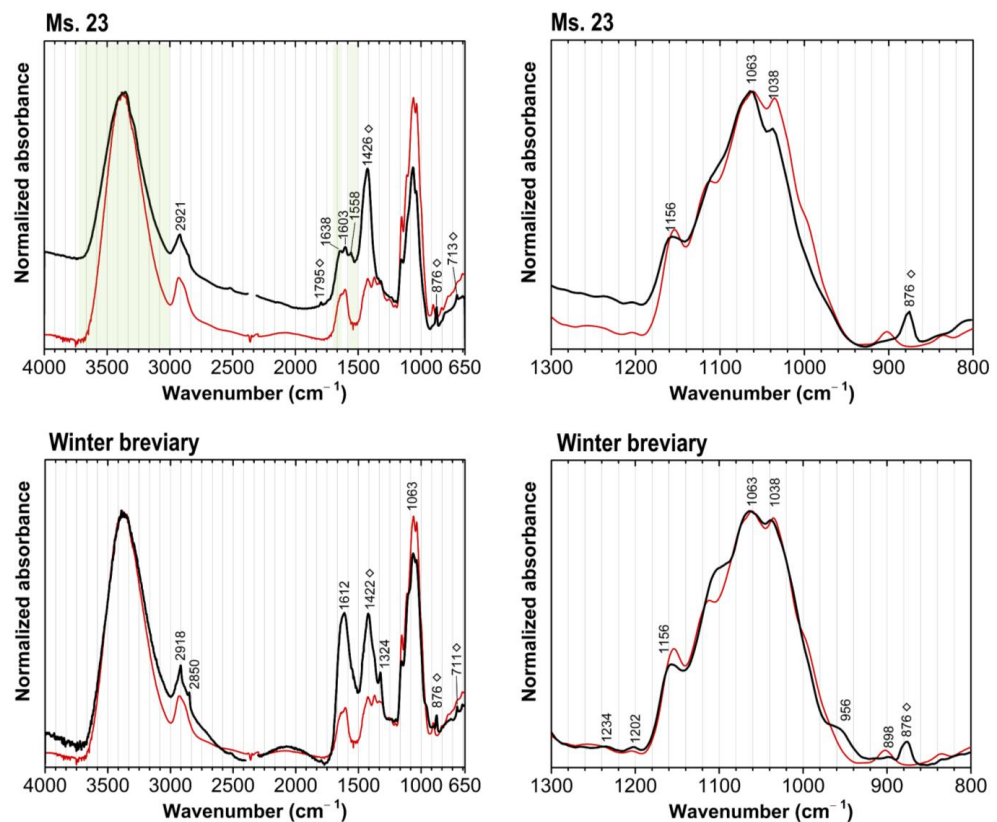
#### 4.3. Red Paints Based on Vermilion and a Mixture of Gums

Gum arabic was identified in a red paint in Ms. 23, Figure 5. It is characterized by the presence of the maximum absorbance band at  $1076\text{ cm}^{-1}$  ( $\nu(\text{CO}) + \nu(\text{OH})$ ), and shoulders at  $1146$  ( $\nu(\text{COC})_{\text{glycosidic bond}}$ ),  $1039$  ( $\nu(\text{CC})(\text{CO})$ ), and  $979\text{ cm}^{-1}$  [51–55].



**Figure 5.** Infrared spectrum of a red paint found in fol. 2 of Ms. 23 (left) and close-up into the fingerprint region (right), compared to the gum arabic reference (blue spectrum and red band). In addition to gum arabic, calcium carbonate (◇) is also detected.

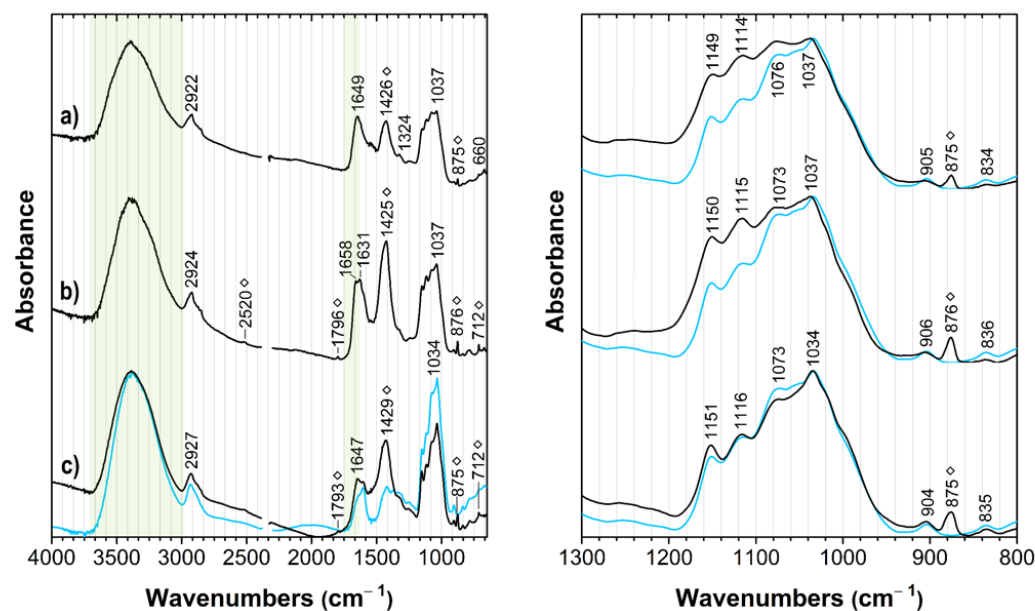
In Ms. 23 and in the winter breviary, the presence of a possible mixture of gum mesquite and a cellulose-based material was detected, Figure 6. The spectra profile does not present a match to gum mesquite alone but with its mixture with a cellulose-based material. Nonetheless, more studies will be needed to confirm the nature of this unknown material.



**Figure 6.** Infrared spectra of Ms. 23 and the winter breviary and close-up into the fingerprint region, compared to the mixture of gum mesquite and a cellulose-based material (red spectrum). In addition, calcium carbonate (◇) and a protein are also detected (green bands).

