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The only surviving Medieval codex of Galician-Portuguese secular poetry: tracing history through luxury pink colors

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Abstract

The Ajuda Songbook is an exceptional illuminated manuscript being the only surviving codex of Galician-Portuguese secular poetry; it was produced in the end of the thirteenth century, beginning of the fourteenth century. The diversity of colors accentuated by the presence of lapis lazuli blue and brazilwood pink, demonstrates the desire to produce a sumptuous manuscript. Pink is, in this context, a luxury color and its identification attests to one of the earliest known occurrences of brazilwood in artworks. Scientific analysis showed, for the light pinks, a different formulation from that found in fifteenth-century books of hours and from all historical reconstructions of these colors prepared to date. This knowledge was used to further expand a database previously built in our laboratory and applied to the characterization of pink shades in the Ajuda Songbook. Thirteen brazilwood recipes were selected from seven Medieval treatises and reference materials were prepared based on such historical information. Three types of colors were achieved, defined as translucent rose, rose, and red. The translucent rose was obtained from recipes where egg white is used for extraction, and no other additives are present; rose from recipes with calcium carbonate; and red from a wider range of recipes, in which these ingredients are not mentioned. These colors were then prepared as paints, and analytical results were thus compared with data from the light pinks seen in the Ajuda Songbook's architectural backgrounds. We were able to reproduce the pink very well using infrared spectroscopy, identifying its main ingredients: calcium carbonate as filler; lead white as the pigment that produces light pink; and the binder as a polysaccharide with a fingerprint similar to mesquite gum. For the chromophore color, the application of chemometrics approaches to molecular fluorescence spectra highlighted a high degree of similarity with the paint reconstructions.

Keywords Brazilwood recipes, Paint formulations, Molecular characterization, Ajuda Songbook

Introduction

Color in Medieval illuminated manuscripts

Medieval illuminated manuscripts stand as one of the most important testimonies of Medieval art, as much for their intellectual content as for the material science behind their manufacture [1–3]. Therefore, tracing the history of these manuscripts is vital to appreciate their cultural value and to understand the degradation processes causing their partial or complete loss [4, 5]. Among the main constituting materials of Medieval illuminated manuscripts, organic colorants bear especially valuable information. As dye recipes and paint formulations can be specific to a certain *scriptorium*, these materials are

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key to provenance and dating studies, Fig. 1 [5–8]. In this context, it is essential to establish the paint formulation in terms of inorganic pigments, additives, and binders and to determine the exact recipes used in the production of lake pigments [6–11]. Microspectrofluorimetry supported by an extensive spectral database acquired on historically accurate reconstructions and chemometrics has proven to be a highly sensitive tool, able to correlate specific features found in organic colors to the different recipes that were used in their manufacture [6–11]. This approach has been often complemented with analysis by vibrational techniques such as infrared and Raman spectroscopies, which provide information on any other pigments, additives, and binders present [9, 11–13].

Following this methodology, this article illustrates the construction of a database of brazilwood recipes and paint formulations, whose characterization sheds light on the composition of pink colors in the Ajuda Songbook [14]. In this illuminated manuscript, the only surviving Medieval codex of Galician-Portuguese secular poetry [15], brazilwood pinks are different from any other previously found in books of hours [11, 14]. Since its discovery in the "Colégio dos Nobres", Portugal, it has been the subject of several studies, as essential questions about its identity, including the location of its production, remain unanswered [14]. Recently, our team conducted the first scientific study of the colorants used in the *Songbook* illuminations; by means of a multidisciplinary approach, the materiality of the Ajuda Songbook allowed us to establish a chronology through color [14]. The use of both orpiment and mosaic gold points to an overall dating between the end of the thirteenth and beginning of the fourteenth century.

Based on the results of previous research on this topic [12, 16], in-depth knowledge of the pink colors and their conservation condition must rely on the preparation and systematic characterization of a series of references. Seven manuscripts were selected for this purpose out of several Medieval texts (Table 1): Ms. Sloane 1754, Ms. Trésorier, Ms. Illuminandi, Ms. Lebègue, Ms. Alphabetum Romanum, Ms. Montpellier, Ms. Strasbourg (Additional file 1) [17–24]. The reproduction and characterization of recipes described in these texts allowed the research team to gain insight into the most common processes, extraction methods, and ingredients used in the production of brazilwood lakes and to identify the aspects that can differentiate one recipe from another. The so-obtained pigment lakes were then applied on parchment and filter paper within a complex paint formulation including lead white, calcium carbonate, and a polysaccharide-based binder, as found in the Ajuda Songbook's pinks [14]. The wealth of knowledge gathered from this experimental work is not only essential for the present investigation of the Ajuda Songbook, but also for future materials and provenance studies of similar manuscripts.

Brazilwood paint formulations and their characterization

Brazilwood, corresponding to *Biancaea sappan* and similar redwood species, was extensively imported to Europe from Asia, and more specifically from Sri Lanka, India, and Southeast Asia [25–27]. The oldest record of its importation dates back to the thirteenth century [28]. When the Portuguese landed in the country that is now known as Brazil, in the fifteenth century, a new species named *Paubrasilia echinata* became the primary source of brazilwood in Europe [29]. Even though the term "sappanwood" has been typically employed to describe *Biancaea sappan* species, here onwards "brazilwood" will be used for both *Biancaea sappan* and *Paubrasilia echinata* species for consistency [30].

Albeit the wood was also widely used, the principal value of brazilwood lay in its dye, Fig. 2 [26]. The main chromophore of this redwood is brazilein, which according to the literature results from the oxidation of the major component of the heartwood, brazilin [29–31]. It has been shown that, in Medieval times, dye extraction from the wood was based on alkaline or slightly acidic to neutral solutions; the dye found extensive application in textile dyeing and in the production of lake pigments, through the complexation with a metal ion, typically Al^{3+} [11, 26, 32]. Brazilwood has been identified in several objects of artistic and historical significance, mainly textiles and paintings [30–54]. For instance, Roger, Melo,

Fig. 1 Main components present in a Medieval paint

Table 1 Information on the seven treatises and recipe books selected to produce the historically accurate reconstructions, with a color indication of the resulting pigments, as follows: translucent rose (yellow), rose (blue) and red (red)

Title: *Liber de coloribus illuminatorum sive pictorum - Liber de tincturis pannorum* (13th-14th century) Abbreviation: Ms. Sloane 1754 Translation/critical edition: Sylvie Neven 2016; Daniel V.Thompson 1926 Ms. Sloane 1754 is a book of recipes from a wide collection of manuscripts covering several subjects, which were gathered by physician Han Sloane. Its recipes deal with the art of illuminating. According to Neven, the manuscript was written in Anglo-Norman language around the 13th century, while Thompson indicates that it was written in Latin in the 14th century and may be attributed to Northern Europe due to textual evidence of French origin. It contains three recipes for brazilwood and one observation on the quality of redwood for illuminating and dyeing cloths.	V1. *Egg white* Brazilwood Alum V2. *Egg white* Brazilwood Alum
Title: *Trésorier de philosophie naturelle des pierres précieuses, quatrième livre* (1390) Abbreviation: Ms. Trésorier Translation/critical edition: Anne-Françoise Cannella 2006 The treatise belongs to the Bibliothèque nationale de France, and was written in 1390. One of the three best-known works by writer Jean d'Outremeuse focuses on technical recipes for dyeing glass, gilding, lapidary, ivory, and pigment recipes. It contains four recipes of brazilwood in which gypsum replaces traditional calcium carbonate as an additive.	V1. *Urine* Brazilwood Alum Gypsum V3. *Urine* Brazilwood Alum Gypsum
Title: *De arte illuminandi* (14th century) Abbreviation: Ms. Illuminandi Translation/critical edition: Franco Brunello 1992 *De Arte illuminandi* is a 14th-century treatise, written by an unknown author. As the title indicates, it is entirely dedicated to themes and techniques related to the art of illuminating and is strictly organized according to the manufacture of each book and illumination. It was allegedly written in Italy, in Latin, and includes two recipes for brazilwood.	V1. *Lye* Brazilwood Alum Calcium carbonate V2. *Egg white* Brazilwood Alum Gum Arabic
Title: *Experimenta de coloribus* (1431) Abbreviation: Ms. Lebègue Translation/critical edition: Mary P. Merrifield 1849 The Lebègue manuscript was written by Jehan Le Begue in 1431. The author based his work on a compilation of writings entitled *De diversis coloribus* (1382-1441) by Jehan Alcherius. According to Clarke, it also contains earlier information from the works of Theophilus and Heraclius. Seven recipes for brazilwood are described in *Experimenta de coloribus*. Two of these are not related to brazilwood paint, but rather describe the preparation of a colored varnish and pellets made out of flour and brazilwood extract.	V20. *Egg white* Brazilwood Alum V304. *White wine* *Water* Brazilwood Alum Gypsum
Title: *Alphabetum Romanum* (1433-1479) Abbreviation: Ms. Alphabetum Romanum Translation/critical edition: Sylvie Neven 2016 Ms. Alphabetum Romanum, Vat. Lat. 6852, from the Bibliotheca Apostolica Vaticana, was written between 1433 and 1479 by Felice Feliciano of Verona. It is a treatise with illustrations on writing Roman capital letters and on inks (including magic ink), colors, and coloring parchment. Neven proposes that it was written in a mixture of Latin and Italian languages. It contains two recipes related to the making of brazilwood colors.	V1. *Vinegar* Brazilwood Alum Calcium carbonate
Title: *Liber diversarum arcium* (15th century) Abbreviation: Ms. Montpellier Translation/critical edition: Mark Clarke 2011 Ms. Montpellier is presently part of Ms. H277 in the Bibliothèque Inter-Universitaire de la Sorbonne, France. It was written in Latin in 15th-century Venice; however, according to Clarke, part of its core illustrates techniques of earlier tradition, that are closer to the 14th century, as well as practices from Northern Europe. It includes three recipes for brazilwood.	V.1.8.2. *Urine* Brazilwood Alum V1.8.3. *Gum Arabic* *Water* *Egg white* Brazilwood Alum
Title: Ms. Strasbourg (15th century) Abbreviation: Ms. Strasbourg Translation/critical edition: Viola & Rosamund Borradaile 1966; Sylvie Neven 2016 Ms. Strasbourg is a recipe collection from 15th-century Northern Europe. According to Neven, this work is the oldest German-language source enabling the study of painting techniques from Northern Europe. The translations currently available derive from a 19th-century copy, commissioned by Sir Charles Eastlake, as the older manuscript was lost in the Strasbourg Library fire in 1870. It includes three recipes for brazilwood.	V1. *Egg white* Brazilwood Alum Calcium carbonate V2. *Lye* Brazilwood Alum

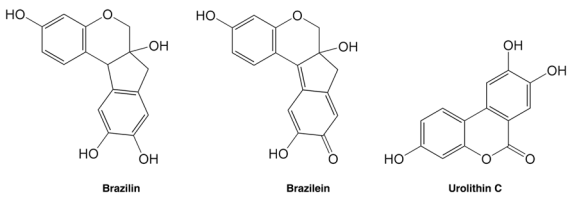

Fig. 2 Chemical structures of brazilin, brazilein, and urolithin C

and coworkers found brazilwood as a characteristic dye in fifteenth-century books of hours of French and Flemish production [11, 55].

