



Plant and animal molecules non previously identified in Maya mural paintings: First results from Acanceh

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ABSTRACT

The debate regarding the Maya painting technique has been extensive since its discovery. Initially, due to their similarity to the Pompeian frescoes, these paintings were mistakenly referred to as "Maya fresco wall paintings". However, archaeometry studies applied to the knowledge of pre-Hispanic Maya murals have frequently detected organic binders, which indicates that Maya painters mainly applied dry techniques in their development. But what organic binders did these artists use for this purpose? After many decades of optimizing physicochemical protocols to detect them, the results are only conclusive for a few plant-based binders. Previous analytical methods applied for other authors (Magaloni, 1998a, 2001; Vázquez de Ágredos Pascual, 2006; Guasch-Ferré, 2016; Guasch-Ferré et al., 2019) have ranged from stratigraphic observation using optical microscopy to more advanced techniques focusing mainly on plant-derived sugars. This article describes the results obtained by applying a methodology for the extraction of medium polarity organic components using Gas Chromatography-Mass Spectrometry (GC-MS) to Maya mural paintings. This methodology has identified new molecules suggesting plant and animal origin in mural paintings remains from Acanceh, dating from the Late Classic Period (ca. 600–900). These results not only provide new evidence on pictorial binders, but also invite replication of this study on other murals from the Maya area to expand our knowledge of painting technique of these works, which are of great interest to archaeology, art history, conservation and restoration sciences.

1. Introduction

For centuries, Maya architecture has captivated observers with its grandeur, diverse forms, and remarkable beauty, showcased through intricate artistic elements such as sculptural details, modelled stuccoes and vibrant mural paintings. However, the architecture looks very different today, as much of the original colours have been lost. Painting buildings not only served to protect the surfaces and accentuate the ornaments but also imbued them with meaning and conveyed messages. Despite the fact that the external facades may have been painted with red stucco, other colours such as blue, yellow or green were also used.

The interplay shapes, lines, volumes and colour gave rise to a distinct visual language. This underscores the importance of studying the application of colour (Staines, 1999).

The technical, plastic, and aesthetic quality of the Maya architectural claddings, particularly the mural paintings, was initially compared to the claddings of other great cultures such as the Romans, who used the fresco technique. As a result, it was believed that the Maya also painted using fresco. Scholars such as Tentori (1961), Villagra (1949) or Hanau et al. (1966) proposed the use of fresco, while others like Ruppert et al. (1955) suggested the use of dry painting, and Breton (1906) and Morley (1925) proposed a mixed technique. Over time, the debate was expanded with the

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study of historical sources such as Sahagún (1981) and Hernández (1943), who mentioned the use of gums and plants for stuccos and colour application. Finally, the application of archaeometry techniques allowed the identification of organic elements both in the stuccos and in the pictorial layers. For example, Hansen et al. (1995) identified organic components in the stuccos of Nakbé. Particularly significant were the studies employing gas chromatography coupled to-mass spectrometry (GC-MS) and high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS) studies carried out by Magaloni (1996, 1998a, 1998b, 2001, Magaloni et al., 1995a, 1995b), Vázquez de Ágredos (2006, 2007, 2008, 2010) and Guasch-Ferré (2016; Guasch-Ferré et al., 2019). These analyses made possible to demonstrate the use of plant-derived components focusing on the identification of sugars. Although the same authors proposed the use of animal fats, the methodology employed did not allow for their identification. Consequently, this hypothesis was dismissed in favour of the successfully identified plant-base components. However, Thompson (1932) through his ethnohistorical research, proposed that the ancient Maya may have used animal binders, specifically pheasant eggs whites, to produce blue colour.

To further explore these hypotheses, studies have focused on various Maya sites where polychrome stucco remains have been preserved. One such site is Acanceh, located in the Northern Maya Lowlands (Fig. 1). Its occupation dates back to the Middle Preclassic period (900 BCE–300 BCE AD) and continued through the Classic (250–950 CE) into the Postclassic (950–1541 CE). The site held great political significance, maintaining connections with major Maya cities of Yucatán, in the Northern Maya Lowlands. Spanning approximately 3 km², it contained

over 300 archaeological structures, several of which were monumental in scale. Nevertheless, many of these structures have since fallen into disrepair. Despite this, traces of painted stucco have been identified in multiple buildings. Polychrome remains in red, black, and yellow, dating to the Late Preclassic period, have been found on the main pyramid. From the Classic period, the Palace of the Stuccos (Fig. 2) stands out as the best-preserved example, featuring a modelled and polychrome frieze with red, yellow, green, and blue hues (Quintal, 1999). The frieze was discovered in 1906 during the dismantling of an ancient structure by the inhabitants of the site. Shortly afterwards, in 1908, the first scientific report was made by Adela Breton and since then the frieze has undergone several archaeological, stylistic and iconographic documentation and studies due to its eclectic style (Miller, 1991). Recently, within the framework of the Acanceh Project, led by archaeologist Beatriz Quintal, it was decided to apply a synthetic polymer to consolidate the pictorial layers as a conservation measure.

Vázquez de Ágredos (2006) and Guasch-Ferré (2016) have conducted studies on the organic components in the Acanceh stuccos, both in the pictorial layers and in the mortars. Initially, the identification of indigoid compounds related to the indigo dye used in the blue colour was achieved through pyrolysis/sililation and GC-MS (Py-GC-MS) (Doménech et al., 2014) and Microparticle Voltammetry (VMP) (Doménech et al., 2007, 2011). These studies also identified indigoid components in yellow samples from the site (Doménech et al., 2011). Subsequently, sugars of plant origin were also identified, being possibly used as binders in the pictorial layer and as an aggregate in the mortars (Guasch-Ferré et al., 2019).



Fig. 1. Location of Acanceh, indicated with a red point. Modified from Gendrop and Heyden (1989).