The cellulose-based material in Ms. 23 paint possibly contains a protein due to the bands corresponding to amides I and II at  $1638\text{ cm}^{-1}$  and  $1558\text{ cm}^{-1}$ , respectively. The paint in the winter breviary is more complex, with bands corresponding to an unidentified component at  $1612\text{ cm}^{-1}$  and  $1324\text{ cm}^{-1}$  (a degraded proteinaceous tempera?). Both paints show bands corresponding to calcium carbonate, which was most likely used as an additive.

In the red paints from IL 12, IL 21, and the *Charter of Vila Flor*, there was a clear correlation with the gum mesquite reference, Figure 7. The spectrum obtained for the paint found in the Charter is more defined than the other two, which is influenced by a compound that is raising the baseline. Vermilion red does not absorb in the mid-infrared but its presence usually raises the baseline of the spectra, as displayed in the spectra of IL 21 and IL 12. Interestingly, two other compounds are common to the three manuscripts: calcium carbonate and, possibly, a proteinaceous binder. In the Charter, the intensity of the amide I band is considerably low, which can indicate that the protein might have been applied as a thin varnish layer or added in a small quantity to the main binder. Calcium carbonate, indicated by its intense characteristic bands, points to the addition of this compound as an additive, a common practice in vermilion paints [9,10,57].



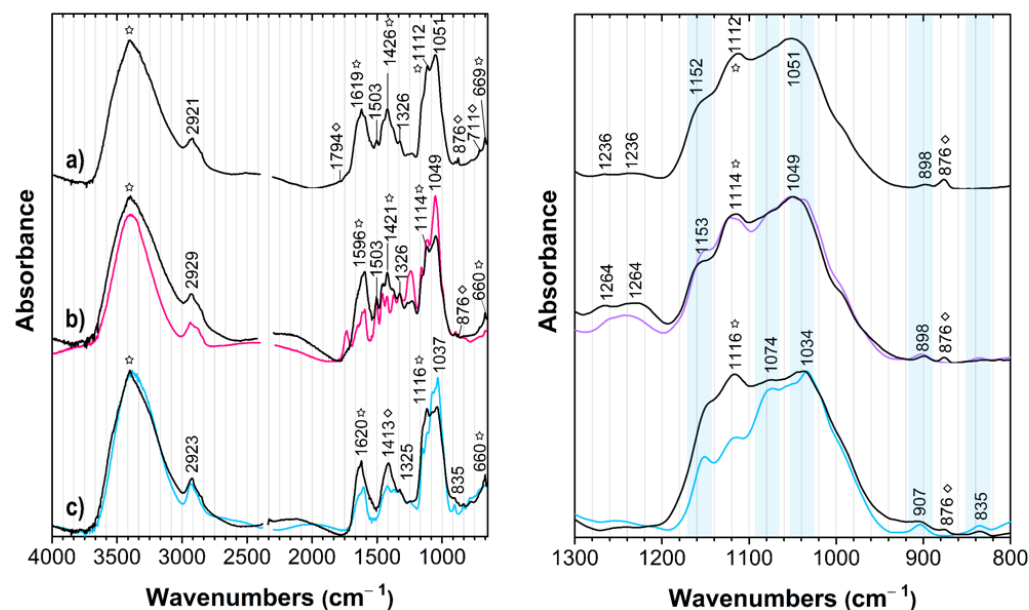
**Figure 7.** Infrared spectra of red paints with a similar fingerprint to that of gum mesquite (blue spectrum) (**left**) and close-up into fingerprint region (**right**): (a) IL 21, fol. 88; (b) IL 12, fol. 18; and (c) *Charter of Vila Flor*. In addition to gum mesquite, calcium carbonate (◇) and a protein (green bands) are also detected.

#### 4.4. Pink Paints

The pink paints were the systems that presented a wider range of variations, either in tempera, additives or the presence of degradation products, Figure 8 and Figures S17–S20 (Supplementary Material). The spectra of IL 15, IL 42, IL 21, IL 36, IL 12, and IL 18 correspond well with the gum mesquite reference. The first typology detected is pink paint that contains calcium carbonate and gypsum. Calcium carbonate is present in many paints. Gypsum now appears in eight of the twelve examples of pink paints. At least four examples show good correspondence with a reference of brazilwood's wood, Figures 8 and S18 (folio 1).

Previous research on the reconstruction of brazilwood recipes from the *Book of All Colors* has shown that adding calcium carbonate in the presence of alum produces gypsum [58]. This is due to the formation of calcium ions from the dissociation of calcium carbonate and the formation of sulfate ions from the dissociation of alum, resulting in the formation of  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  [58]. Both calcium carbonate and gypsum are present in IL 15, IL 36, IL 42, and IL 21. IL 42 is particularly interesting for understanding this process, as shown in

Figures 8a and S18 (Supplementary Material). In the presence of high quantities of gypsum, the bands at  $1112\text{--}6\text{ cm}^{-1}$  deformed the fingerprint region for identifying the polysaccharide.



**Figure 8.** Infrared spectra of pink paints with similar fingerprint to gum mesquite (blue spectrum and bands) (left), brazilwood's wood (pink spectrum), and the sum of brazilwood's wood, gum mesquite, and gypsum (purple spectrum) and the close-up fingerprint region (right): (a) IL 42, fol. 133; (b) IL 15, fol. 9; and (c) IL 15, fol. 84. In addition to the binder, calcium carbonate (◇) and gypsum (☆) are also detected.