Methodologies to identify brazilin and brazilein, as well as a distinctive minor compound named urolithin C [35], rely on HPLC–DAD, SERS, FT-SERS, LC-HRMS/MS, and NMR, Fig. 2 [31, 36–38, 40, 42–45, 50]. Although these techniques enable the unequivocal identification of the colorant, an in-depth study of the exact paint formulation and recipe would require a multi-analytical approach based on UV–Visible (UV–VIS) reflectance, microspectrofluorimetry, μRaman, μFTIR, and chemometrics, combined with comparison against a database of historical reconstructions for colorants, binders, and color paints [6–11]. Such reconstructions must be prepared with as much historical accuracy as possible, following procedures described in Medieval written sources; upon preparation, historically accurate reconstructions must be characterized at the molecular level and the results compared with those obtained from Medieval colors [12, 13, 56, 57]. The reconstructions are then validated by correlation and can be used to improve the application of this analytical approach or for degradation studies [58].

Preparation of brazilwood pigments in technical written sources

From a total of twenty-eight treatises and recipe books formulated in Europe from the ninth to the seventeenth century, seven were here selected for an in-depth study of their constituting materials, Table 1 and Additional file 1 [17–24]. The documents selected are all dated between the thirteenth and fifteenth century, based on a criterion of date proximity with the Ajuda Songbook, and a critical edition exists for most of them. Recipes were also chosen to ensure the highest possible representativeness and variability among them.

The majority of brazilwood recipes describe how to make a lake pigment, while very few illustrate the preparation of colored varnishes. Briefly, the production of brazilwood lake pigments unfolds through the following steps: a) extraction of the chromophore in alkaline or acidic solution—in some recipes, this solution is then filtered to remove rasps; b) precipitation of the lake pigment through the addition of alum; and c) addition of ingredients to adjust pH to an optimal value (neutral or weakly acidic) for lake formation. The latter step is not present in all the recipes. The type of extraction solution used, the order of steps described, and the additives included appear to vary from recipe to recipe. It is worth noting that, in the written sources examined, the most common extraction reagent reported for brazilwood was egg white (glair), mentioned in six of the seven treatises and recipe books; glair has a pH around 9. To the best of our knowledge, egg white was not used to extract other red dyes, although in a recipe from Ms. Montpellier it is employed for saffron yellow. In this work, brazilwood reconstructions using egg white extractions were prepared for the first time.

Key aspects in the making of historically accurate reconstructions of brazilwood-based recipes

The choice of pH, complexing ions, and additives plays a key role in determining the chromophore's extraction times and final pigment hue. A previous publication focused on reproducing recipes from *The book on how to make all the color paints for illuminating books (*from here onwards referred to as *The book of all colors)* and a nineteenth-century Winsor & Newton archive proved that it is much easier to replicate nineteenth-century recipes than Medieval ones [5, 12]. In Medieval recipes, extraction times range from minutes to a few hours when basic solutions such as lye, an ash extract, or lime water are used [4]. With neutral or slightly acidic solvents like fresh urine, whose pH is around 6, the extraction process can take days [12]. In *The book of all colors*, brazilwood lakes are the only pigments that can be obtained with four recipes, leading to a translucent carmine (Chapter 44), which would have been applied as a glaze, and three pink colors of dark opaque, bright, and very light appearance [5]. Carmine/red, dark opaque pink, and bright pink were identified in fifteenth-century books of hours. Lab* color coordinates were used to describe carmine and to differentiate among pinks that were characterized by a lighter tone (higher L*) together with a component of yellow ($b^* > 0$) or blue ($b^* < 0$); all colors had a red component ($a^* > 0$). The higher the quantity of calcium carbonate or gypsum present in the formula, the greater the opacity (higher L* value). The most opaque, and also the lighter, pigments are those where lead white, a basic lead carbonate, or calcium carbonate were added. The ions released from these additives, namely, Ca^{2+} and Pb^{2+}, which can also serve as complexing ions, are responsible for colors with a bluer shade [12].

It should be noted that, of the two urine-based recipes ($pH \leq 7.5$), one presents a component of yellow and the other one of blue (Chapters 8 and 27, respectively). The

same is observed when moving to extractions in a basic medium: one is characterized by a blue (Chapter 9) and one by a yellow component (Chapter 44) [5]. All recipes recommend scrapping brazilwood very finely, except for one in Chapter 44 that suggests grinding it instead. In all recipes, the colorant is complexed with an aluminium ion, deriving from alum, and this would yield an insoluble brazilwood lake pigment. Due to Al^{3+} being a Lewis acid when added to the extraction solutions, the pH decreases. To control and improve the lake pigment formation, most recipes examined mention the use of buffers that help stabilize the pH to neutral values for pigment precipitation. Buffers most commonly used are calcium carbonate and lead white.

Another important aspect to take into consideration is the omitted or missing information. When working with Medieval written sources, two main problems may arise: erroneous translation and unknown/incorrect terminology, as well as a lack of accuracy in the instructions provided. Regarding the first issue, Castro et al. describe, as an example, the case of the word *"asado"* in *The book of all color*s, initially translated as "fried meat" and later interpreted as linked to a pot with two handles [59, 60]. As in this case the second translation was more adequate to the context of the recipe, this demonstrates the importance of comparing different sources and interpretations whenever possible.

Frequent omissions and inaccuracies in Medieval recipes pose another significant challenge to scholars. Information related to ingredients' required quantities is often missing or given in measurement units that differ from region to region, highlighting the value of knowing the exact origin of the written source under study. In addition, some essential steps in the recipe, such as filtration, are not precisely described. It is possible that this information was given in previous recipes or was expected to be known by practitioners of the time; such procedures, however, may not be obvious to current researchers.

Assessing the condition of brazilwood colors in books of hours through historical reconstructions

The colors obtained following instructions of *The book of all colors* resulted in different hues: light pink (L* 62; a* 17; b*− 13), reddish-pink (L* 32; a* 35; b*− 5), and red (L* 39; a* 42; b* 17). These were compared with shades found in books of hours examined in previous studies [11], along with their emission and excitation spectra [7, 11, 12]. This study provided interesting information for the evaluation of their color, which may be helpful in conservation-related discussions. For example, the hues of the pigment reconstructions were consistent with the pink and red brazilwood colors found in French books of hours [11]. This comparison shows that, despite often being described as relatively unstable and subject to fading, brazilwood-derived pigments in books of hours sometimes appear to be well preserved (possibly, because they were protected from light).

Results and discussion

In-depth research into Medieval brazilwood recipes dated to the fourteenth and fifteenth centuries

Extensive research on treatises and recipe books allowed us to collect eighty-five recipes related to brazilwood. The oldest brazilwood recipe was found in the treatise of Ibn Badis, dated to circa 1025, which describes the production of a lake pigment using water as the extraction solution. Most of these recipes report the characteristic process used to produce a pigment lake, i.e. addition of a metal ion to prompt complexation with the organic dye. Due to the high number of recipes found, thirteen of the most representative were chosen and are shown in Additional file 1.

As it was verified for recipes in *The book of all colors*, important details appear to have been omitted in the recipes here examined as well, especially in regards to exact quantities of the main ingredients: only five recipes, indeed, include this piece of information for one or more of the components, while all others provide imprecise directions, such as "a little", "however much alum is sufficient", "moderate dose" or "in proportion to the quantity of brazilwood", Additional file 1. Brazilwood is the ingredient for which fewer details on the needed amount are given; however, recipes typically indicate that it should be reduced to scrapings prior to use.

Another challenge lies in the presence of unknown or biased terms. For example, in Ms.Trésorier, both recipes describe the use of *"crete"* or *"craie de pelletier"* after the addition of alum. A similar term, *"creta"* is described by Vitruvius and by Davidovits as "terra" or "argila" [61, 62]; however, according to Cannella, both terms are synonyms and correspond to calcium sulfate [18, 61]. To the authors' knowledge, these two works are the only ones containing an explanation of the terms mentioned above. Furthermore, as Medieval sources originate from different regions in Europe, measurement units may vary significantly and can thus be difficult to correlate. For example, in Ms. Strasbourg, the second brazilwood recipe refers to the use of one *"settin"* of alum: this amount, according to scholar Sylvie Neven, corresponds to ½–¼ of *"lot"*, though one *"lot"* would be ± 14.61 g in Berlin or ± 17.59 in Innsbruck [23].

In light of all these setbacks, in the present work, recipes were reproduced at least twice, following a consistent methodology, varying the quantities systematically, including steps that were possibly omitted such as filtration, and taking doubtful ingredients into careful

consideration to achieve the widest possible understanding of the final products obtained (for more information, please see Additional file 1: Table S1 and Figs. S1 and S2).

Characteristics of brazilwood recipes defining the final color

The main ingredients and steps used to produce historically accurate brazilwood lake pigments are summarized in Tables 1 and 2. All recipes consistently mention the following three components: brazilwood, alum, and an extraction solution. According to the recipes selected, the brazilwood dye is extracted with egg white, lye, urine, vinegar, and white wine, and then complexed with an aluminium ion. Contrary to recipes found in *The book of all colors*, the use of acidic solutions is reported not only in recipes listed in Table 2, but also in other sources. Nevertheless, it is notable that basic and neutral extraction solutions, especially those based on egg white, are cited in all the Medieval sources examined, while acidic solutions are mentioned in only thirteen recipes.