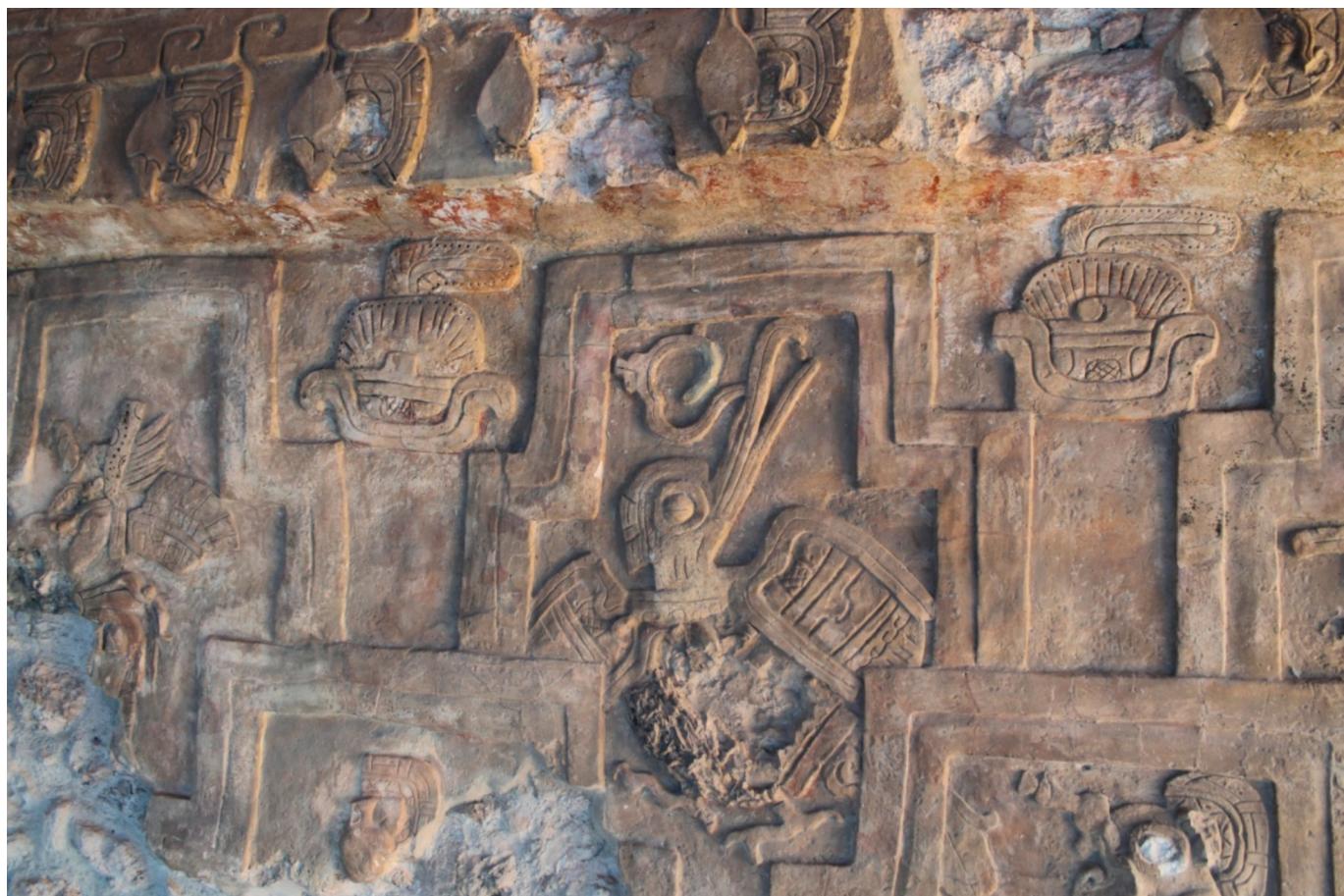


Fig. 2. Photograph of the stucco surfaces showing the loss of its original colour. Image courtesy of the Acanceh Modeled Stucco Conservation Project 2016.

Unlike previous analyses that focused on identifying sugars and Maya blue dye, this article aims to uncover medium polarity components in the materials used as binders within the pictorial layer of the polychrome frieze at the Palace of the Stuccoes in Acanceh. This type of compounds has not been identified in Maya mural paintings so far. This was the first time that this methodology was applied in Maya pictorial layers with the aim of identifying a broad spectrum of compounds of both plant and animal origin. Furthermore, the identification of these organic compounds was carried out using a sensitive technique, as GC-MS, with samples taken from areas of different colours, which led to the detection of cholestanol derived steroids, products from animal metabolism, and used exclusively in the recipe of the blue and green colour beautifully applied in the polychrome frieze of Acanceh. Additionally further lipids and fatty metabolites from plant origin were identified in the remaining colours confirming the presence of organic binders in their preparation.

2. Materials and methods

2.1. Materials and samples

Samples (Fig. 3) were sourced from the databases of two institutions and did not contain synthetic polymers, as they were stored prior to the application of any conservation treatments. Four samples with different colours from the Palace of the Stuccoes (Classic Period) were selected from the mural painting and architectural finishes database of the Laboratory of Analysis and Diagnosis of Works of Art of the Universitat de València (UV). Moreover, two samples from the database of the Conservation Section of the National Institute of Anthropology and History (INAH Yucatán) were also analysed: a blue and a red sample

which may have originated from the same palace. Both samples were collected during excavations carried out by archaeologist Beatriz Quintal and were initially stored in the site camp. They were subsequently rescued and incorporated into the colour sample base of the INAH Yucatán. The blue sample most likely came from the Palace of the Stuccoes of the Classic period, as the Pyramid of the Masks does not present this colour. However, it remains uncertain whether the red sample comes from the masks or from the polychrome frieze. The samples were selected based on the range of colours tones visible on the frieze of the Palace of the Stuccoes. Following the principle of minimal intervention, one sample was selected for each distinct colour tone observed. A summary of the samples is presented in Table 1.

2.2. Methods: Extraction of medium polarity metabolites

A methodology was developed for this purpose. To obtain the sample powder, mechanical cleaning was performed using a soft-bristle brush. Abrasion was used to extract the powder, yielding between 2 mg and 14 mg depending on material. The powder obtained was placed in glass vials containing 300 µl of Sigma® deuterated chloroform and subjected to an ultrasonic bath for 15 min. Afterwards, the samples were left to precipitate, and the supernatant was pipetted and placed in a Sigma® chromatographic vial equipped with 200 micro litre inserts.