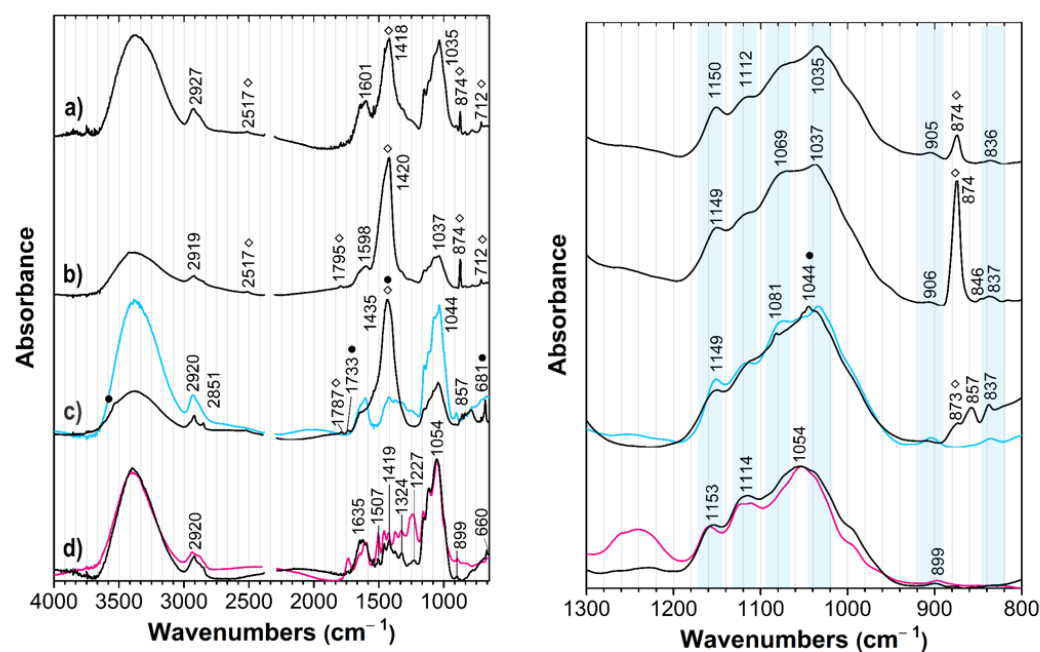
In IL 18 and IL 12, the pink paints possibly presented signs of degradation, Figure S19 (Supplementary Material). In addition to the gum mesquite reference, calcium soaps (IL 12) and oxalates (IL 18) were also identified. Calcium oxalate was also detected in a pink paint of the IL 36. These compounds can result from the degradation of tempera.

Finally, the last typology of pink paints observed is presented in Figure S20 (Supplementary Material), where there is a good correspondence between the pink paints of Ms. 22 and Ms. 23 and proteinaceous and polysaccharide binders. For Ms. 23, the band at  $1113\text{ cm}^{-1}$  corresponds well with a reference to a complex between brazilein and alum. This paint was possibly made following a recipe in which the dye is extracted with egg white and complexed with alum, previously established as a transparent rose color [43].

#### 4.5. Purple and Blue Paints

The purple paints were made by adding a brazilwood lake pigment to a blue pigment. Figure 9 shows the spectra of blues and purples in IL 42, Ms. 22, and the *Ajuda Songbook*. The spectra matched the gum mesquite reference well, except for the spectrum of Ms. 22, which needs more in-depth consideration. The spectra in IL 42 were acquired from two purples in different folia, showing a match with a polysaccharide binder similar to gum mesquite and calcium carbonate in more significant quantities in the purple paint of folio 133, Figure 9a,b.

Two blues are presented, one from the *Ajuda Songbook* and the other from Ms. 22. The blue from the *Ajuda Songbook* is in a mixture of lead white and calcium carbonate, Figure 9c. The blue of Ms. 22 is described as dark blue and is based on azurite. However, it corresponds well with a brazilwood's wood reference. As brazilwood's wood overlaps gum mesquite between  $1200\text{ and }950\text{ cm}^{-1}$ , it is not easy to assert that the paint has this last component. Using brazilwood applied over other colors, mainly blue, is common in medieval illuminations.



**Figure 9.** Infrared spectra of purple and blue paints with similar fingerprint to gum mesquite (blue spectrum and bands) and brazilwood's wood (pink spectrum) (left) and close-up into the fingerprint region (right): (a) IL 42, fol. 9; (b) IL 42, fol. 133; (c) *Ajuda Songbook*, fol. 16; and (d) Ms. 22, fol. 76v. In addition to the binder, calcium carbonate (◇) and lead white (●) are also detected.

#### 4.6. Overview

Overall, it is possible to verify that gum mesquite is the preferred material for tempera in the manuscripts. Gum arabic was only identified in the red paint of the book of hours, Ms. 23 in folio 2; Figure 5. However, we need to consider that only some invisible micro-samples were collected, with the aim of being representative of the main colors. When a proteinaceous tempera is detected, three typologies can be considered: (1) it is applied in a mixture with the polysaccharide binder, as are examples of the red paints in the books of hours IL 21 and IL 12 and in the *Charter of Vila Flor*; (2) it is applied as a varnish over the paint, Figures S24 and S28; and (3) it is found in brazilwood paints as exemplified in the books of hours Ms. 22 and Ms. 23. As recently studied, there are several examples of brazilwood recipes where egg white is used as an extraction solution. The presence of glair in brazilwood paints may come from the manufacture of the lake pigment [43].

## 5. Natural Polysaccharides Exuded from *Prosopis* spp. and *Senegalia* spp. Trees

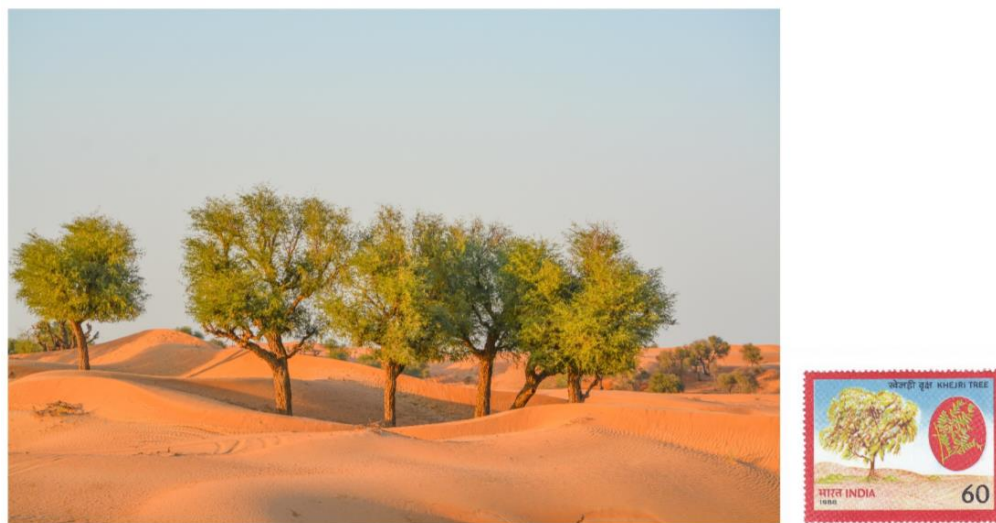
### 5.1. Ethnobotanical Uses of Gum Mesquite