Recipes can be divided into three types based on paint color: translucent rose, rose, and red. This observed chromatic difference does not only depend on the pH of extraction or precipitation, but also on the type of additives used and on the treatment undergone by the extraction solution, namely, the chromophore extraction time, as well as the heating or boiling steps. These paints were applied on filter paper and parchment as a single and

Table 2 Main steps and ingredients of the brazilwood recipes, with a description of original and used quantities

Source	Recipe	Brazilwood[a]	Alum[a]	Additive[a], quantity	Brazilwood; Alum; Additive (g)	Extraction	Filtration	Final pH
Ms. Sloane 1754	1	n.a	*proportion to the quantity of brazilwood*	n.a	5;5	Egg white, 100 mL	b	4.1
	2	*small scrap*	*moderate dose*	n.a	1;1	Egg white, 50 mL, 3 days, RT	No	4.3
Ms. Trésorier	1	*1 weigh*	*half of 1 weigh*	Gypsum, quarter of 1 weight	2.5; 1.25; 0.625	Urine, 60 mL, Boil	No	4
	3	n.a	n.a	Gypsum, n.a	5; 0.223; 0.625	Urine, 50 mL, 1 day, RT	b	5.4
Ms. Illuminandi	1	n.a	*as much as* (brazilwood)	Calcium carbonate, as much as (brazilwood)	2.5;2.5; 2.5	Lye, 50 mL, 1 day; Heated ca. 90 °C	b	6.8
	2	*two, or, …, three common beans*	*half an ounce*	n.a	5; 0.5	Egg white (50 mL) and gum Arabic (10 mL), 3 days, RT	b	5.3
Ms. Lebègue	20	n.a	*a little*	n.a	5; 0.6	Egg white, 50 mL, 1 day, RT	c	5.8
	304	n.a	*a little*	Gypsum, put in some	5; 0.5; 0.34	White wine and water, 25 mL each; Boil	c	2.7
Ms. Alphabetum Romanum	1	n.a	*sufficient*	Calcium carbonate, n.a	5; 0.5; 1.5	Vinegar, 100 mL, 6 days, RT; Boiling and reduced to ¼	b	4.8
Ms. Montpellier	1.8.2	n.a	*however much alum is sufficient*	n.a	1; 0.04	Urine, 10 mL, 1 day, RT;Water, 10 mL, 1 day, RT	b	6.7
	1.8.3	n.a	*quantity of a chickpea*	n.a	1;0.31	Egg white (10 mL), Gum Arabic (5 mL) and water (2,5 mL)	No	4.9
Ms. Strasbourg	1	*½ ounce*	*½ ounce*	Calcium carbonate, ½ ounce	2.5; 2.5; 2.5	Egg white, 50 mL, 8 days, RT	b	7.2
	2	*½ or 1 oz*	*one settin*	n.a	5:0.4	Lye, 50 mL; Heated	b	5.7

n.a non applicable

[a] English translation of the expressions given in the recipes

[b] Filtration was conducted at the end of the recipe, after addition of all the components

[c] Filtration was conducted after chromophore extraction and before addition of alum and additives

double paint layer (Additional file 1: Table S2); results obtained on one single paint layer will be discussed in this section. The L* a* b* color coordinates in Table 3 show the difference between rose, translucent rose, and red paints.

Translucent rose

Translucent rose pigments are always obtained from recipes where egg white was used as the extraction solution and only alum was added. As brazilein is a weak organic acid, its protonated and deprotonated forms show different colors ($pK_a = 7$), [12]. In egg white, due to a basic pH of 9, the molecule present in the highest quantity should be the deprotonated form of brazilein, of red color. Some authors also state that not only brazilein, but also other minor yellow compounds, are extracted in the process, which can affect the solution color [34, 43, 52]. These factors, as well as the semi-transparent nature of egg white, yield a reddish-rose tone to the paint, similar to red colors, yet with a translucent aspect that only pigments from these recipes display.

Within the translucent rose reconstructions, darker and browner shades were also obtained for extraction times as long as several days [L* 77–57; a* 39–17; b* 29–4], Table 3. Unlike lye and lime, whose recommended extraction times range between a few minutes and a few hours, one or more days are typically advised for egg white recipes, with the exception of recipe 1.8.3 from Ms. Montpellier. This might be due to the impossibility of heating egg white, since that would induce changes in the structure and conformation of the globular proteins, resulting in denaturation or coagulation [63]. The only noticeable difference in the color values of translucent rose and red lies in the paint luminosity, with egg white recipes mostly displaying higher L* values than red colors, Table 3.

Rose

Rose pigments result from a wider range of solutions and factors, with the addition of a carbonate source as a common element. Typical carbonate sources are calcium carbonate and lead white, which in acidic media will dissociate into $CO^2{}_3{}^-$ and Ca^{2+}/Pb^{2+} ions. After

Table 3 Extraction method, extraction pH, and final pH for brazilwood lake pigments, as well as L*, a*, b* color values for brazilwood lake paints of different colors applied on parchment

Color	Extraction method	Extraction pH	Final pH	Colorimetry			Source
				L*	a*	b*	
Translucent rose	Egg white	9.3	4.1	57.4 *53.7*	38.5 *57.4*	27.1 *31.5*	Ms. Sloane 1754 V1
	Egg white	9.0	4.3	70.5 *68.9*	25.4 *27.5*	28.7 *30.2*	Ms. Sloane 1754 V2
	Egg white	9.0	5.3	76.5 *34.9*	17.3 *47.3*	6.1 *13.6*	Ms. Illuminandi V2
	Egg white	9.0	5.8	73.6 *36.9*	23.7 *53.4*	3.6 *10.3*	Ms. Lebègue V20
	Egg white, gum Arabic, water	8.4	4.9	75.3 *41.7*	20.3 *49.9*	5.7 *3.9*	Ms. Montpellier V1.8.3
Rose	Lye	9.6	6.8	62.6 *26.2*	25.1 *19.2*	1.8 *3.3*	Ms. Illuminandi V1
	Vinegar	2.6	4.8	44.3 *20.1*	35.8 *18.2*	0.9 *3.8*	Ms. Alphabetum V1
	Egg white	7.2	7.2	73.9 *48.1*	23.6 *50.2*	0.2 *-7.4*	Ms. Strasbourg V1
Red	Urine	4.2	4	69.2 *32.7*	22.2 *36.3*	9.2 *13.7*	Ms.Trésorier V1
	Urine	5.4	5.4	58.1 *28.9*	32.1 *33.6*	8.2 *13.0*	Ms.Trésorier V3
	White wine	3.0	2.7	69.5 *40.5*	18.4 *35.7*	7.7 *5.9*	Ms. Lebègue V304
	Urine	7.4	6.7	62.9 *41.4*	23.9 *37.8*	8.7 *9.2*	Ms. Montpellier V1.8.2
	Lye	10.6	5.7	31.0 *17.9*	25.8 *13.6*	4.7 *4.6*	Ms. Strasbourg V2

Dyes that were not extracted with egg white were applied with the aid of gum Arabic. All paints were applied as a single and double paint layer (Italics), and both sets were analyzed by colorimetry

acidification of the extraction solution by the addition of alum, carbonate ions are added to serve as buffers and increase the solution pH to neutral values. Ca^{2+} and Pb^{2+} ions may also contribute to the color change, as bluer shades are obtained when these ions are present in solution [12]. As expected, paints resulting from recipes like recipe 1 from Ms. Illuminandi, Ms. Alphabetum Romanum, and Ms. Strasbourg present the lowest b* values when compared to other recipes [L* 74–44; a* 36–24; b* 2–0], Table 3. This difference proves that the presence of carbonates and Ca^{2+} ions will confer bluer shades to the product obtained (for more information, please see Additional file 1: Table S2).

Red

Lastly, red lake pigments were obtained from recipes where neither egg white nor carbonate ions are present. In these recipes, the chromophore is extracted with lye, urine, and white wine, showing that the extraction type and pH will not necessarily affect the final color. In all of these recipes alum is then added; however, except for recipe 2 from Ms. Strasbourg and recipe 1.8.2 from Ms. Montpellier, another component is used, i.e. calcium sulfate. While some recipes, such as recipe 8 from *The book of all colors*, offer a choice between calcium carbonate and sulfate as possible additives, those examined here only mention the addition of calcium sulfate. This suggests that these recipes' main aim was the production of a red pigment, in which calcium sulfate would be added as a filler to modify the physical properties of the paint, or to make it more opaque. In general, red colors have darker hues and, within these recipes, the extraction time and presence of calcium sulfate do not affect the measured values as the aforementioned components [L* 70–31; a* 32–18; b* 9–5], Table 3. It is also important to note the color variations observed for one and two paint layers, except for Ms. Sloane 1754 V2.

Multi-analytical characterization of brazilwood lake pigments and paints

Fiber-optics reflectance spectroscopy and UV–VIS microspectrofluorimetry

Reflectance spectroscopy and microspectrofluorimetry were used to unequivocally identify brazilwood in all the paints. Absorbance spectra are characterized by a high-intensity band at 550–555 nm, with a shoulder around 520 nm, and a lower intensity band around 370–85 nm, as seen in Fig. 3a, which is in accordance with the results of Vitorino and coworkers [12]. For translucent rose, the 370 nm band has higher intensity when compared to the other two colors, which might be related to the semi-transparency feature of egg white paints. As the band corresponding to parchment falls around the same wavelength, intensity might include a contribution from the support, considering that, in paints applied on paper, this band has similar intensities as in other colors.

Spectra of rose colors are well defined and show an absorbance maximum within the expected wavelength range (550–555 nm), which is in contrast with spectra obtained for red colors. These spectra normally feature broader and less defined bands, as verified for Ms. Tresórier 1, Fig. 3, that are characteristic of saturated colors and corroborated by their typically darker tones.