Gas Chromatography Mass Spectrometry (GC-MS) was selected as the analytical technique. The equipment used was an Agilent chromatograph (CG 7860) coupled with a 5977B mass spectrometers. The column was an HP-5 ms 30 m x 0.25mm x 0.25 um (Agilent, Santa Clara, Ca., USA). The oven temperature was programmed to start 50 °C (held for 1 min), increase at 7 °C per min up to 300 °C and then remain

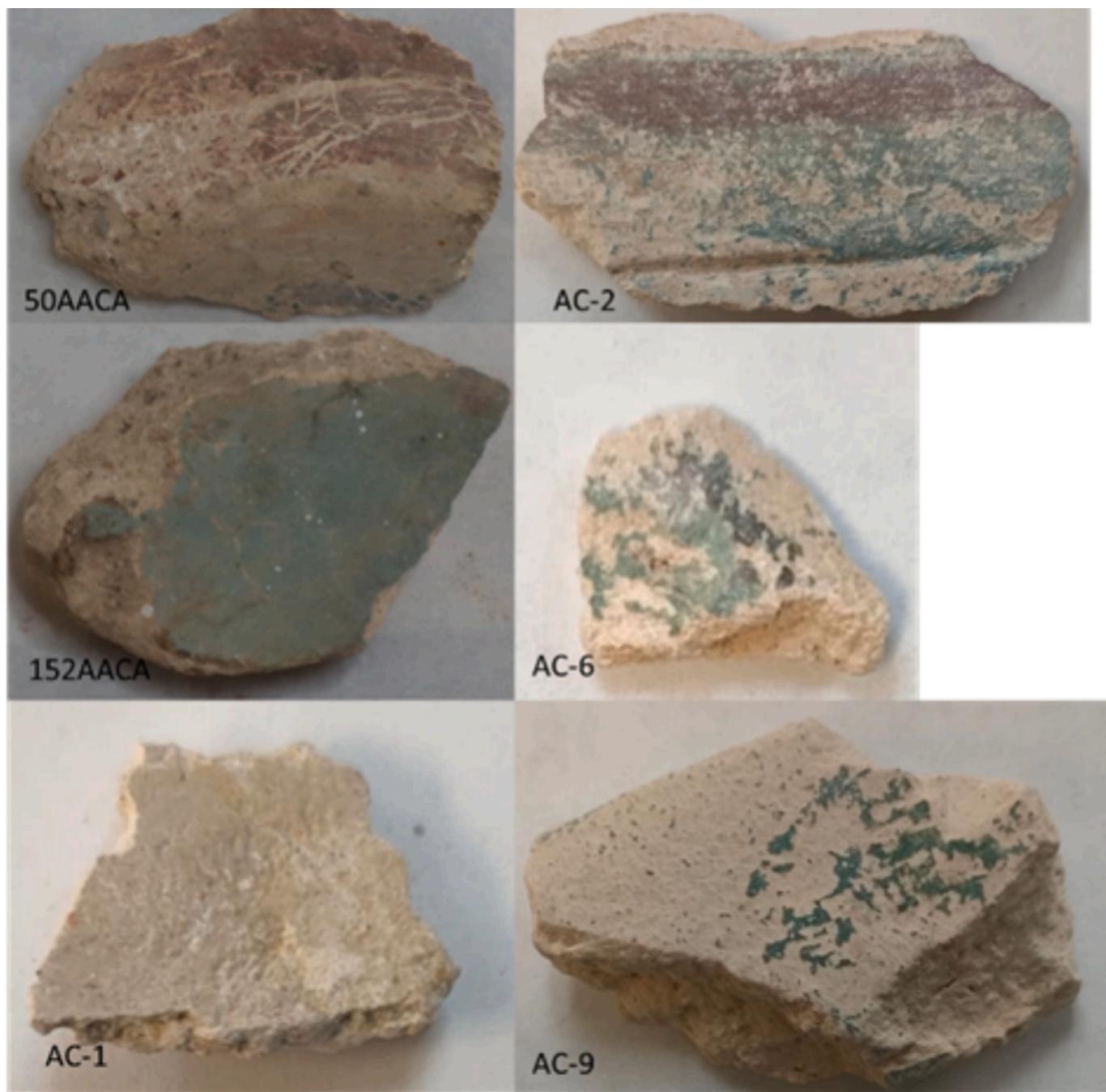


Fig. 3. Photographs of the different colour sampled, along with their corresponding sample codes.

constant for 3 min. The mass detector response in electron impact mode was generated at 70 eV and monitored in TIC SCAN (Total Ion Current) format from 50 to 650 m/z . Helium was used as the carrier gas at flow rate of 1 mL/min. Chromatographic peaks were assigned by comparing the resulting mass spectral with the NIST 2017 library and previously published mass spectra.

Only compounds with at least 70 % similarity to known mas fragmentation patterns were selected, and each compounds fragmentation profile was verified. The identified compounds were listed in an Excel spreadsheet along with their percentage (Table 2). Additionally, the selected molecules were searched in the PubChem Compound Summary database of the US National Center for Biotechnology Information to determinate their origin retrieve their CID (compound ID). If a compound lacked a CIG, additional references were consulted to gather the

necessary information.. This information was recorded in a table relating the compound with the type of molecule and the origin (Table 3). Finally a summary table was created to associate each identified compound with its corresponding sample, allowing for the observation of their distribution by colour (Table 4).

3. Results

GC-MS analysis has enabled the identification of various organic molecules, with the most representative being monoterpenes and medium-polarity compounds such as lipids. The identified molecules are listed in Table 3. It is important to note that the results of the analyses not only allowed the identification of molecules with clear structures and defined fragmentation patterns, but also compounds exhibiting

Table 1

Summary of the analysed samples, indicating colour, sample code, associated institution, origin, chronological period, and quantity of material obtained. Table by the authors.