As with other plant secretions, the production of mesquite gum is mainly dependent on the ecosystem's biotic or abiotic conditions. The gum is an adaptation to the severity of the environment, a reaction to wounds produced after the plant is injured by sharp instruments, animals, such as insects, or under severe heat and dry stress. It is collected and sold locally or through intermediaries that select and process it before mesquite gum reaches the international markets.

The gum nodules are sorted according to their color and impurity content, and ground to obtain crude mesquite gum. In the Western Hemisphere, such as in Mexico and the USA, mesquite gum is used, among other applications, as a food additive, an excipient in pharmaceutical preparations [59,60], and for coating fruits to extend their shelf life [61].

From the Arabian Peninsula to India, the species *Prosopis cineraria* (L.) Druce is used as fuel, animal food, fencing, and to obtain its amber-colored gum; Figure 10, left. In India, mesquite gum is gathered in May and June, and the average production is circa 100 g to 300 g per tree/year. It is used in Ayurvedic medicine, especially in the Rajasthan State, to treat several ailments, as well as in some traditional sweet dishes [62]. In India, the native

species, known as *khejri*, was selected to be represented in a philatelic issue, composed of only one stamp, that celebrated the 1988 World Environment Day. This option denotes the cultural relevance and ecological value attributed to this tree, Figure 10, right.



**Figure 10.** *Prosopis cineraria* (L.) Druce (left—on the red dunes of Dubai, United Arab Emirates; right—Indian postage stamp (1988 World Environment Day) representing the species *Prosopis cineraria* (L.) Druce [*khejri*]).

The New World species *Prosopis juliflora* (Sw.) DC. [= *Neltuma juliflora* (Sw.) Raf.] found ideal conditions in Indian, African, and Arabian ecosystems, where related *Prosopis* species already lived, and now is a major invasive plant. Although having this invasive status, it may be very valuable for humans, as it can be used in the afforestation of arid and saline lands, charcoal production, animal food, and to produce gum mesquite, Figure 11 [63,64].



**Figure 11.** Mesquite gum from *Prosopis juliflora* (Sw.) DC. stem. Reproduced with the gracious permission of Dr. Majeti NV Prasad, University of Hyderabad, India.

### 5.2. Gum Arabic and Mesquite in Plants

Gum arabic and mesquite come from these leguminous trees [65]. There are more than a hundred species that produce gum, commonly called gum arabic. According to Howes (1949), the name of this gum emerged because it was imported into Europe from Arab ports, explaining the name “Arabic”. It is also possible to find other names associated with this same gum, namely “Turkish gum” or “East India gum”, which, as previously explained, were derived from the place from which they were imported [66].

The species that produces the gum arabic considered to be of the best quality is *Senegalia senegal* [8,67]. In addition to this species, the exudate of *Vachellia seyal* is also currently exported as gum arabic; however, it is a lower quality gum and is exported in much lower quantities (approximately 10% of the total) [66,67]. Currently, the largest exporter of gum arabic is Sudan, but it could come from other areas of North Africa and India, namely from what the authors refer to as the “Gum Belt” [67].

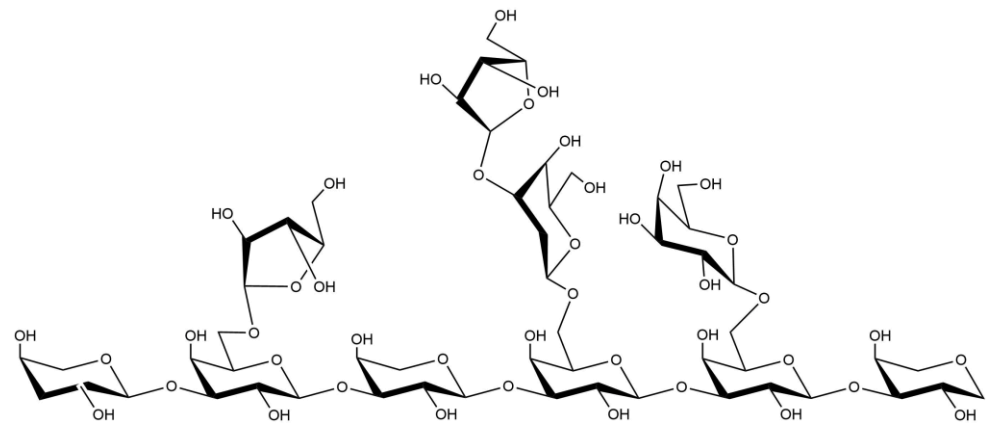
Gum mesquite is now obtained from species of the genus *Prosopis* L. [67,68]. *Prosopis juliflora* is one of the most important sources of gum mesquite and is native to Mexico, Mesoamerica, and North and West South America [66,69]. Other gum mesquite producing species include *P. laevigata*, *P. velutina*, *P. pubescens*, *P. alba*, *P. flexuosa*, and *P. glandulosa* [66,68]. Therefore, in the Middle Ages, which *Prosopis* species were available in European markets? A species of the genus *Prosopis* that would be accessible considering its habitat is the *Prosopis cineraria*. This species has a synonym, *Prosopis spicigera*, and is native to the Arabian Peninsula, Western Asia, the Indian Subcontinent, and northern India. It is an exudate with physical characteristics similar to those of gum arabic [66,69–72]. Other species native to the African and Asian continents were also found. However, little information is available on gum production of the plants, such as *P. africana* and *P. koelziana* [72].