The emission and excitation spectra for translucent rose, rose, and red colors are plotted in Figs. 3b–d. In these spectra, differences between the three types of colors are more evident, Table 4. As microspectrofluorimetry is highly sensitive to the chromophore's environment, it is able to clearly differentiate among paint formulations involving the same chromophore. We have proven before that, despite a similar spectral shape common to all brazilwood paints, this technique is able to distinguish recipes based on small shifts in the excitation and emission maxima, as well as differences in band intensities (see also Microspectrofluorimetry and chemometrics paragraph) [7]. By examining the excitation and emission spectra of all three colors, distinctive traits for each type can be identified. Red, for example, is defined by excitation and emission maxima at ca. 550 and 602 nm, respectively [6, 7]. Translucent rose typically displays a better signal-to-noise ratio, possibly due to the absence of fillers during preparation of the pigment lake, and respective excitation and emission maxima at ca. 557 and 587 nm. The rose color, characterized by the addition of calcium carbonate, yields spectra with a low signal-to-noise ratio and lower signal intensities. This color has an excitation maximum at ca. 552 nm and an emission maximum at ca. 594 nm.

Infrared spectroscopy

Figures 4, 5, 6 present a series of infrared spectra acquired from six reconstructions prepared as paint on parchment (paints without egg white were applied with gum Arabic), divided by the type of color obtained, with two examples per color from different recipes. Infrared spectra obtained for all the pigments can be inspected in Additional file 2: Table S3. In general, infrared spectroscopy proved helpful to characterize the binder and additives of each recipe; in one instance, namely the Ms. Strasbourg 2 recipe, this technique notably detected the brazilwood extract.

Spectra acquired from translucent rose reconstructions are dominated by the contribution of egg white, identified through the amide I band at 1649 cm^{-1} (νC=O), the amide II band at 1533 cm^{-1} (νCN and σ NH), as well as νOH and νNH at 2400–3000 cm^{-1} [64]. For Ms. Montpellier 1.8.3

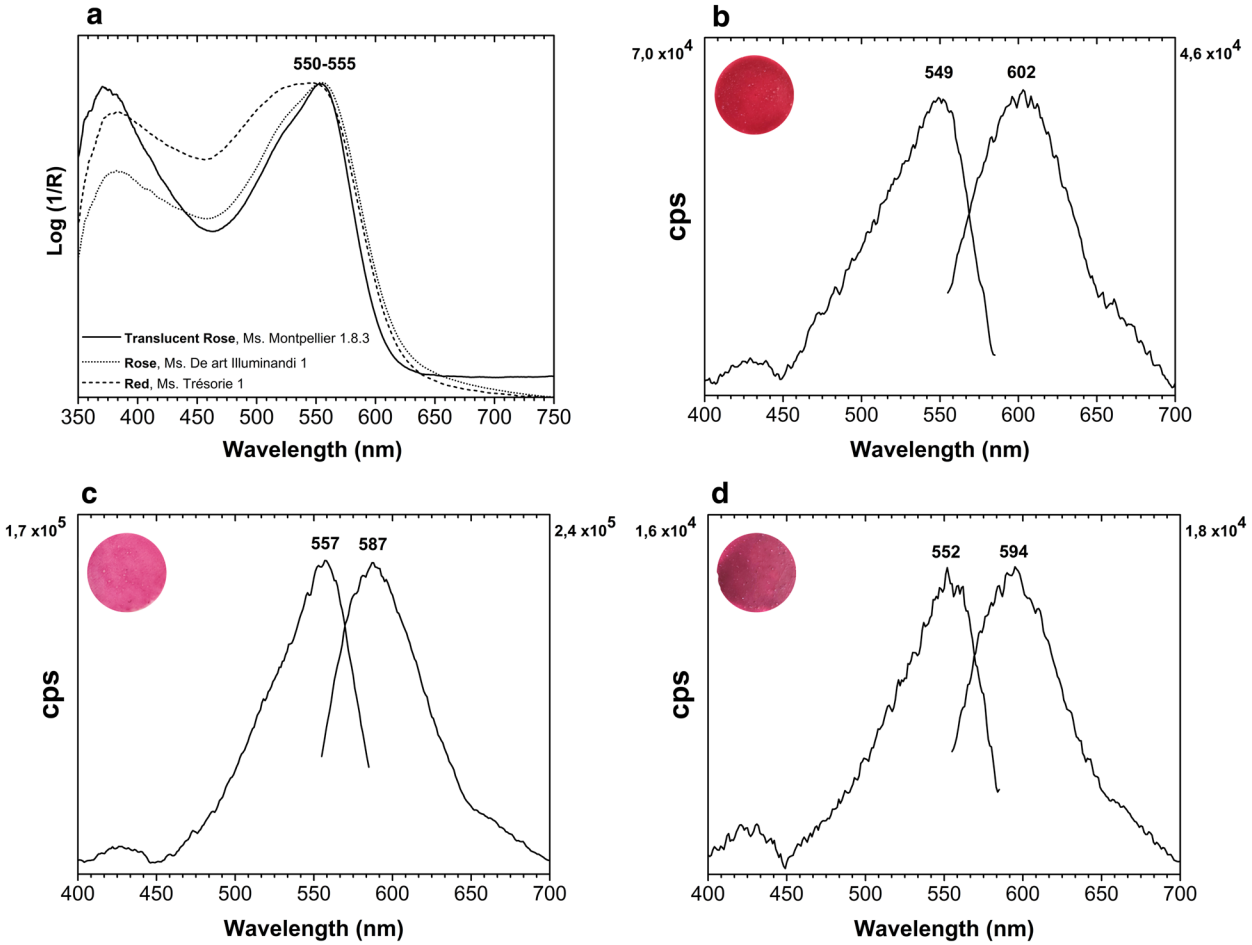

Fig. 3 **a** Absorbance spectra representative of the three types of colors obtained from the recipes, and excitation and emission spectra from **b** red color of Ms. Trésorier 1, **c** translucent rose of Ms. Montpellier 1.8.3, and **d** rose of Ms. Illuminandi. Reconstructions were analyzed as color paints applied on parchment

Table 4 Absorption maxima, as well as fluorescence excitation and emission maxima for: pink historical reconstructions applied as paints using gum Arabic and mesquite gum; pinks seen in the Ajuda Songbook's architectural backgrounds; pinks found in books of hours (Ms.22 e Ms.24, Mafra National Palace collection)

	λ_{abs} (nm)	λ_{exc} (nm)	λ_{em} (nm)	Formulation	References
Pink historical reconstructions	556–565	553–556	581–588, 602		–
Ajuda Songbook *BW1-type spectra*	557–567	552–561	582–586	Lead white, calcium carbonate	[7, 14]
Books of hours	552–559	556–560	585–590		[7, 11]
BW2-type spectra		555–562	594–602	Calcium carbonate, gypsum	[7]
BW3-type spectra		553–564	590–600	Calcium carbonate, higher amounts of gypsum	[7]

Data presented refer to clusters BW1, BW2, and BW3. For more details, please see Fig. 11 and text

gum Arabic, likely used as an additional organic component along with the egg white binder, was detected through its 1076 cm^{-1} signal, typical of glycosidic bonds [65, 66]. Apart from egg white, spectra of these reconstructions display bands at 3400 cm^{-1} (νOH) and 1119 cm^{-1} that possibly correspond to aluminium hydroxide [32, 67].

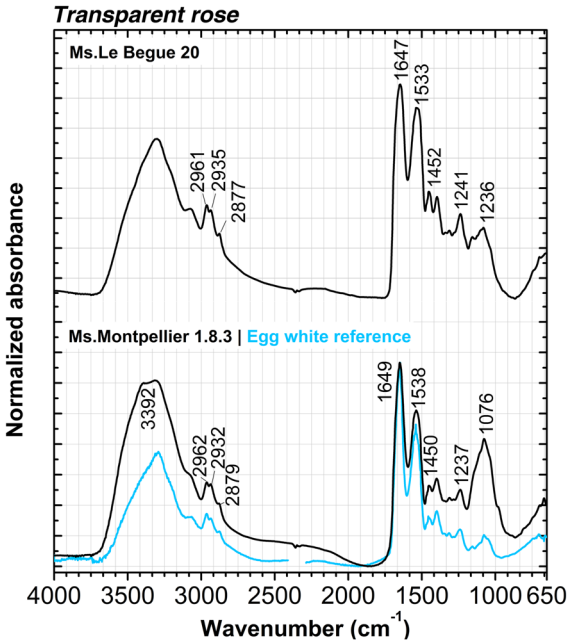

Fig. 4 Infrared spectra of recipes that result in translucent rose colors: Ms. Lebègue 20 and Ms. Montpellier 1.8.3 (black) compared with egg white reference (blue)

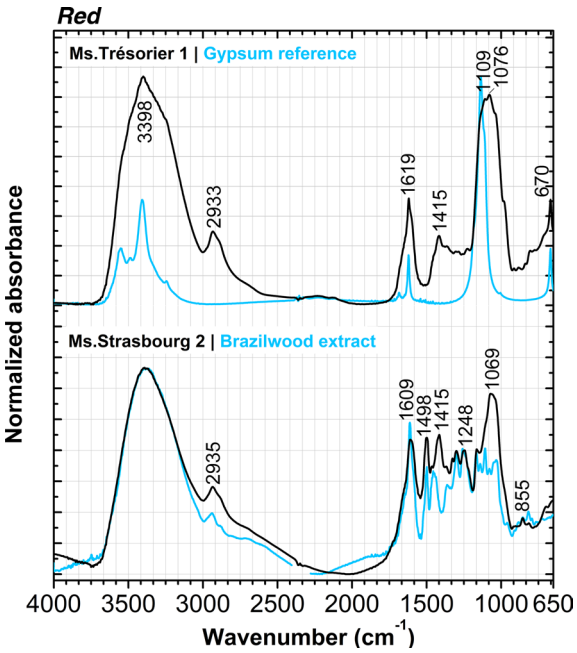

Fig. 6 Infrared spectra of recipes that result in red colors: Ms. Trésorier 1 and Ms. Strasbourg 2 (black) compared with gypsum and brazilwood extract references (blue), respectively