LABEL	COLOR	SOURCE	STRUCTURE	PERIOD	WEIGHT (mg)
50AACA	Red	INAH	Unknown	Unknown	5
152AACA	Blue	INAH	Palace of the Stuccoes	Classic	3
AC1	Yellow	Universitat de València	Palace of the Stuccoes	Classic	12
AC2	Blue	Universitat de València	Palace of the Stuccoes	Classic	4
AC6	Black	Universitat de València	Palace of the Stuccoes	Classic	2
AC9	Green	Universitat de València	Palace of the Stuccoes	Classic	14

similar fragmentation patterns and structures, such as isomers. Examples include pinene ((1R), α -pinene and β -pinene); cymene (o-cymene and p-cymene) or limonene (D-limonene). In addition, some compounds identified may have resulted from degradation over time or from natural oxidation and reduction processes.

Table 3 shows the different classes of identified molecules: styrene is classified as an aromatic hydrocarbon, while other compounds belong to the group of unsaturated hydrocarbons known as alkenes (heneicosene, heptadecene, pentadecene and decene). The largest group are the terpenes (monoterpenes and triterpenes) such as γ -terpinenene, pinene, carene, sabinene, cymene, carvacrol, limonene, squalene and supraene. Finally, there are some fatty metabolites such as lipids (cholestane), fatty acids (octadecenoic acid, erucic acid, oleic acid, *cis*-vaccenic acid) and fatty alcohols (pentadecanol and heptadecanol).

4. Discussion

The compounds identified and listed in the previous section, (**Table 3**) constitute the main group of essential oils. Essential oils can be extracted from plants in temperate and warm countries, such as tropical forests. The compounds of these oils can be synthesized in all parts of the plants (flowers, leaves, stems, seeds, roots and bark). Although some compounds are more characteristic of some species or families of plants, they can also appear in other plants (Bakkali et al., 2008; Grassmann and Elstner, 2003). For example, the name pinene was given by association with several species of *Pinus*, in which this compound had been very well identified. However, this product is very widely distributed in nature, especially in conifers (Simonsen, 1957) but also in other species, such as *Juniperus comitana*, *Juniperus gamboana*, and *Juniperus standleyi*, which are distributed throughout southern Mexico and Guatemala (Adams et al., 1985). The identification of medium polarity metabolites (**Table 3**), such as aromatic components, in archaeological samples after such a long time can be explained in different ways, with the most plausible being the use of clays and pigments capable of absorbing organic molecules into their structure. Clays like palygorskite and montmorillonite can absorb the indigo molecules used to produce Maya blue (Doménech et al., 2007). In this case, absorption is enhanced by the tabular structure of the clay. For other colours, clays identified in some of the analysed samples by Vázquez de Ágredos (2010) may perform a similar function. This supports the hypothesis that organic binders of plant origin may have contributed to the dyeing process, potentially explaining the ability to paint large architectural surfaces with minimal among of pigments. Furthermore, recent studies have shown that interior construction materials, such as plaster and certain paints, are capable of absorbing limonene (Thevenet et al., 2021).

As shown in **Table 4**, most of the samples, except for 152AACA, show a large volume of aromatic compounds derived from plant the essential oils, with limonene, pinene, carene and cymene being particularly noteworthy. This can be seen in the chromatographic profiles of the

Table 2

Identified metabolites and their percentage of similarity based on mass fragmentation spectra, as compared with those reported in the NIST 2017 library. Table by the authors.

COMPOUND	50AACA	152AACA	AC-9	AC-6	AC-2	AC-1
Styrene	93 %	—	93 %	93 %	93 %	93 %
β-Ocimene	—	89 %	—	—	—	—
Octadecenoic acid	84 %	—	—	70 %	—	—
9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	84 %	25 %	—	70 %	70 %	90 %
6-Octadecenoic acid	84 %	44 %	—	—	83 %	72 %
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	96 %	—	96 %	96 %	96 %	96 %
Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl-	94 %	—	94 %	96 %	38 %	91 %
α-Pinene	94 %	—	—	94 %	93 %	91 %
β-Pinene	90 %	—	87 %	87 %	81 %	81 %
2-Carene	—	86 %	—	—	—	—
3-Carene	90 %	—	91 %	91 %	87 %	90 %
(+)-4-Carene	81 %	86 %	83 %	91 %	90 %	83 %
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	87 %	—	76 %	64 %	—	76 %
γ-Terpinene	97 %	—	97 %	97 %	97 %	97 %
α-Cymene	95 %	—	94 %	94 %	94 %	94 %
p-Cymene	93 %	—	87 %	93 %	94 %	93 %
D-Limonene	97 %	70 %	97 %	97 %	97 %	97 %
Limonene	94 %	—	97 %	94 %	97 %	94 %
1-Decene	93 %	—	95 %	87 %	94 %	95 %
1-Undecanol	91 %	—	—	—	91 %	91 %
1-Dodecanol O dodecene	91 %	—	—	91 %	93 %	91 %
n-Pentadecanol	91 %	—	91 %	91 %	91 %	91 %
1-Pentadecene	91 %	60 %	—	91 %	91 %	—
Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	83 %	—	—	72 %	87 %	—
n-Heptadecanol-1	91 %	—	—	91 %	—	91 %
1-Heptadecene	—	93 %	—	87 %	90 %	95 %
Phenol, 2,5-bis(1,1-dimethylethyl)-	90 %	—	—	90 %	94 %	93 %
Erucic acid	90 %	—	—	55 %	60 %	64 %
Oleic Acid / <i>cis</i>-13-Octadecenoic acid.	81 %	—	—	—	87 %	64 %
Squalene	98 %	—	—	98 %	99 %	89 %
Supraene	97 %	—	—	93 %	94 %	91 %
Heneicosane	—	—	—	—	—	97 %
10-Heneicosene (c,t)	78 %%	—	—	25 %	94 %	—
Thiocyanic acid, 5. alpha.-cholestan-3. beta.-yl ester	—	—	90 %	90 %	—	—

(continued on next page)

Table 2 (continued)

COMPOUND	50AAC	152AAC	AC-9	AC-6	AC-2	AC-1
Cholesta-4,6-dien-3-ol, (3. β)-	—	—	—	—	93	—
					%	
Cholesta-3,5-diene	—	96 %	—	—	—	—
Cholesterol	—	91 %	—	—	—	—

samples (Table 4; Figs. 4–9), where limonene is the most clearly visible compound. The use of organic substances of plant origin in the pictorial layers had already been proposed for mural painting by Magaloni (1996, 1998b, 2001; Magaloni et al., 1998a) and subsequently confirmed by Vázquez de Ágredos (2006, 2007, 2008, 2010). While identification of organic compounds by the mentioned authors (Magaloni 1996, 1998a, 1998b; Vázquez de Ágredos 2006, 2007, 2008, 2010; and Guasch-Ferré et al., 2019)) focused on the analysis of sugars, the methodology employed in this study also enabled the detection of essential oil molecules, thereby suggesting their use in mural painting. However, the analyses conducted do not provide conclusive evidence regarding whether these molecules originated from a resin, a gum or an aqueous extract, such as one obtained by immersing bark in water and using the enriched water for painting. Further research is required in this area, including comparisons with extracts of plants local plant species, which would help to identify specific metabolites or marker molecules unique to each species.