Physically, mesquite is slightly darker than gum arabic but has similar characteristics such as viscosity and solubility [68].

### 5.3. Polysaccharides in Gum Arabic and Mesquite

To understand the formation of gums, it is necessary to understand their role and relation with the plant, specifically its cell walls. Cell walls are composed of complex chains of highly organized polymers, including polysaccharides. These are responsible for various functions, including rigidity, cellular communication, food storage, maintenance of cell shape, and, more importantly, protection against pathogenic organisms or factors [73]. The gums applied as tempera in medieval illumination are exudates [65,73]. These compounds are formed through gummosis (transformation of the highly organized chains of the cell wall into an amorphous substance), which is, as already described, caused by the incision of a mechanical wound or insect attack on the plant itself [65,68,73].

In structural terms, gums are composed of polysaccharides [68]. These structures comprise monosaccharide residues connected by glycosidic bonds [68,73]. There can be several differences between them, namely, the type of monosaccharide (one or more types), the kind of glycoside bond, whether the polysaccharide is composed of linear chains or linear chains with branches, and whether it is neutral or positively/negatively charged. These factors are essential for differentiating between gums, as will be discussed. The polysaccharides that compose the medieval gums are arabinogalactan proteoglycans type II, which also have a small percentage of protein (less than 10% or up to ~24%). Type II arabinogalactans comprise a backbone chain composed of  $\beta$ -D-galactose, with a  $\beta$ -(1  $\rightarrow$  3) bond, although they have also been found with a  $\beta$ -(1  $\rightarrow$  6) bond, Figure 12. Side chains of varying numbers are linked to this main chain. These side chains are linked to the main one by the  $\beta$ -(1  $\rightarrow$  6) bond, and to these are connected to the arabinoses (Ara) by carbon 3 and 6. These units of arabinose can be replaced by galacturonic acid (GalA), glucuronic acid (GlcA), 4-O-methyl-glucuronic acid (4-O-MeGlcA), and rhamnose (Rha) [73].



**Figure 12.** Chemical structure of an arabinogalactan type II.

#### 5.4. Structures of Gum Arabic and Gums of the Genus *Prosopis*

As previously mentioned, it is essential to understand the structure of these gums to differentiate them in analytical terms. Table 1 presents the sugars identified for each gum (without species specification) and for different species.

**Table 1.** Monosaccharides identified for gum arabic, gum mesquite, and the corresponding species. GlcA, glucuronic acid.

	<i>A. senegal</i>	<i>A. seyal</i>	<i>Acácia</i>	<i>Gum arabic</i>	<i>Gum mesquite</i>	<i>P. velutina</i>	<i>P. juliflora</i>
Refs	[53]	[53]	[68]	[66]	[68]	[55]	[69]
Galactose	x	x	x	x	x	x	x
Arabinose	x	x	x	x	x	x	x
Rhamnose	x	x	x	x			
4-O-Metil-GlcA	x	x				x	x
GlcA	x	x	x	x	x	x	
Mannose					x		
Xylose					x		

According to Coimbra and colleagues, gum arabic (from *Senegalia senegal*) comprises approximately 90% arabinogalactans and less than 10% protein [73]. Howes describes sugars in more detail (without reference to the species) as consisting of 1*d*-glucuronic acid, 3*d*-galactose, 2*l*-arabinose, and 1*l*-rhamnose [66]. Murthy described another set of monosaccharides from the gum of a *Senegalia* species with some similarities to the previous one, being composed of a main chain of  $\beta$ -1,3- and  $\beta$ -1,6- bonds of D-galactose with bonds  $\beta$ -1,6- to *d*-glucuronic acid units. The side chains include  $\beta$ -D-glucuronic acid,  $\alpha$ -L-rhamnose,  $\alpha$ -L-arabinose, and  $\beta$ -*d*-galactose. Proteins have the following amino acids: proline, hydroxyproline, and serine [68].

For gum mesquite (without reference to the species), Murthy described it as being made up of the following monosaccharides: L-arabinose, D-galactose, D-mannose, D-glucuronic acid, and D-xylose. The author also states that gum mesquite does not contain D-rhamnose, which structurally distinguishes it from gum arabic [68]. However, this theory was refuted by Vernan-Carter and colleagues (2000), who identified rhamnose in the structure of gum mesquite [59]. On the other hand, Nussinovitch described the monosaccharides of *Prosopis juliflora* as L-arabinose, D-galactose, and 4-O-methyl-D-glucuronic acid (4:2:1) [69].

By comparing the identification of each author, it can be verified that for gum arabic and the species that produce it, four common monosaccharides stand out: galactose, arabinose, rhamnose, and glucuronic acid. Only Lopez-Torres and colleagues described the presence of 4-O-methyl-glucuronic acid in *Senegalia senegal* and *Vachellia seyal* [53]. In

the case of gum mesquite, only galactose and arabinose are common in the three studies. Murthy identified mannose, xylose, and glucuronic acid for gum mesquite without defining the species, Nussinovitch identified 4-O-methyl-glucuronic acid for *P. juliflora* and Vernan-Carter and colleagues (2000) identified the presence of rhamnose [59,68,69].

Table S1a,b (Supplementary Material) present the available percentage values. Only two species were identified entirely, and the percentages of each monosaccharide and the protein content are presented. Most studies provide percentages of the overall amount of sugar and protein. However, with the available data, there appear to be differences between both genders, as denoted by the monosaccharides identified in Table 1 and the percentages of protein.

Therefore, we know that it will be possible to distinguish between these two gums, but more precise information on their composition is still needed.

## 6. Why It Is Possible to Distinguish Medieval Gums Using Infrared Spectroscopy

The identification of gums is based on their structure, taking into account the following quote: “The precise chemical and molecular structure differs according to the botanical origin of the gum, and these differences are reflected in some of the analytical properties of the gum” [67]. Fourier Transform Infrared Spectroscopy (FTIR) is essential for answering this question. It allows qualitative and quantitative analysis, and as demonstrated in the study by Synytsya and colleagues for the analysis of pectins, it enables the quantification of functional groups, such as methyls, esters, acetyls, and amides, among others [51]. The differentiation of the gums is based on their functional groups, which, as shown in Figures 12, S29 and S30 (Supplementary Material), are placed into utterly different structures.