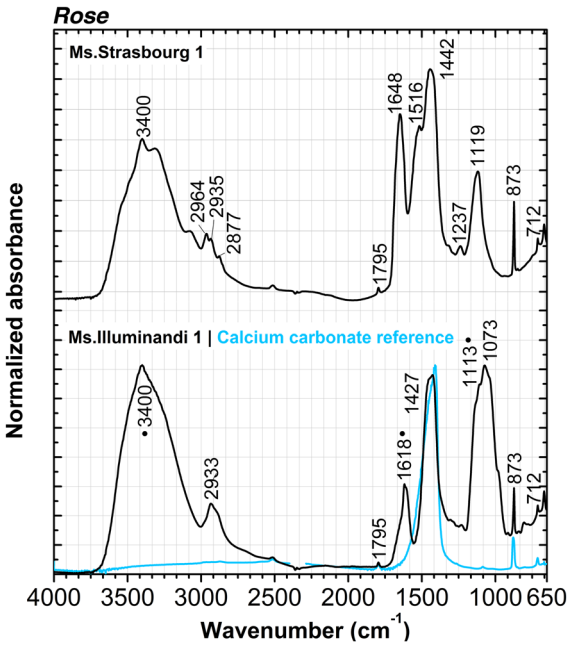

Fig. 5 Infrared spectra of recipes that result in rose colors: Ms. Strasbourg 1 and Ms. Illuminandi 1 (black) compared with a calcium carbonate reference (blue). Signals attributed to gypsum in the formulation are marked with (●)

Infrared spectra of rose colors are mainly characterized by the presence of calcium carbonate, identified through a series of distinctive bands at 2515, 1795, 873 (σ_{as} CO_3^{2-}), and 712 cm^{-1} (σ_s CO_3^{2-}) [68]. In Ms. Strasbourg 1 and Ms. Illuminandi 1, the highest intensity band of calcium carbonate appears broadened and shifted towards higher wavenumbers, i.e. 1442 cm^{-1} and 1427 cm^{-1} for the two reconstructions. This may be due to the influence of the egg white's amide II band and the asymmetric stretching of the gypsum sulfate ion, respectively. In this category of colors, gypsum is never listed as a recipe ingredient, so its presence—frequently observed for recipes containing calcium carbonate—likely results from a side reaction during paint preparation. In particular, gypsum forms from the dissociation of calcium carbonate into CO_3^{2-} and Ca^{2+} ions and subsequent reaction with SO_4^{2-} ions from alum; the carbonate ion is converted into CO_2, while Ca^{2+} is free to react with the sulfate ions, resulting in gypsum formation. Interestingly, gypsum formation is detected in all recipes using urine, lye, or white wine as extraction agents, regardless of heating conditions, but not in Ms. Strasbourg 1, which employs egg white.

Red colors yielded the most heterogenous spectra, which is explained by a wide variety of processes that can lead to this typology. Signals most commonly detected belong to gypsum, as seen for Ms. Trésorier 1, giving rise to absorption bands at 3398 (νOH), 1619, 1109 (ν_{as} SO_4^{2-}), and 670 cm^{-1} (σ_{as} SO_4^{2-}) [68]. Dry pigment lakes obtained from these recipes were applied with gum Arabic, which for Ms. Montpellier 1.8.3 was detected at 1076 and 2933 cm^{-1}. The highest intensity band for the polysaccharide binder is further broadened by the presence of gypsum. Ms. Strasbourg 2 exhibits the most different spectra, which contain, besides the binder signals, the typical absorption bands of brazilwood extract. As opposed to spectra collected from Ms. Strasbourg 1, aluminium hydroxide is not detected here and signals from brazilwood match those of the uncomplexed dye.

As a final remark, the types of colors identified yield infrared spectra that can be grouped according to the components detected, with only a few discrepancies within each type.

Rose paints and the Ajuda Songbook pinks

Light pinks from the architectural background

A detailed visual examination of the *Songbook*'s architectural elements shows that all final details, including some of the highlights created with white and other colors, are completed and varnish is applied as a protective layer. It is also important to note that eleven folios of the Ajuda Songbook, composing a quire, were found loose in the Public Library of Évora in 1842, and were incorporated in the codex at a later time [14]. This section compares the rose paint reconstructions with a selection of paints from the Ajuda Songbook illuminations, Fig. 7.

Infrared and Raman spectroscopy identified two additives in the *Ajuda*'s pinks, lead white and calcium carbonate. The first was likely used to adjust the color shade to a light pink, while the latter serves as a filler to improve the physical and chemical properties of the paint. Infrared spectra contain bands of lead white at 3534 cm^{-1-} (νOH), 1422 cm^{-1} (νCO_3^{2-}), 1045 cm^{-1} (σ CO_3^{2-}), and 685 cm^{-1}, Fig. 8 [69]; and bands of calcium carbonate at 1422 and 874 cm^{-1} (CO_3^{2-} stretching and bending). Typical Raman signals of these two compounds are respectively found at 1052 (νCO_3^{2-}) and 1086 cm^{-1} (νCO_3^{2-}) (Additional file 2: Fig. S3) [69].

The binding medium was identified as a polysaccharide, specifically an arabinogalactan proteoglycan of type II; mesquite gum and gum Arabic both belong to this class [70, 71]. More information on their composition is included in Additional file 2. Several authors have shown that each of these polysaccharides has a specific fingerprint in the 1200–1000 cm^{-1} region of their infrared spectra [72–74]. This region is dominated by skeletal C–O and C–C vibrations of glycosidic bonds and presents a well-defined structure. Certain identification is possible when these compounds are pure; because paints are often complex mixtures, however, it is difficult to make this assignment. The highest intensity bands for gum Arabic and mesquite gum are at 1075 and 1034 cm^{-1}, respectively. This difference is very clear

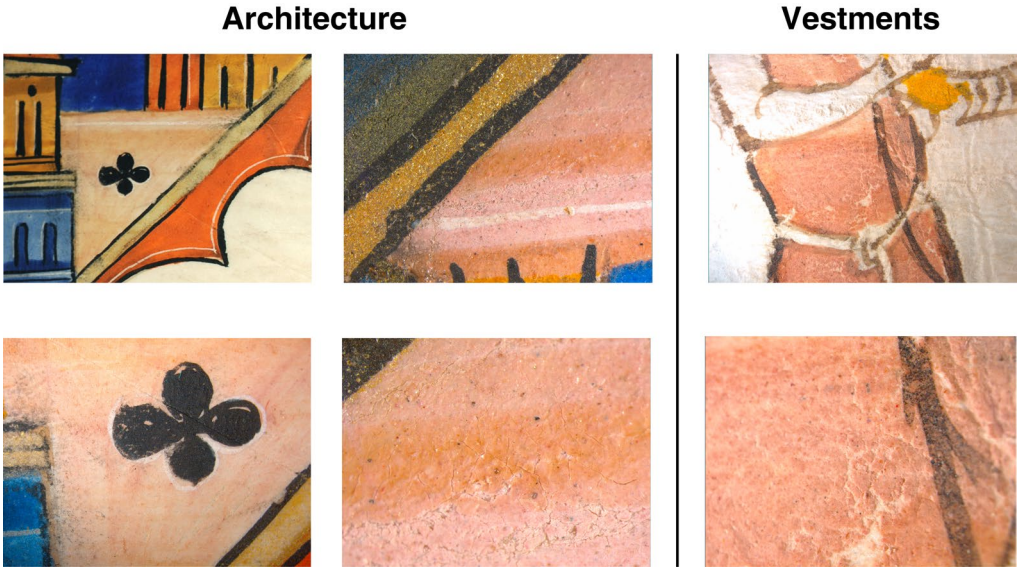

Fig. 7 Details of brazilwood pinks from some of the architectural elements and vestments found in the Ajuda Songbook illuminations; macroscopic (top) and microscopic (bottom)

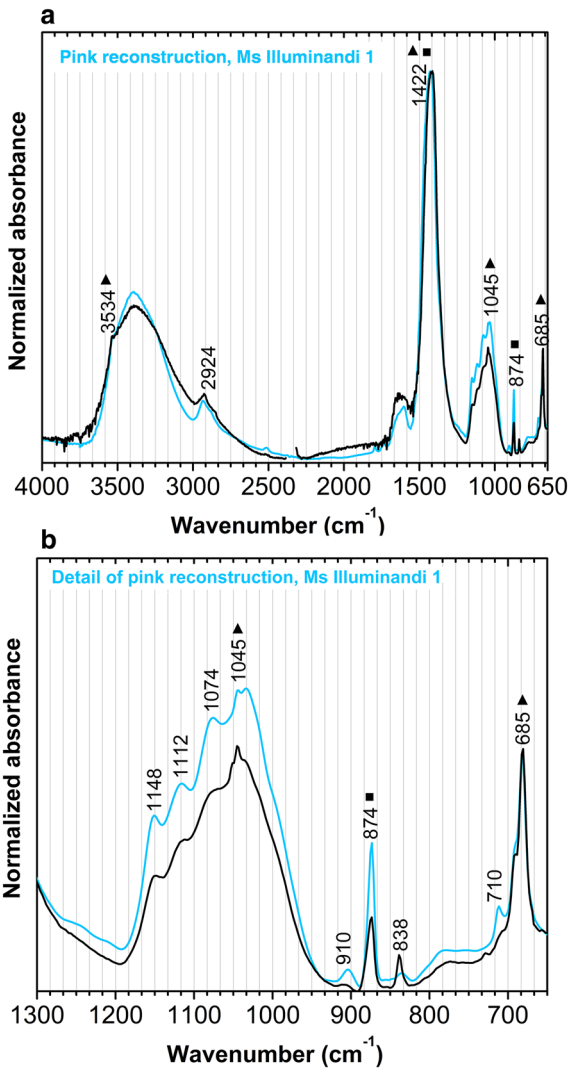

Fig. 8 Infrared spectra acquired from **a** the Ajuda Songbook's architectural pink (black) compared with a historical reconstruction (blue) prepared following recipe 1 of Ms. Illuminandi and **b** detail of the polysaccharide bands in the 1300–650 cm^{-1} region. The main bands detected are assigned to calcium carbonate (■) and lead white (▲)

when inspecting infrared spectra within this region of interest (Additional File 2: Figs. S4 and S5). A compound with a mesquite gum-like fingerprint was previously identified by our research group in a Portuguese manuscript dated to 1512 (The Charter of *Vila Flor*) [75]; the same type of gum was also observed in books of hours, but not further discussed [11]. Among all spectra of binding media acquired on the Ajuda Songbook pinks, we have selected for display one in which the gum is present in high quantity (Additional file 3: Fig. S5). Nevertheless, even in this case, traces of gypsum were detected, which do not enable a perfect match with the available mesquite gum reference. On the other hand, a pink reconstruction based on recipe 1 of Ms. Illuminandi yielded a very good match that could not be achieved using gum Arabic, Fig. 8. In addition, it must be taken into account that our reference was likely obtained from *Prosopis velutina*, and other possible sources of *Prosopis* exist. Also, processing of the gum may cause a slight alteration of the fingerprint region, but not of its specific band maximum. For this reason, paint reconstructions were prepared using a mesquite gum binder. Several reconstructions were produced using all the additives previously identified, with different relative proportions of brazilwood, lead white, and calcium carbonate. This methodology allowed us to provide a full characterization of the paint formulation using infrared spectroscopy, Fig. 8. Quantities of each ingredient are described in the Experimental section, "Preparation of historical pink reconstructions" paragraph.