The identification of fatty compounds warrants closer attention. Based on the information available in the PubChem database, it can be concluded that most of the identified fatty acids (Table 2) originated from both animal and plant sources, while others are exclusively plant or animal derived. For example, undecanol (Burdock, 1997; Zhang et al., 2021), octadecenoic acid (Patti et al., 2021), oleic acid (Nutter et al., 1943; Lalman and Bagley, 2001), pentanoic acid and heptadecanol (Pfeuffer and Jaudszus, 2016; Kumari et al., 2024; Pradhan and Dubey, 2021) are among the compounds identified. Other fatty components, such as erucic acid (Vetter et al., 2020; Wang et al., 2022), are of plant origin. Cholestane lipids and their derivatives (cholesta-3,5-diene and thiocyanic acid, 5. α -cholest-3. β -yl ester) (Derewiaka, 2019) are exclusive to animal products. In addition to cholesterol, two triterpenes (squalene and supraene) were also identified. These compounds are primarily of animal origin, although they can also be derived from plants (Lozano-Grande et al., 2018) and served as intermediate metabolites in the synthesis of cholesterol (Popa et al., 2015).

Furthermore, the analysis of these samples highlights the importance of studying stored materials as a consequence of the cultural and heritage loss caused by the rapid deterioration of the friezés colours.

Since this is the first time they have been detected in paint layers of architectural finishes from the Maya region, their presence opens up a new and important line of research. The results obtained not only validate the methodological approach application of this methodological proposal used for the identification of binders in Maya mural paintings, but also serve as a starting point for expanding the analysis of these works in the Maya area. Cholesterol, cholesta-3,5-diene and cholesta-4,6-dien-3-ol, (3 β)-, compounds of animal origin, have been identified specially in the blue-coloured samples (152AAC and AC-2) (Table 4; Figs. 5 and 7). This could be interpreted as an intentional use of animal fat in a particular colour, for the blue colour. The use of an animal-based-binder was previously suggested by Thompson (1932), who through ethnohistorical sources, documented that the ancient Maya may have used animal fats, particularly proposed pheasant egg whites for the colour blue. The identification of fats in Acanceh does not confirm the use of eggs but suggest the use of some animal binder. The almost exclusive use of animal fat in blue may have a symbolic explanation that it is not yet fully understood. There is also a practical explanation: in order to produce the blue shade, the dye must be heated with the clay. This heating process could improve workability of the binder in

Table 3

Medium polarity metabolites identified in coloured samples by GC-MS, including molecular class and common sources. The information was primarily obtained from the PubChem database from National Library of Medicine, National Center for Biotechnology Information (USA). Table by the authors.

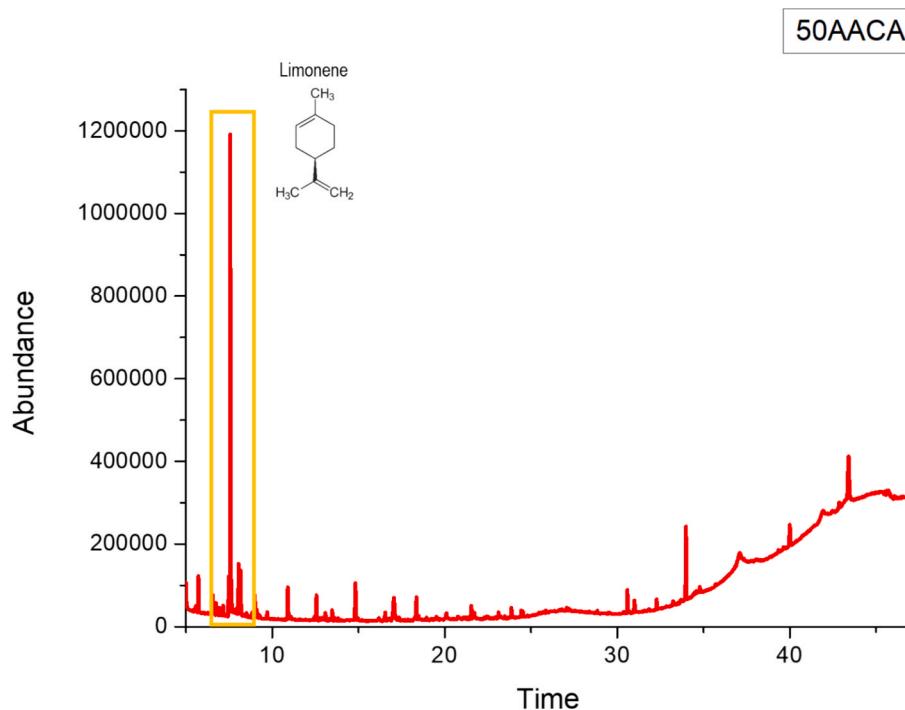
COMPOUND	TYPE OF MOLECULE	SOURCE
Styrene	Aromatic hydrocarbon	Plants, Altingiaceae family
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	Monoterpene	Plant with perennial flowers of the Valeria officinalis.
Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl-	Monoterpene	Stereoisomer of α -pinene. Plants. Stereoisomer of α -pinene
α -Pinene	Monoterpene	Plants
β -Pinene	Monoterpene	Plants
2-Carene	Monoterpene	Plants
3-Carene	Monoterpene	Plants
(+)-4-Carene	Monoterpene	Plants
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	Monoterpene	Plants. As known as sabinene.
γ -Terpinene	Monoterpene	Plants
β -Ocimene	Monoterpene	Plants
α -Cymene	Monoterpene	Plants
p-Cymene	Monoterpene	Plants
Carvacrol, TBDMS derivative	Monoterpene	Phenol naturally occurring monoterpene derivative of cymene
Limonene	Monoterpene	Plants
D-Limonene	Monoterpene	Plants
1-Undecanol	Fatty alcohol	Plants (fruits) and animals (eggs, butter and cooked pork).
Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	Fatty acid ester	Perennial flowering plant of Valeria officinalis.
Octadecenoic acid	Fatty alcohol	Fatty acid of animal and vegetable origin. Also named stearin acid.
6-Octadecenoic acid	Fatty alcohol	Fatty acid of animal and vegetable origin
9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	Fatty alcohol	Fatty acid of animal and vegetable origin
Erucic acid	Fatty alcohol	Plants, family Brassicaceae
Oleic Acid	Fatty alcohol	Animal and plants
n-Heptadecanol-1	Fatty alcohol	Saturated fatty acid, plant or animal
n-Pentadecanol	Fatty alcohol	Plants
1-Pentadecene	Alkene	Mammalian metabolite, also identified in plants
1-Heptadecene	Alkene	Saturated fatty acid, plant or animal
10-Heneicosene (c,t)	Alkene	Plants
Phenol, 2,5-bis(1,1-dimethylethyl)-	Triterpene	Phenol, plant metabolite and mammalian metabolite
Squalene	Triterpene	Mainly animal, may also come from vegetables. Intermediate metabolite in the synthesis of cholesterol.
Supraene	Triterpene	Mainly animal, may also be derived from plants. Product of biosynthesis. Squalene Stereoisomer
Thiocyanic acid, 5. α -cholest-3. β -yl ester	Lipid	Animal
Cholesta-4,6-dien-3-ol, (3. β)-	Lipid	Animal
Cholesta-3,5-diene	Lipid	Animal
Cholesterol	Lipid	Animal