The most intense band is the broad absorption from the O-H stretching of the hydroxyl groups at  $3384\text{ cm}^{-1}$ . The most important region with similar intensity is at  $1200\text{ to }1000\text{ cm}^{-1}$ . In this region, it is possible to distinguish these gums based on their differences using the skeletal vibration bands of glycosidic bonds, Figure 13.

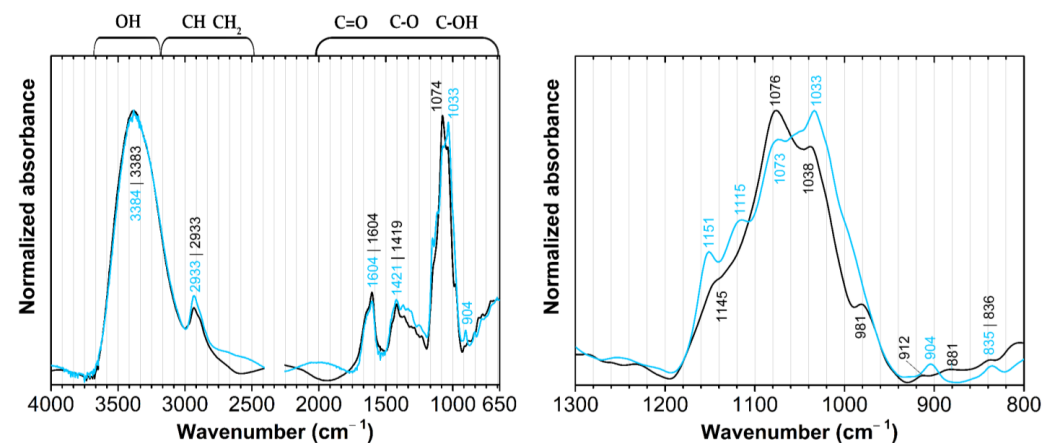


Figure 13. Infrared spectra of gum arabic (black) and gum mesquite (blue).

Figure 13 shows the spectra of gum arabic and mesquite. Table 2 describes the main peaks for each gum and their attribution. The region between  $1334\text{ and }1253\text{ cm}^{-1}$  is the most significant region for discrimination. However, we will discuss the relevant areas of the infrared spectrum from its beginning.

In the region corresponding to the stretching of OH ( $\nu\text{OH}$ ) and CH ( $\nu\text{CH}$ ), similar values are observed for both gums,  $3384\text{ cm}^{-1}$  and  $2933\text{ cm}^{-1}$  for gum mesquite and  $3383\text{ cm}^{-1}$  and  $2933\text{ cm}^{-1}$  for gum arabic, at high intensities, similar to the fingerprint region.

**Table 2.** Wavenumbers of infrared bands identified for gum mesquite and gum arabic and respective attributions, based on [51].

Gum Mesquite	Gum Arabic	Attributions
3384	3383	$\nu(\text{OH})$
2933	2933	$\nu(\text{CH})$
1604	1604	$\nu(\text{C}=\text{O})_{\text{amide I}}$ *
1460	1455	-
1421	1419	$\nu(\text{C}-\text{OH})_{\text{COOH}}$
1334	-	$\delta(\text{CH})$
1253	-	$\delta(\text{CH})$
-	1235	$\delta(\text{OH})_{\text{COOH}}$
1151	1145	$\nu(\text{COC})_{\text{glycosidic bond}}$
1115	-	$\nu(\text{CC})(\text{CO})$
1073	1076	$\nu(\text{CO}) + \text{d}(\text{OH})$
1033	1038	$\nu(\text{CC})(\text{CO})$
-	981	$\gamma(\text{COOH})_{\text{dimers}}$
904	912	-
-	881	$\delta(\text{CCH})(\text{CO}), \text{d}(\text{COH})$
835	836	$\gamma(\text{C}-\text{OH})_{\text{ring}}$

\* This may be attributed to the protein content on the gums.

The region between 1300 and 800  $\text{cm}^{-1}$  presents the most significant number of differences and allows these gums to be distinguished, Figure 13. It includes the vibrations of the glycosidic and pyranose bonds, Table 2. The bands of higher intensity are found at 1076  $\text{cm}^{-1}$  and 1033  $\text{cm}^{-1}$  for gum arabic and gum mesquite, respectively. The band at 1076  $\text{cm}^{-1}$  corresponds to the sum of the stretching of C-O ( $\nu\text{C}-\text{O}$ ) and the bending of O-H ( $\delta\text{OH}$ ) bonds, while the band at 1033  $\text{cm}^{-1}$  corresponds mainly to the stretching of C-O ( $\nu\text{C}-\text{O}$ ). It should be noted that gum arabic also has a band at 1038  $\text{cm}^{-1}$ , and gum mesquite presents a band at 1073  $\text{cm}^{-1}$  but with lower intensities. For gum mesquite, two bands were also identified at 1151  $\text{cm}^{-1}$  and 1115  $\text{cm}^{-1}$ . The first band corresponds to the stretching of the glycosidic bond ( $\nu\text{C}-\text{O}-\text{C}$ ) present in both gums. However, due to differences in the composition and arrangement of the structure, the band corresponding to this vibration in the spectrum of gum arabic is shifted to 1145  $\text{cm}^{-1}$ . The band at 1115  $\text{cm}^{-1}$  for gum mesquite, attributed to the stretching of C-O/C-C, is not detected in gum arabic. The same phenomenon occurs in the opposite situation. Gum arabic presented a weak band at 981  $\text{cm}^{-1}$ , corresponding to the out-of-plane stretching of carboxylic acid ( $\gamma\text{COOH}$ ), which is not detected in gum mesquite.

Both gums have a band at 1604  $\text{cm}^{-1}$  that may or may not correspond to the stretching of amide I ( $\nu\text{C}=\text{O}$ ), deriving from the low protein content in these gums (Table S1a,b, Supplemental Material).