Pinks from the musical scenes

Unlike the architectural background, musical scenes are unfinished. Pink colors of five illuminations were studied by infrared spectroscopy and molecular fluorescence (ff. 16, 17, 21, 40v and 59), including three from the loose folios found in Évora (ff. 16, 17, 40v) (Figs. 9 and Additional file 2: Fig. S4,). All these paints share the same binding medium as the architectural background, Fig. 9. On f. 59, gypsum was identified instead of lead white, Fig. 10. A lighter pink was prepared by diluting the lake pigment with gum; the same gum was also used as a glaze to saturate the color (Additional file 3: Table S4 and Fig. S6). In all the other pink colors, calcium carbonate was identified: in f. 17, together with gypsum, while in f. 16 combined with lead white.

The consistency in terms of formulation observed in the light pink paints from the architectural elements is not encountered in the musical scenes. It is important to note that in all illuminations on the loose Évora folios, calcium carbonate is used as a filler: this could be a relevant clue that will be further explored in situ using handheld Raman equipment.

Microspectrofluorimetry and chemometrics

To fully exploit the remarkably high sensitivity of microspectrofluorimetry, allowing the distinction of different chromophore environments (affected by factors like pH, additives, and the presence of alum), a chemometric clustering method was applied to the rose paints. The excitation and emission spectra were inserted in a previously created dendrogram, generated by means of hierarchical cluster analysis (HCA), Fig. 11 [7]. The development and testing of this model, using data from

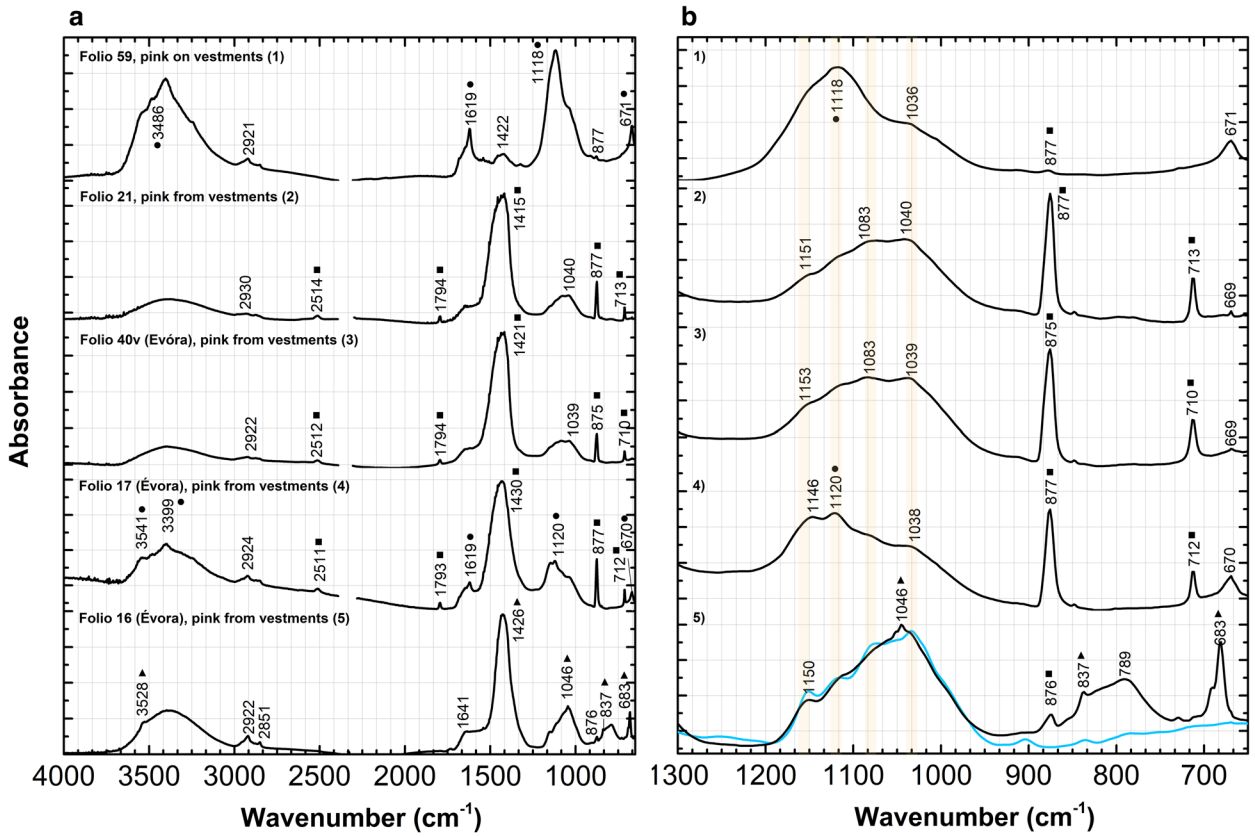

Fig. 9 **a)** Infrared spectra acquired from pink vestments in the illuminations on folios 59 and 21, and three folios from Évora, namely, folios 16, 17 and 40v; **b)** detail of the polysaccharide bands and additives' characteristic signals in the 1300–650 cm^{-1} region. The main bands detected are assigned to gypsum (●), calcium carbonate (■), and lead white (▲). Matching polysaccharide bands among spectra shown in **a)** are marked in **b)** with yellow strips

Fig. 10 Illuminations on folio 59 (*left*) and one of the folios found in Évora, folio 16 (*right*)

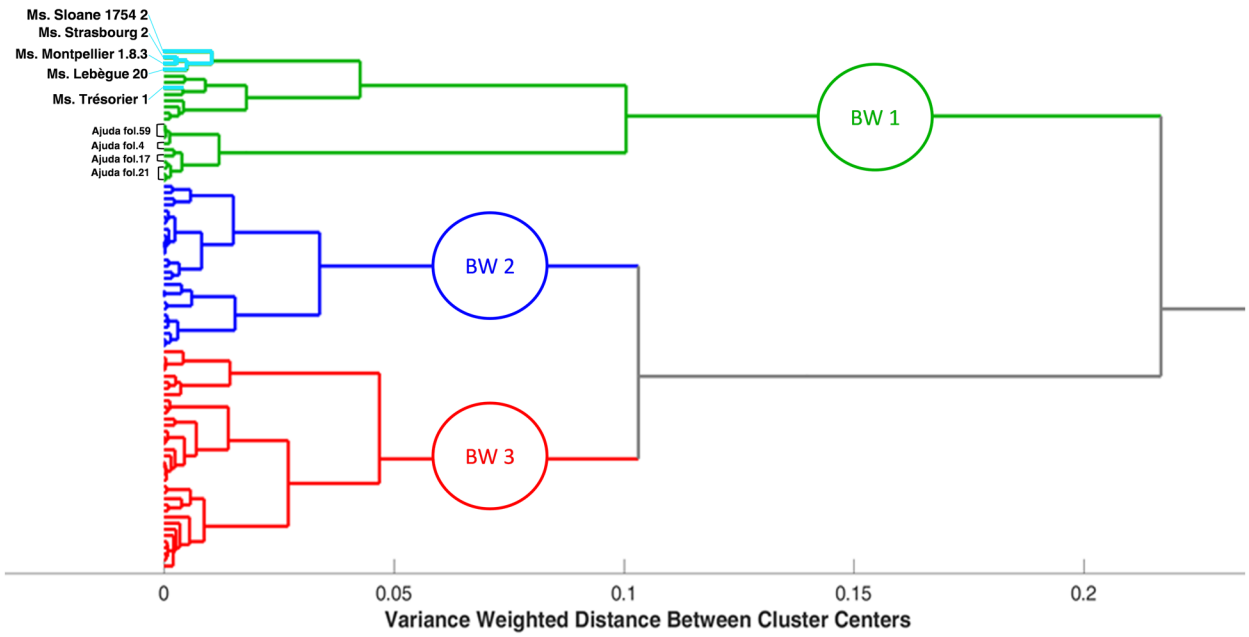

Fig. 11 Dendrogram generated by HCA from the processing of excitation and emission spectra of brazilwood-based paints and historical pink reconstructions prepared using five brazilwood recipes. Three main clusters are evident: Ajuda Songbook (green, BW1), books of hours (blue, BW2), and books of hours (red, BW3). For more details, please see Table 4 and [7]

Medieval manuscripts, have been discussed elsewhere [7]. The statistical treatment of the data allows the disclosure of patterns, uncovering similarities and differences in the molecular profiles, which would be quite difficult, if not impossible, to do if hundreds of spectra were analysed one by one, without a statistical approach. Within the data used, are those acquired from the Ajuda Songbook' pinks allowing us to correctly place the reproductions in comparison with the historical paint.

The dendrogram separates brazilwood paints into three clusters: BW1, BW2, and BW3. Cluster BW1 is composed of paints that contain both lead white and calcium carbonate in their formulation; in BW2 and BW3, paints with calcium carbonate and gypsum are numbered; BW3 includes paints with calcium carbonate and higher amounts of gypsum. In cluster BW1 all the spectra collected from the Ajuda Songbook and folio 60 from the book of hours IL 15 (fifteenth century, France) are represented, while clusters BW2 and BW3 assemble data from brazilwood paints found in books of hours of French or Flemish production. What enables the differentiation among different paints is the presence of specific extenders, which affects the excitation and emission spectra, being detected by this statistical approach.

The rose reconstructions fit in cluster BW1, which is in accordance with their formulation with lead white and calcium carbonate, as do the Ajuda Songbook paints, Fig. 11. Moreover, the dendrogram differentiates between two reds (Ms. Trésorier 1 and Ms. Strasbourg 2) and the translucent rose colors (Ms. Montpellier 1.8.3, Ms. Lebègue 20, and Ms. Sloane 1754 2). Red colors are found at one end of the dendrogram, while translucent rose colors are located next to data from historical artworks.