combination with the colour. Furthermore, the use of fat could be extended to green (Table 4; AC-9) (Fig. 9) and black (Table 4; Fig. 8). The same colour technology for green was used for its production and the compound Thiocyanic acid, 5. α -cholest-3. β -yl ester was

Table 4

Molecules identified in relation to the analysed samples. Table by the authors.

COMPOUND	50AACa (red)	152AACa (blue)	AC-9 (green)	AC-6 (black)	AC-2 (blue)	AC-1 (yellow)
Styrene	x	—	x	x	x	x
(1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-ene	x	—	x	x	x	x
Bicyclo [3.1.1] hept-2-ene, 3,6,6-trimethyl-	x	—	x	x	x	x
α -Pinene	x	—		x	x	x
β -Pinene	x	—	x	x	x	x
2-Carene	—	x	—	—	—	—
3-Carene	x	—	x	x	x	x
(+)-4-Carene	x	x	x	x	x	x
Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)-	x	—	x	x	—	x
I-Terpinene	x	—	x	x	x	x
β -Ocimene	—	x	—	—	—	—
o-Cymene	x	—	x	x	x	x
p-Cymene	x	—	x	x	x	x
Carvacrol, TBDMS derivative	x	x	—	—	x	x
Limonene	x	x	x	x	x	x
1-Undecanol	x	—	—	—	x	x
Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	x	—	—	x	x	—
Octadecenoic acid	x	—	—	x	—	—
9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	x	x	—	x	x	x
6-Octadecenoic acid	x	x	—	—	x	x
Erucic acid	x	—	—	x	x	x
Oleic Acid / cis-13-Octadecenoic acid.	x	—	—	—	x	x
n-Heptadecanol-1	x	—	—	x	—	x
n-Pentadecanol	x	—	x	x	x	x
1-Pentadecene	x	x	—	x	x	—
1-Heptadecene	—	x	—	x	x	x
Squalene	x	—	—	x	x	x
Supraene	x	—	—	x	x	x
Heneicosane	—	—	—	—	—	x
10-Heneicosene (c,t)	x	—	—	x	x	—
Thiocyanic acid, 5 α -cholest-3 β -yl ester	—	—	x	x	—	—
Cholesta-4,6-dien-3-ol, (3 β)-	—	—	—	—	x	—
Cholesta-3,5-diene	—	x	—	—	—	—
Cholesterol	—	x	—	—	—	—

**Fig. 4.** Gas chromatogram of the chloroform extract from sample 50AACa. Limonene, the main aromatic compound in this extract, is marked in orange and its chemical structure is shown. Source authors.

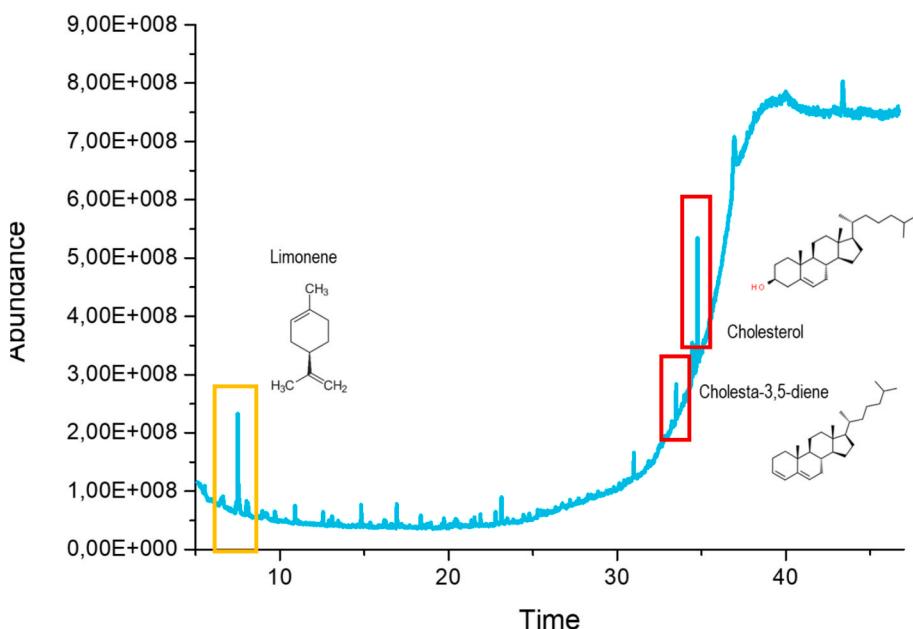


Fig. 5. Gas chromatogram of the chloroform extract from sample 152AACa. Limonene, the main aromatic compound in this extract, and cholestanone compound are marked in orange and red. Their chemical structure are also shown. Source authors.