Other regions of the spectra include additional information. For gum mesquite, the bands identified at 1334 and 1253  $\text{cm}^{-1}$  correspond to the bending of the CH groups ( $\delta\text{CH}$ ). On the other hand, both present bands at 1421 and 1419  $\text{cm}^{-1}$  corresponding to the stretching of the hydroxyl ( $\nu\text{C}-\text{OH}$ ) of the carboxylic acid

In the region between 950  $\text{cm}^{-1}$  and 800  $\text{cm}^{-1}$  gum mesquite has one band at 904  $\text{cm}^{-1}$  and gum arabic has two bands at 912  $\text{cm}^{-1}$  and 881  $\text{cm}^{-1}$ . The latter is attributed to the bending of CCH and CO ( $\delta\text{CCH}, \text{CO}$ ) and the bending of the hydroxyl group ( $\delta\text{COH}$ ). Both exhibit out-of-plane stretching of the pyranose hydroxyl groups ( $\nu\text{C}-\text{OH}$ ) at 835  $\text{cm}^{-1}$  and 836  $\text{cm}^{-1}$  for gum mesquite and gum arabic, respectively.

After a detailed analysis of the infrared spectrum, it was concluded that the two gums can be differentiated. The next step will be to verify whether it is possible to distinguish species within the same genus, taking into account the different percentages attributed to each monosaccharide and the protein component, which will affect the structure of these compounds.

## 7. Perspectives

This micro review is for the special issue “Sustainability in Cultural Heritage Conservation”, seeking to strengthen efforts to protect and safeguard the world’s cultural and natural heritage, as described by the editors. This new look into the medieval tempera aims to increase sustainability in Cultural Heritage, specifically for protecting medieval manuscript illuminations. We have revisited very complex colors used in outstanding manuscripts to achieve this goal. Our chronological timeline encompasses the period from the 12th to the 15th centuries, starting with the monastic scriptoria and concluding with secular workshops, Figure 14 [4–6,9,23,44,74]. The Renaissance Charter was possibly produced in a Portuguese royal workshop, and the *Ajuda Songbook* was produced in an aristocratic or royal workshop. It is interesting to consider the coexistence of a royal workshop with the monastic scriptoria, such as the one in Alcobaça monastery, which produced the winter breviary. A selection of books of hours by art historians was also included in our study and found in the collections of the Portuguese libraries and archives (c.80 mss) [38,40]. They were produced in large numbers for private use and were ordered in France, Flanders, and Italy. We aim to explore and compare the influence of different production contexts, starting with a closer look at the tempera, the invisible and essential component of color.



**Figure 14.** Timeline of the manuscripts studied in this micro review.

The proteinaceous tempera used in the Portuguese monastic scriptoria in the 12th–13th centuries was progressively replaced by polysaccharides obtained from the *Prosopis* species (arabinogalactan proteoglycan of Type II) in the previously described manuscripts. In the *Ajuda Songbook*, we identified gum mesquite, which is used by itself and combined with protein-based tempera; it can also be applied as a colored or uncolored varnish. Gum mesquite is found in colors based on organic dyes (pinks and purples) as well as on lead white and yellow ochre. Among the colorants, red lead, lapis lazuli, and yellow ochre were found in a mixture with lead white in the *Ajuda Songbook*, Ms. 23, Ms. 24, and IL 19, possibly to control the hue and enlighten the final color. Carbon-based blacks in the winter breviary and IL 19 were identified in a mixture with yellow ochre. As in the *Ajuda Songbook*, varnish layers were also found in the other analyzed manuscripts. In IL 12, two types of varnish were used in different folios: starch and gum mesquite. Starch was found as an extemporaneous material and varnish in samples from Ms. 23, Ms. 24, Ms. 29, and the IL 36. Both Ms. 23 and Ms. 24 also contain a proteinaceous binder in addition to starch.

We find a similar pattern in the books of hours, winter breviary, and Charter, although the tempera used in the books of hours is much more variable. A gum mesquite-based tempera is identified in most colors studied in these illuminated manuscripts. Gum arabic was accurately detected in a vermilion-based paint in Ms. 23, Figure 5.

Thus, we are observing a progressive replacement of protein-based tempera by gums. The manuscripts studied in Portuguese collections are essentially based on gum mesquite tempera. However, proteinaceous tempera is still applied as varnish or in colors based on

vermilion. What led to this change? This passage will undoubtedly impact the way colors were seen at the time. What was the reason behind it? We will analyze these questions in the future with humanities researchers, particularly experts in art history.