In fact, Ajuda data are characterized by excitation maxima between 552 and 561 nm, closer to the translucent rose (557 nm) than to the red (550 nm); similarly, emission maxima of the translucent rose (587 nm) are closer to Ajuda values (between 582 and 586 nm) than those of the red colors (602 nm).

Even though the dendrogram shows a great similarity between the reconstructions' formulation and the historical paints, they are located at opposite sides of the cluster, which indicates that the chromophore's environment (i.e. exact recipe) differs from that of the Ajuda Songbook. Nevertheless, this approach clearly highlights a closer proximity of translucent rose colors to the Ajuda Songbook compared to red colors.

Conclusions

Heritage materials such as the Ajuda Songbook's pink paints are highly complex systems of intrinsically heterogeneous composition, often subject to unmonitored long-term changes. The light pinks found in this illuminated manuscript are based on a different dye formulation when compared to colors identified in books of hours. A Medieval color based on a brazilwood lake

pigment can be revealed by combining information from its infrared spectra with molecular fluorescence spectra, Figs. 8 and 11 [6–11]. The former enables one to quantify the binders and additives, as well as to assess their conservation condition [7, 11–13]. Microspectrofluorimetry, on the other hand, allows the acquisition of emission and excitation spectra in the same microscopic spot, *in situ*, which is crucial when using molecular fluorescence for dye identification. These molecular spectra carry essential information on the specificities of the recipe, which can be deciphered by comparison with a database of historically accurate reconstructions and with the aid of chemometrics, as shown in this work. A database of historically accurate reconstructions of brazilwood lake pigments and paints was here created based on a selection of Medieval recipes and paints were fully characterized using colorimetry, reflectance spectroscopy, microspectrofluorimetry, and infrared spectroscopy. New types of extraction methods, based for instance on egg white and acidic solutions, were found, as well as previously unknown additives and processes. Interpreting this information and reproducing these recipes was one of the main difficulties encountered, as already described in previous works, as these can be subjective or omit important details, which makes the comparison with original paints a fundamental step to validate the references. Based on the new reconstructions prepared, we were also able to provide a detailed characterization of the Ajuda manuscript's paint formulation using infrared spectroscopy, with the following results: calcium carbonate as filler; lead white to adjust the hue to a lighter shade; and a polysaccharide-based binder with a fingerprint similar to mesquite gum. While the exact recipe used in the historical manuscript under study is yet to be unraveled, the application of chemometrics to molecular fluorescence spectra showed a high degree of similarity with the paint reconstructions, Fig. 11. Ultimately, the wealth of information gathered from this approach can help address questions related to the location, date, and fabrication technique of the manuscripts under study.

Experimental

Materials and methods

All reagents are analytical grade, with the exception of the *Paubrasilia echinata* brazilwood and gum Arabic in grains from A. Senegal that were acquired from Kremer, as well as mesquite gum (*Prosopis spp.*), 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.

Preparation of historical reconstructions of brazilwood recipes

The following paragraph describes the processes adopted to produce brazilwood reconstructions based on the historical recipes selected.

Ms. Sloane 1754, recipe 1: 50 mL of egg white (pH = 8.9) were added to 5 g of scraped brazilwood. The solution was left to rest for 1 h and then filtered through a thin threaded plastic tissue. The same quantity of alum (5 g) was added to the filtered solution, which was stirred for another hour.

Ms. Sloane 1754, recipe 2: A mixture of 1 g of scraped brazilwood with 50 mL of egg white (pH = 9.1) was left to rest for three days. After that, 1 g of alum was added to the solution that was then stirred for 1 h. After dissolution of the alum, the solution was left to rest for another four days.

Ms. Trésorier, recipe 1: 2.5 g of scraped brazilwood, 1.25 g of alum, and 0.625 g of gypsum were mixed in an agate mortar and then transferred to a glass Erlenmeyer flask. 50 mL of urine (three days, pH = 7.2) were added to the mixture, which was heated to 100 °C and left to boil for 1 h under constant stirring. After boiling, 10 mL of urine were added, and the solution was stirred again for an hour. Then, the solution was poured into a gypsum bowl and left to dry. Even though the recipe does not refer to filtration, when pouring into the bowl, the solution was filtered through a thin threaded plastic tissue to remove any scrapings of brazilwood that might interfere with paint application. The resulting dry lake pigment was then washed twice by adding water and centrifuging, then left to dry again.

Ms. Trésorier, recipe 3: 0.5 g of brazilwood and 0.625 g of gypsum were poured into a glass container with 50 mL of urine (three days, pH = 7.6) and stirred for 1 h. Then, 0.223 g of alum were added to the solution, which was stirred for another hour to dissolve the gypsum and alum, then left to rest for twenty-four hours (close to a window—"put it in the sun"). After that, the solution was filtered through a thin threaded plastic tissue into a gypsum bowl and left to dry. The resulting dry lake pigment was then washed twice by adding water and centrifuging, then left to dry again.

Ms. Illuminandi, recipe 1: A solution of lye was first prepared using 7.5 g of vine ash and 100 mL of water. This solution was heated to 100 °C and left to boil for 30 min. It was then left to rest until reaching room temperature and filtered using filter paper. 50 mL of this lye (pH = 10.5) were added to 5 g of scraped brazilwood. The extraction solution was left to rest for twenty-four hours, then heated to 90 °C (almost until its boiling point) for

one hour under constant stirring. After heating, the extraction solution was filtered through a thin threaded plastic tissue. 5 g of calcium carbonate and 5 g of powdered alum, previously mixed and ground together, were then added. The solution was stirred for one hour to dissolve all the components, then centrifuged to obtain a lake pigment. The resulting product was washed twice by adding water and centrifuging, then left to dry.

Ms. Illuminandi, recipe 2: 50 mL of egg white (pH = 9.1) were added to 5 g of scraped brazilwood, and the resulting mixture was left to rest for three days. Then, a solution of 20% gum Arabic was prepared using 2 g of freshly powdered material in 10 mL of water. The solution was stirred until complete dissolution of the gum Arabic and filtered through filter paper. To accelerate this process, the solution was heated up to 50 °C. 0.5 g of alum was added to the gum Arabic. After dissolving completely, the mixture was added to the initial solution of brazilwood and left to rest for twenty-four hours. After that, the solution was filtered through a thin threaded plastic tissue.

Ms. Lebègue, recipe 20: 5 g of scraped brazilwood and 0.6 g of alum were mixed with 50 mL of egg white (pH = 9.2), stirred for 1 h, then left to stand for twenty-four hours. After that, the solution was filtered through a thin threaded plastic tissue. The resulting ink is ready to use.

Ms. Lebègue, recipe 304: A solution with equal quantities of white wine and water (25 mL) was prepared and to that 5 g of scraped brazilwood were added. The solution was heated to 100 °C and left to boil for 1 h. Then it was filtered through a thin threaded plastic tissue and 0.5 g of alum was added. The mixture was stirred for 1 h to dissolve the alum and, then, 0.34 g of gypsum was added. The solution was stirred for another hour to dissolve all the components, then it was centrifuged to obtain a lake pigment. The resulting product was washed twice by adding water and centrifuging, then left to dry.

Ms. Alphabetum Romanum, recipe 1: 5 g of scraped brazilwood and 0.5 g of alum were poured into 100 mL of vinegar (pH = 2.5) and left to stand for six days. After that, the solution was heated to 100 °C and left to boil until it was reduced by a quarter. The remaining solution was filtered through a thin threaded plastic cloth and 1.5 g of calcium carbonate was added to it. It was left again to rest for two days and, according to the recipe.

Ms. Montpellier, recipe 1.8.2: 10 mL of urine (three days, pH = 7.1) were heated up to 80 °C and placed inside a seashell circa 20-cm wide and 5-cm deep. 1 g of scraped brazilwood was added to the urine and left to stand for twenty-four hours. Then 0.04 g of alum and 10 mL of water were added to the solution, which was then filtered through a thin threaded plastic cloth into a similar seashell. The solution was left to rest until the lake pigment was deposited at the bottom of the seashell, while the supernatant was removed. The lake pigment was washed twice by adding water and centrifuging, then left to dry.

Ms. Montpellier, recipe 1.8.3: 10 mL of egg white, 5 mL of a solution of 20% gum Arabic (prepared via the same method as in Ms. Illuminandi, recipe 2), and 2.5 mL of water were mixed and poured into a seashell circa 20-cm wide and 5-cm deep. 1 g of scraped brazilwood was added to this mixture and the solution was stirred for 1 h. 0.31 g of alum were then added and the solution was stirred again for 1 h.

Ms. Strasbourg, recipe 1: 2.5 g of alum and 2.5 g of calcium carbonate were ground together to a very fine powder, and 2.5 g of scraped brazilwood were added in a glass container. 25 mL of egg white (pH = 9.2) were also added and the solution was stirred for 1 h, at the end of which another 25 mL of egg white were added; the solution was then stirred for another hour and left to stand for eight days. After that, the product was filtered through a thin threaded plastic cloth. The resulting ink can be readily used or be left to dry and, when necessary, dissolved with water to paint.

Ms. Strasbourg, recipe 2: 50 mL of lye (prepared via the same method as in Ms. Illuminandi, recipe1) was heated up to about 50 °C and 5 g of scrapped brazilwood were added to it. The solution was stirred for one hour, maintaining the temperature. Then 0.4 g of alum was added and the solution was stirred for another hour. The resulting solution was filtered through a thin threaded plastic cloth and left to stand overnight. After that, the solution was centrifuged to obtain a lake pigment. The resulting product was washed twice by adding water and centrifuging, then left to dry.

The historically accurate reconstruction paints were applied onto filter paper and parchment, on 2×2-cm circles. Paints produced from recipes that used egg white for the extraction were applied directly, without adding any additional binder, while others that resulted in a dry lake pigment were applied with the aid of a solution of 20% gum Arabic, in relative proportions of approximately 5–7% pigment and 93–95% binder. The historic reconstructions were prepared and measured with each analytical technique at least three times each to assess reproducibility.