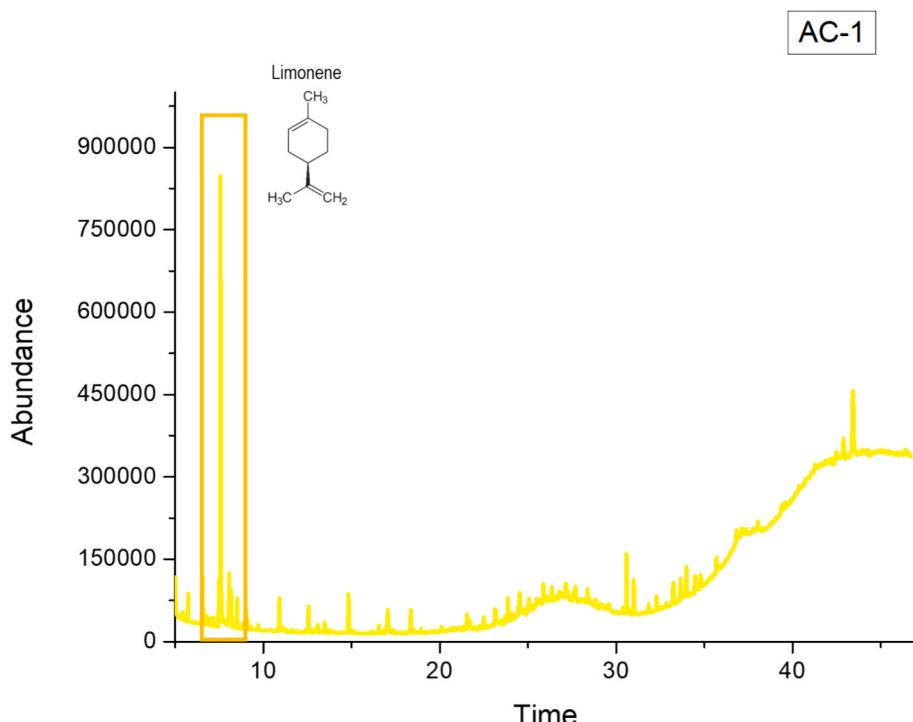


Fig. 6. Gas chromatogram of the chloroform extract from sample AC-1. Gas chromatogram of the chloroform extract from sample 50AACa. Limonene, the main aromatic compound in this extract, is marked in orange and its chemical structure is shown. Source authors.

identified. This technical similarity goes beyond the share use of the same dye, the ancient Maya also gave both colours the same name 'Yax' (Houston et al., 2009). The appearance of the compound in black may be explained by the superposition of black over blue during the application of colour, as can be seen on the surface (Fig. 3). At the time of obtaining the black colour, it was not possible separate black from blue. Thus, it could explain why this compound appears in black analysis.

Regarding other warm-range colours such as red (50AACa) (Table 4; Fig. 4), yellow (AC-6) (Table 4; Fig. 6) and black (AC-1) (Table 4, Fig. 8),

several fatty compounds are present. Nevertheless, these compounds are not exclusively of animal origin; they can also originate from plants. Notably, squalene and supraene, found in red (50AACa), black (AC-1), blue (AC-2) and yellow (AC-6), can be of both animal and plant origin and are part of the cholesterol metabolic pathway. This suggests that, while the use of fat in these colours is possible, we cannot definitively conclude that animal fats were used as binders, as we can in the case of the blue and green colours.

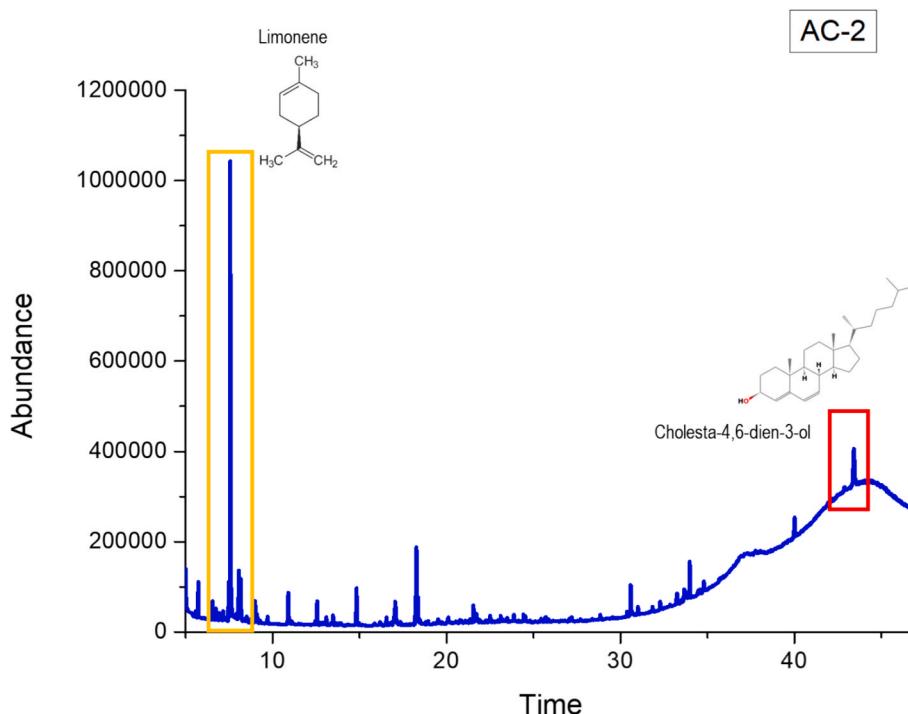


Fig. 7. Gas chromatogram of the chloroform extract from sample AC-2. Limonene, the main aromatic compound in this extract, and cholestanane compound are marked in orange and red. Their chemical structure are also shown. Source authors.