The next step is to learn more about the complexity of polysaccharide tempera by using sophisticated analytical techniques. Recently, by studying lac dye colors produced in Portuguese monastic scriptoria, we have appreciated the incredible heterogeneity of these invisible micro-samples (publication in progress). This complexity and heterogeneity are essential for its resilience and durability. This analysis uses soft radiation for UV–VIS multispectral luminescence at the Soleil Synchrotron. Therefore, we will use this technique to assess whether the heterogeneity we detected in lac dye colors is found in brazilwood lake pigments. It will also allow us to discuss whether the protein is used as mixed tempera or a varnish.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su16125027/s1>, Figure S1. Winter breviary Alc. 54 (National Library, 14th–15th c.); Figure S2. *Ajuda Songbook* (Palace of Ajuda Library, 13th–14th c.); Figure S3. Book of hours Ms. 22 (Mafra National Palace, 1400–20); Figure S4. Book of hours Ms. 23 (Mafra National Palace, 1410–1430/1460–1470); Figure S5. Book of hours IL 15 (National Library, ca.1450); Figure S6. Book of hours IL 42 (National Library, ca.1470); Figure S7. *Charter of Flower Town*, National Archives; Figure S8. References for (a) calcium carbonate ( $\text{CaCO}_3$ ) and (b) gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ); Figure S9. References for (a) lead white ( $2\text{PbCO}_3 \cdot \text{Pb}(\text{OH})_2$ ) and (b) yellow ochre; Figure S10. Reference for cellulose-based material; Figure S11. Reference spectra for egg white (black spectrum) and parchment glue (green spectrum) with zoom into OH and CH regions that allows a possible distinction between both; Figure S12. Infrared spectra of the black paints in winter breviary fol. 92v, IL 19 fol. 20v, and Ms. 22 fol. 76v, matching gum mesquite (blue bands). The last manuscripts also present bands that possibly correspond with cellulose-based material. Calcium carbonate ( $\diamond$ ), yellow ochre ( $\bullet$ ), and carbon black ( $\bullet$ ) were also identified; Figure S13. Infrared spectra of white paints found in *Ajuda Songbook* and the *Charter of Flower Town* matching a mixture of gum mesquite (blue bands) and lead white ( $\bullet$ ) (purple spectrum). Calcium carbonate ( $\diamond$ ) was also identified; Figure S14. Infrared spectra of red paints in folios 12, 73 and 35 of IL 36, Ms. 31 and Ms. 29 matching gum mesquite (blue line and bands). Ms.29 also has correspondence with a calcium soap (orange spectrum). Calcium carbonate ( $\diamond$ ), gypsum ( $\star$ ), lead white ( $\bullet$ ), and a calcium soap (calcium palmitate used as reference - $\dagger$ ) were also identified; Figure S15. Infrared spectra of orange paint in Ms. 22 fol. 76v, matching gum mesquite (blue line and bands). Calcium carbonate ( $\diamond$ ) was also identified; Figure S16. Infrared spectra of yellow paints matching gum mesquite (blue line and bands): (a) *Ajuda Songbook*, fol. 59 (b) *Ajuda Songbook*, fol. 4; (c) Ms.24, fol. 60; (d) IL 15, fol. 84. Calcium carbonate ( $\diamond$ ), lead white ( $\bullet$ ), and yellow ochre ( $\bullet$ ) were also identified; Figure S17. Infrared spectra of carmine paints in folios 12 and 88 of IL 36 and Ms. 21, matching gum mesquite (blue bands). Calcium carbonate ( $\diamond$ ), calcium oxalate ( $\boxtimes$ ), and gypsum ( $\star$ ) were also identified; Figure S18. Infrared spectra of carmine paints in folios 1, 23 and 133 of IL 42 matching gum mesquite (blue bands). Folio 1 also presents bands that might correspond to a brazilwood-based material (pink bands). Calcium carbonate ( $\diamond$ ) and gypsum ( $\star$ ) were also identified; Figure S19. Infrared spectra of carmine paints in folios 54 and 18 of IL 18 and IL 12, matching gum mesquite (blue bands) and a protein (green line and orange bands). Calcium carbonate ( $\diamond$ ), calcium oxalate ( $\boxtimes$ ), and a calcium soap (calcium palmitate used as reference - $\dagger$ ) were also identified; Figure S20. Infrared spectra of carmine paints in folios 76v and 2 of Ms. 22 and Ms. 23, matching a protein (green line). Calcium carbonate ( $\diamond$ ) and a possible correspondence to a complex between brazilein and alum ( $\dagger$ ) were also identified; Figure S21. Infrared spectrum of a green paint in IL 12 f. 1v, matching gum mesquite (blue line and bands). Azurite ( $\circ$ ), malachite ( $\square$ ), and lead white ( $\bullet$ ) were also identified; Figure S22. Infrared spectra of gold preparation in IL 19 f. 20v, matching gum mesquite (blue line and bands). Calcium carbonate ( $\diamond$ ) and lead white ( $\bullet$ ) were also identified; Figure S23. Infrared spectra of glazes applied over blue paints in folio 59 of the *Ajuda Songbook* and folio 76v of Ms.22, matching mesquite gum (blue line). In the *Ajuda Songbook*, the presence of a protein (orange bands) was identified, while in Ms. 22, the fingerprint region indicates the possible use of a brazilwood-based material (pink bands); Figure S24. Infrared spectra of extemporaneous material or varnishes, matching starch (orange line and bands): (a) transparent extemporaneous material found in folio 116v of Ms. 24; (b) drop found in blue paint in folio 24 in Ms. 23; (c) drop found in folio 61 of IL 36; (d) varnish applied in folio 35 of Ms. 29. Calcium carbonate ( $\diamond$ ) and protein

(green bands) were also identified; Figure S25. Infrared spectra of extemporaneous material IL 19 fol. 21, matching starch (orange line), a protein (orange bands), and possibly mesquite gum (blue band). Calcium carbonate ( $\diamond$ ) and a calcium soap (calcium palmitate used as reference - $\star$ ) were also identified; Figure S26. Infrared spectra of varnishes in folios 1v and 18 of IL 12, matching mesquite gum (blue line) and starch (orange line). Calcium carbonate ( $\diamond$ ) and gypsum ( $\star$ ) were also identified; Figure S27. Infrared spectra of extemporaneous material in the winter breviary fol. 9 and IL 36 fol. 12, matching gum mesquite (blue bands) and cellulose-based materials (brown bands) and brazilwood (pink bands). Calcium carbonate ( $\diamond$ ) was also identified; Figure S28. Infrared spectra of extemporaneous material in folios 116v of Ms 24 and folio 24 of Ms. 23, matching a proteinaceous tempera namely egg white (green line). Calcium carbonate ( $\diamond$ ) was also identified; Figure S29. Structure of gum arabic proposed by Nie and colleagues (2013); Figure S30. Structure of mesquite gum proposed by Vernon-Carter and colleagues (2000); Table S1a. Percentage of each compound identified for gum arabic and respective species. Additional references are given in the percentage value; Table S1b. Percentage of each compound identified for gum mesquite and respective species. Additional references are given in the percentage value. References [36,43,53–55,59,60,68,69,75–85] are cited in the Supplementary Materials.

**Author Contributions:** M.J.M. conceived this research in close collaboration with M.V., L.M.d.C. and M.J.M. coordinated the access and analysis of the manuscripts. M.J.M. and M.V. performed the data acquisition and discussion. L.M.d.C. coordinated the ethnobotanic study of the gum. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** All data generated during this study are either included in this published article or available from the corresponding author upon reasonable request.

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