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RESEARCH PAPER

Disclosing the composition of unknown historical drug formulations: an emblematic case from the Spezieria of St. Maria della Scala in Rome

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Abstract

This paper reports a pioneering study of an unknown historical drug formulation preserved in the *Spezieria* of *Santa Maria della Scala* in Rome, founded at the end of the seventeenth century by the Discalced Carmelites. Due to limited literature related to pharmaceutical remedies and drugs of the Early Modern Era (between the XV and XVIII centuries) and the complexity in their formulations, the study of these drugs represents a great challenge. The untargeted nature of the selected drug required a multi-analytical approach with complementary techniques to formulate a compositional hypothesis: FT-IR spectroscopy, gas chromatography-associated/mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) were successfully employed to identify different organic compounds. Systematic archaeobotanical research was performed as well, allowing us to acquire data related to the possible *genus* of plants from which these natural compounds derive and their geographical origin. The unknown drug formulation turned out to be a complex mixture used as an ointment with an anti-inflammatory purpose. It mainly contains a mixture of Venetian turpentine; a Pine resin (colophony) from the *Pinaceae* family; an exudate of a plant from South America, whose identified components are triterpenic compounds such as alpha- and beta-amyrins, betulin and lupeol; and saturated fatty acids which act as carriers and/or to reduce the viscosity of abovementioned exudates and resins. The study of historical drugs is important not only in order to know the practices handed down by the *speziali* in the past but also to reconstruct historical recipes, which can inspire new dermatological, cosmetic, hygienic and current healing products.

Keywords Historical drugs · FT-IR · GC-MS · NMR · Venice turpentine · Pine resin · Betulin · Amyrin · Santa Maria della Scala

Introduction

For over 13,700 years, humans have used medicinal plants to treat diseases [1]. Medicinal plants were exploited as fuel, clothing, shelter and a source of nutrition; their use on a large scale allowed people from different cultures to learn about their properties and therefore to use them as products for body care, cosmetics and as a medical resource to treat diseases [2]. Knowledge about medicinal plants has been passed down from generation to generation becoming one of the oldest sciences in countries such as China, Greece, Egypt, Middle East, the Arabian Peninsula and India.

The study of these compounds is important from a historical, anthropological and social point of view. The chemical analysis of these materials can provide crucial information regarding both pharmaceutical and technological knowledge and the practices in their use. In particular, the analyses relating to organic substances and mixtures seem to be of

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fundamental importance to identify the materials, understand their origin and their use and provide further information also relating to everyday life [1].

Different parts of medicinal plants can be used such as seeds, fruits, flowers, roots, leaves, bark, exudates or even the entire plant. The compounds present in most medicinal plants can have direct or indirect therapeutic effects and the components of the plants can interact with each other [2]. Generally, ancient drugs are rare to find because of the degradation processes occurring to the organic fraction of the compounds contained in them and the storage/conservation conditions. Some examples of historical medicines refer to unknown substances, of very complex formulation, including mixtures of various organic and inorganic compounds. Thus, the study of historical drugs represents a great analytical challenge and requires the application of complementary analytical techniques to identify the ingredients of such complex mixtures. Furthermore, it is essential that the analytical results are translated and interpreted to understand the functionality of the identified compounds in the drug formulations.

In this study, we present an in-depth physical-chemical study of an unknown historical drug preserved in the main showcase of the *Spezieria di Santa Maria della Scala* in Rome. This *Spezieria* was founded at the end of the seventeenth century by the Order of Discalced Carmelites and is located on the first floor of the convent of the Discalced Carmelite friars, a religious order of Spanish origin that at that time controlled trade with the East and West Indies [3, 4]. As reported by Vázquez de Ágredos-Pascual et al. (2018) [3], thanks to this commercial activity, the Carmelite friars of Santa Maria della Scala (to which this conventual pharmacy belonged) had the possibility to use vegetal substances and mineral resources both from the Far East and the New World. In particular, the friars were known to use and mix organic compounds (especially from botanical sources) for their pharmaceutical preparations, in accordance with the most important pharmacopoeias. The preliminary analysis performed on organic substances from the New World (e.g. Mechoacan and Guaiaco Resin) and from India (e.g. Gumin Kui) proves the combination of knowledge and materials coming from East and West that met at this Carmelite pharmacy in Rome [5]. These previous studies underline that the substances manipulated by the Friars were numerous and of various nature and the great importance that the organic substance of vegetable origin had in Santa Maria della Scala to prepare drugs.