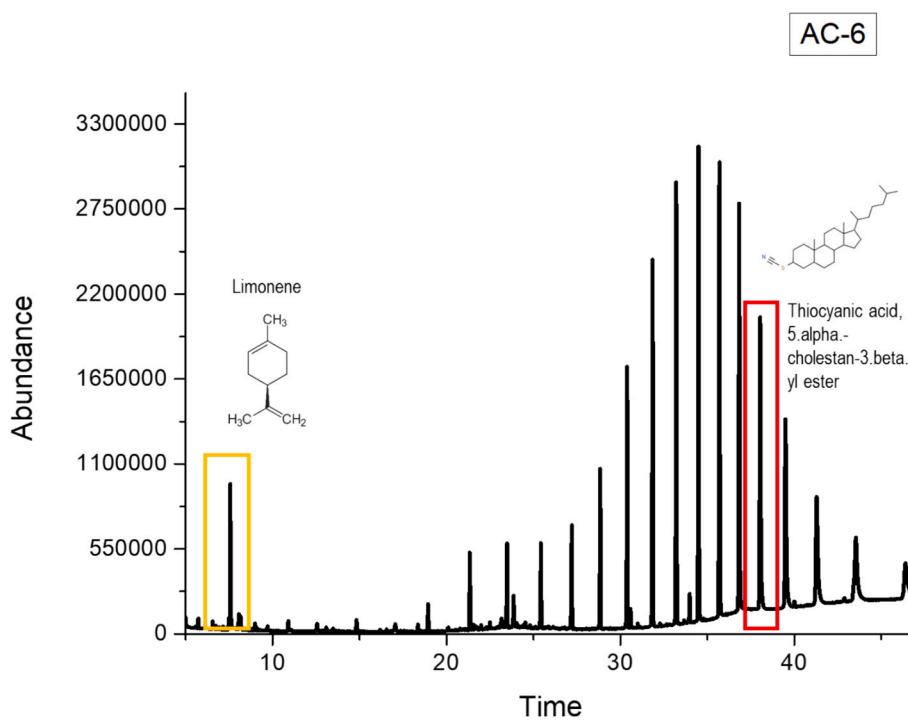


Fig. 8. Gas chromatogram of the chloroform extract from sample AC-6. Limonene, the main aromatic compound in this extract, is marked in orange and its chemical structure is shown. Source authors.

5. Conclusions

This study highlights the scientific value of re-examining previously analysed samples, employing different methodologies and objectives that build upon the information from earlier analyses. The methodology used for the study of the Acanceh samples enabled the detection of

additional classes of metabolites derived from plant or animal fats. The identification of these compounds suggests their use as binders in the pictorial layers of various colours, a finding not previously reported. Furthermore, the analyses of the materials by colours, appears to indicate a preference for the use of animal binders in the blue and green colours in Acanceh. This could represent either an isolated case, or on

AC-9

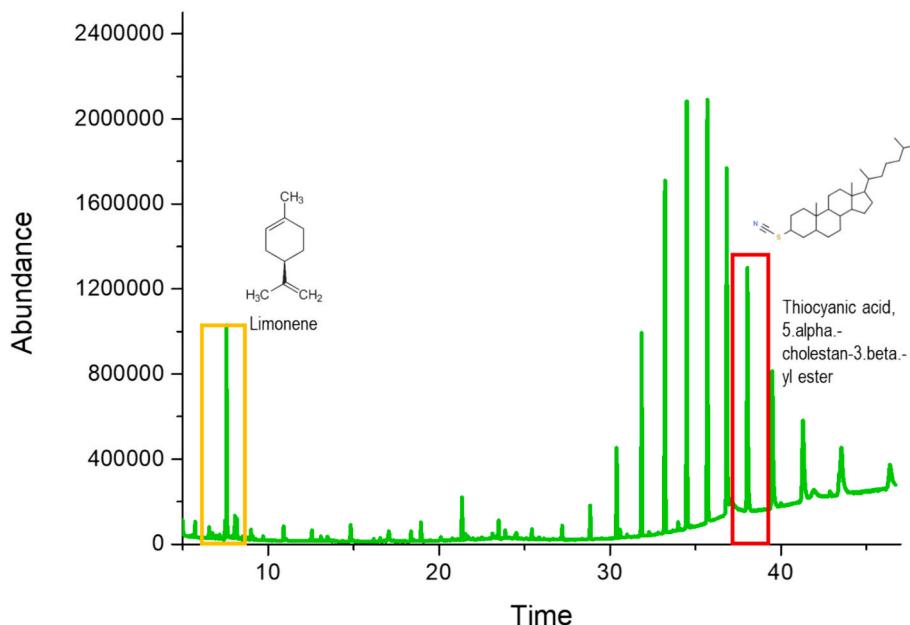


Fig. 9. Gas chromatogram of the chloroform extract from sample AC-9. Limonene, the main aromatic compound in this extract, is marked in orange and its chemical structure is shown. Source authors.

the contrary, new evidence withinin the technical tradition of mural painting in the Maya Lowlands, which, according to authors such as Magaloni (1996) or Vázquez de Ágredos (2006, 2007), changed very little over time. Therefore, this study opens much needed line of research in the field of colour archaeometry in Maya mural painting, of interest of various disciplines, including archaeology, art history, materials science, and conservation and restoration sciences. On the other hand, the identification of compounds mainly associated with plants in the colour red, yellow and black, may suggest to an exclusive use of plant-based materials as binders.

While Thompson (1932) suggested the use of white pheasant eggs, the identification of fats does not confirm this, as fats are not present in eggs white. Instead, the findings suggest the use of another type of animal binder.

Finally, as mentioned, the plant and animal components identified using this novel methodology should be sought in more coloured samples from other Maya sites. In particular Chichén Itzá due to the previously documented use of eggs by Thompson (1932), and sites in the northern part of the state of Campeche, where technical similarities have been noted by Vázquez de Ágredos (2010), could be two promising areas to explore the presence of animal compounds in blue pictorial layers. Moreover, further research should include the analyses of reference samples containing plant and animal fats using the same methodology, which would help determine whether the compounds in question are of animal or plant origin. These efforts could contribute to a deeper understanding of the Maya pictorial techniques.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jasrep.2025.105348>.

Data availability

The data that has been used is confidential.

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