Preparation of historical pink reconstructions

The historical pink reconstructions were produced with different relative proportions of brazilwood, lead white, calcium carbonate, and gum Arabic or mesquite

gum as binders. A comparison of the infrared spectra obtained from the reconstructions and the original paints allowed us to propose what quantities were used in the formulation of the original paints, matching with the reconstructions' known quantities. The established quantities in weight were as follows: 74.8% binder, 19.9% lead white, 4.9% calcium carbonate, 0.4% brazilwood paint. Historical pink reconstructions were then produced following this formulation and the brazilwood recipes described above. The reconstructions were applied on parchment and filter paper, on 2 × 2-cm circles.

Colorimetry

L*a*b* coordinates were measured using a Microflash mobile DataColor International colorimeter equipped with a Xenon lamp over an 8 mm-diameter measuring area. The CIELAB system was used, selecting a D65 illuminant and 10° observer. The instrument was calibrated with a white tile and a black trap, and measurements were performed on top of filter paper. The described values are expressed as average value of three measurements, which proved to be sufficient to ensure reproducibility. In the Lab* cartesian system, L*, relative brightness, is represented on the z-axis. Variations in relative brightness range from white (L* = 100) to black (L* = 0). On the red-green y-axis, a* is usually found between − 60 (green) and + 60 (red). On the yellow-blue x-axis, b* ranges from − 60 (blue) to + 60 (yellow). The (a*, b*) pair represents the hue and chroma of the object studied.

pH measurements

pH measurements were carried out with a Sartorius Docu-pHMeter. Calibration was performed with pH4 and pH7 buffer solutions (Panreac).

Micro-Fourier transform infrared spectroscopy

Infrared analysis was performed by means of two different instruments. For the Ajuda Songbook paints, a Nicolet Nexus spectrophotometer coupled to a Continuμm microscope (15 × objective) with an MCT-A detector was used. Spectra were collected in transmission mode, using a Thermo diamond anvil compression cell, on 50 μm^2 areas, at a 4 or 8 cm^{-1} resolution and as a result of 128 scans. For the references, a Bruker Vertex 70 spectrophotometer (Bruker, Billerica, MA, USA) coupled to a Hyperion 3000 microscope (15 × objective) equipped with an MCT-FPA detector was employed. Spectra were collected in transmission mode, using a Thermo diamond anvil compression cell, at a 4 cm^{-1} resolution and as a result of 64 scans. Spectra were acquired in the 4000–650 cm^{-1} spectral range. CO_2 absorption at ca. 2400–2300 cm^{-1} was removed from the acquired spectra. At least three spectra were collected from different sample spots to ensure reproducibility.

Micro-Raman spectroscopy

Raman spectroscopy was carried out using a Horiba Jobin–Yvon LabRAM 300 spectrometer, equipped with a diode laser with 785 nm excitation and a maximum laser power of 37 mW at the sample. The laser beam was focused through a 50 × Olympus objective lens and the spot size is 4 μm. Laser power at the sample surface was between 9.5 and 0.37 mW. No evidence of paint degradation was observed during spectra acquisition. More than three spectra were collected from the same sample to ensure reproducibility, and a silicon reference was used to calibrate the instrument.

Portable Raman spectroscopy

Handheld Raman spectroscopy was carried out with a Raman Mira DS, equipped with a laser emitting light at 785 nm with a maximum power of 100 mW, within a spectral range of 200–2300 nm. This equipment provides a spectral resolution of 8–10 cm^{-1} and features a measuring spot of 0.042–2.5 mm. The detection technique used goes under the name of Orbital Raster Scan (ORS) and involves averaging the signal collected from relatively large sample areas while maintaining the desired resolution. All spectra were acquired with the maximum laser power and averages, varying the integration time according to the target material and working distance (the higher the distance, the higher the acquisition time). A minimum of three spectra were collected from the same sample to ensure reproducibility.

Fiber-Optics Reflectance Spectroscopy

UV–VIS reflectance spectra were obtained with an Ocean Optics, MAYA 2000 Pro reflectance spectrophotometer equipped with single beam optical fibers and a Hamamatsu linear silicon CCD detector collecting spectra in a 200–1060 nm spectral range. The light source is an Ocean Optics HL-2000-HP halogen lamp, with 20-W output and 360–2400 nm spectral range. Analysis was conducted with a 8-ms integration time, 15 scans, 8 box width, and 45°/45° reflection angle to the bearing surface, with a 2-mm spatial resolution. A Spectralon® white reference was used for calibration. While being acquired from 350 to 800 nm in reflectance, spectra are shown as apparent absorbance, A′ = log10 (1/R).

UV–VIS microspectrofluorimetry

Fluorescence excitation and emission spectra were recorded with a Jobin–Yvon/Horiba SPEX Fluorog 3-2.2

spectrofluorometer coupled to an Olympus BX51M confocal microscope, with spatial resolution controlled by a multiple-pinhole turret, corresponding to minimum 2 μm and maximum 60 μm spot, equipped with a 50 × objective. Beam-splitting is obtained with standard dichroic filters mounted at a 45° angle in a two-place filter holder. For a dichroic filter of 570 nm, excitation may be carried out up to about 560 nm and emission collected after 580 nm. Optimization of the signal was performed daily for all pinhole apertures through mirror alignment, following the manufacturer's instructions, using a rhodamine standard (or other adequate references). Fluorescence spectra were corrected only for the wavelength dependence of the excitation source intensity. For the study of red dyes, two filter holders with two sets of dichroic filters are employed: for lac dye a set of 500 and 600 nm, and for brazilwood a set of 540 and 600 nm. This enables both the emission and excitation spectra to be collected with the same filter holder. Emission from a continuous 450-W xenon lamp, providing an intense broad spectrum from the UV to near-IR, is directed into a double-grating monochromator, and spectra are acquired after focusing on the sample (eye view) followed by signal intensity optimization (detector reading). The pinhole aperture that controls the measurement area is selected based on the signal-to-noise ratio. For weak to medium emitters, it is set to 8 μm; however, in this work, for very weak signals, a 30-μm spot was also used (pinholes 5 and 8, respectively) with the following slits set: emission slits = 3/3/3 mm (6 nm bandpass) and excitation slits = 5/3/0.8 mm (final bandpass of 2 nm). Emission and excitation spectra were acquired on the same spot. With our experimental set, usually, excitation spectra are acquired with a higher signal-to-noise ratio than emission spectra.

Spectra were acquired 5 months and 12 months after the paints were applied. Spectra obtained after 5 months are discussed in the main text. Spectra after 12 months are included in Additional file 4, along with a commentary.

Data analysis

Spectral pre-treatments

Excitation and emission spectra were tested both separately and in combination. Each spectrum was pre-processed by normalization to unit area. Spectra obtained from different samples were then arranged vertically to form two data blocks (excitation and emission) and two-way matrices (samples versus wavelength). These data blocks were analyzed individually and in combination. In the latter case, the two blocks were merged by horizontally concatenating the matrices. The best results were achieved through a combination of the excitation and emission spectra. Further details on the selection of pre-processing methods can be found in reference [76].

Chemometric methods

Hierarchical cluster analysis (HCA) was applied to the spectral data towards the discrimination and classification of artworks using different dye families. Dendrograms were developed considering data from the entire artworks' dataset and including the whole wavelength range. The HCA method was applied not directly to the fluorescence excitation and emission spectra, but rather to the result of a principal component analysis of these data. The first three components encompassing slightly more than 95% of the total variance were used by the HCA method. Ward's algorithm was used to perform the clustering approach and the Euclidean distance selected. Prior to the application of all chemometric methods, the datasets were mean centered. All chemometric analyses and data manipulations were performed using Matlab Version 8.6 (R2015b) (The Mathworks, Natick, MA) and the PLS Toolbox Version 8.2.1 (Eigenvector Research, Manson,WA).

List of abbreviations

Illuminated Medieval manuscripts

PNM	Palácio Nacional de Mafra in Portuguese; Mafra National Palace
BNP	Biblioteca Nacional de Portugal; National Library
Ms.22	French book of hours, manuscript 22 (1400–1420) of the collection of Mafra National Palace.
Ms.24	Book of hours used by Autun, manuscript 24 (1420–1470) of the collection of Mafra National Palace.
IL 15	Flemish book of hours, dated *ca.* 1450, BNP collection

Medieval treatises

The book of all colors	*The book on how to make all the color paints for illuminating books*
Ms. Sloane 1754	*Liber de coloribus illuminatorum sive pictorum—Liber de tincturis pannorum*
Ms. Trésorier	*Trésorier de philosophie naturelle des pierres précieuses, quatrième livre*
Ms. Illuminandi	*De arte illuminandi*
Ms. Lebègue	*Experimenta de coloribus*
Ms. Alphabetum Romanum	*Alphabetum Romanum*
Ms. Montpellier	*Liber diversarum arcium*

Other abbreviations

A. senegal	*Acacia senegal*
A.C (C.I.A.D., A.C.)	Laboratory of Biopolymers—Centro de Investigación en Alimentación y Desarrollo
BW	Brazilwood
HCA	Hierarchical cluster analysis
μFTIR	Micro Fourier-transform infrared spectroscopy
HPLC–DAD	High-performance liquid chromatography with diode-array detection
HRMS/MS	High Resolution Mass Spectrometry with high resolution mass spectrometric detection
NMR	Nuclear Magnetic Resonance
μRaman	Micro Raman spectroscopy
SERS	Surface-enhanced Raman spectroscopy
UV	Ultraviolet
UV–VIS	UV–Visible

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40494-023-00863-1.

Additional file 1: Brazilwood paint recipes, color paints, and color coordinates.

Additional file 2: Molecular data on brazilwood paints (infrared and Raman spectra).

Additional file 3: Details of pink vestments from the Ajuda Songbook.

Additional file 4: Fluorescence emission and excitation spectra acquired after 1 year.

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Author contributions
MV, MJM, PN, and FP coordinated the acquisition and analysis of Raman and infrared data as well as colorimetric measurements. PN and MJM coordinated the acquisition and interpretation of the molecular fluorescence measurements. The selection of Medieval treatises and recipes was coordinated by MV and RJDH, with the collaboration of MJM and PN. All authors contributed to the content, revision, and approval of the article's final version.

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Availability of data and materials
All data generated during this study are either included in this published article or available from the corresponding author upon reasonable request.

Declarations

Competing interests
The authors declare that they have no competing interests.

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