If the reconstruction of the original recipes starting from labelled samples is relatively simple, for those without labels, it can be very complex and therefore more interesting from an analytical point of view. The sample named LS225, having a totally unknown composition, possesses these features and is the selected object of the current research.

The untargeted nature of the sample required a multi-analytical approach with complementary techniques to formulate a compositional hypothesis of the unknown compound(s). The analytical approach we used to identify the composition of the unknown historical formulation is summarized into the workflow depicted in Fig. 1.

The first technique used was Fourier transform infrared spectroscopy (FT-IR), because it allows to distinguish the organic or inorganic nature of compounds within a sample without destroying the sample itself [6, 7]. FT-IR reveals intermolecular bonds and the presence of specific functional groups; for this reason, it is a rapid methodology to highlight the characteristic fingerprint of different classes of products and to distinguish them from one another based on absorption [8]. The Attenuated Total Reflectance FT-IR spectroscopy (FT-IR-ATR) has the advantage of not requiring any preparation for the sample. In literature, it has been successfully used to understand the formation of lead soaps in ancient cosmetics and to follow the transformation of lipids during the preparation of pharmaceutical formulations [9] or of the lipids that are contained in the fatty substance found inside ceramic vessels from the sixteenth century [10].

The next analysis used was gas chromatography coupled with mass spectrometry (GC-MS) because it allows the

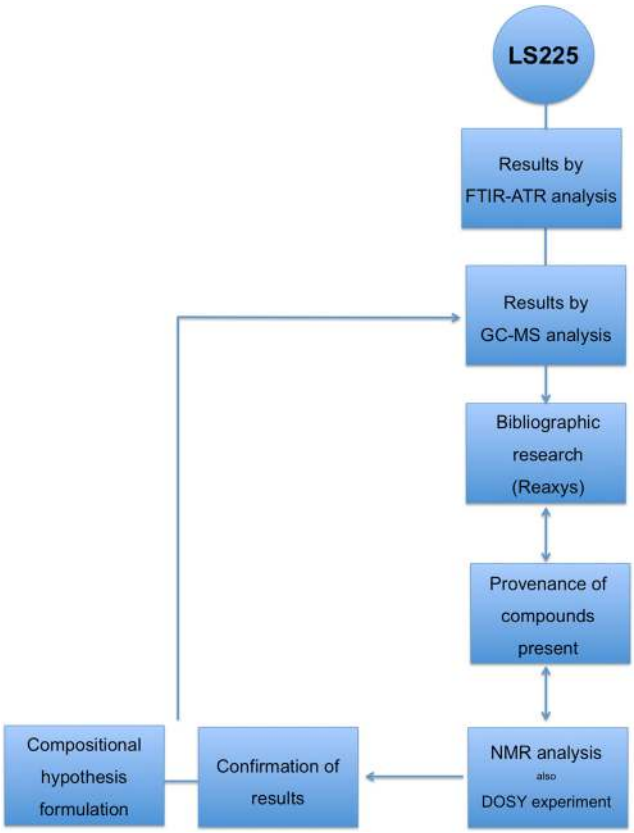

Fig. 1 The workflow followed to characterise the unlabelled sample denotes how the complementarity of the techniques is important to obtain the greatest amount of information and also confirmations

separation and the qualitative and quantitative determination of complex matrices, providing results in a relatively short time and consuming few micrograms of sample. In organic archaeology and Heritage Science, chromatographic identification is often based on the detection of biomarkers. These diagnostic molecules are presently intact in the original material, or they are formed as a result of oxidation due to ageing [6]. An example of this approach was developed by Colombini et al. (2005) in the characterization of natural resins, waxes, bitumen, pitch and lipid materials in Egyptian ceramic vessels [6], but also ancient cosmetics [9, 11] and residues found in Etruscan [8, 12] and Egyptian [13] containers have been studied. Moreover, using this analysis, the chemical nature of these biomarkers can be hypothesised by comparison on robust NIST libraries.

Finally, in this paper, we used nuclear magnetic resonance (NMR) spectroscopy to confirm the structural results obtained by GC-MS analysis. The NMR technique is used in various fields of organic and analytical chemistry, because it is the elective method to determine structure of molecules in solution even when they are present as complex mixtures. Due to its low sensitivity, it is not very usually employed in Heritage Science since NMR analysis requires milligrammes scale samples and this quantity is generally too large for archaeological or historical samples [14]. Nevertheless, this technique has already been successfully employed to study fossil resins and archaeological wood, or polymerized materials, but also to study alterations [9, 15].

The data recorded by the analysis above described were compared with a bibliographic research (based on archaeobotanical and historical studies) in order to better identify the original composition of the drug. As shown in the workflow of Fig. 1, this process was interactively repeated until the hypothesis became scientifically robust.

This stage of research is particularly complicated due to limited literature related to pharmaceutical remedies and drugs of the early modern era.

To the best of our knowledge, in this paper, we present a pioneering physicochemical approach to study an unknown historical drug formulation in the absence of any historical documentations as the unlabelled LS225 sample abovementioned.

Material and methods

Material: Sample and raw materials

The LS225 sample comes from the main window of the sales room of the Antica Spezieria della Scala in Rome (Fig. 2a and b) and the container does not have a label.

The sample, reported in Fig. 3, was composed by compact yellowish-coloured fragments with a fairly smooth and slightly opaque surface. The conditions of storage of the drug were rather optimal, as the container (a dark and thick glass vase) was intact and well closed.

FT-IR-ATR analysis

FT-IR-ATR analyses were performed with a Thermo Nicolet Nexus 670 FT-IR spectrophotometer combined with a Smart Orbit Single Reflection Diamond ATR accessory, from 4000 to 400 cm^{-1} for 128 scans with 4 cm^{-1} resolution. Spectra were elaborated with Omnic 8.0.

Because this analysis is non-destructive, the sample fragments were used for further investigation through GC-MS.

GC-MS analysis

According to a derivatisation method already successfully tested by the authors on archaeological pitch [16], fatty acids from historical and modern drying and non-drying oils [17–21], natural waxes and terpenic resinaceous materials from artistic and archaeological contexts [16, 22, 23], small amounts of sample (~0.35 mg) were transformed into their corresponding methyl esters by a one-spot transesterification using 30 μL quaternary ammonium salt, namely m(trifluoromethylphenyl)trimethylammonium hydroxide, 2.5% in methanol, overnight reaction at room temperature. One microliter of derivatised sample was directly injected in the chromatographic column's head using helium as the carrier gas (flow rate 1.2 mL/min). The GC-MS runs were performed on an Agilent Technologies 6890N (Network GC System) equipped with a mass spectrometric detector Agilent 5973 with a quadrupole analyser. The chromatographic separation was performed on a chemically bonded fused silica capillary DB–5MS Column (30 m length, 0.25 mm internal diameter, 0.25 μm film thickness of stationary phase—5% phenyl methyl polysiloxane).

The inlet temperature was 290 °C, and the MS interface was at 250 °C. The transfer line was at 240 °C and the MS source temperature was 230 °C. The temperature program was set from 100 to 320 °C with a ramp of 10 °C/min, held the temperature for 15 min. The MS was run in Full Scan mode (m/z 50–650), 1.9 scans/s. Electron ionisation energy was 70 eV.

The compounds were identified by comparison with the NIST and MS Search 1.7 libraries of Mass Spectra and a library created by the authors.

NMR analysis

^{1}H NMR, ^{13}C NMR, and 2D spectra were recorded with a NMR Bruker Avance III working at 400 and 100 MHz respectively. Resonance frequencies are referred to tetramethylsilane. The parameters d20 = 50 msec, p30 =

Fig. 2 **a** Sales room of the Antica Spezieria di Santa Maria della Scala in Rome. **b** Main window of the sales room

1.2 msec related to the DOSY experiment were calibrated following the procedures described in literature [24]. The concentration of the samples was 15 mg in 0.5 mL of deuterated chloroform.

The data were treated with MestreNova® software.

Results

FT-IR analysis

As shown in Fig. 4, the FT-IR-ATR spectrum provides an analytical screening result related to the composition of the unknown sample: the remedy exhibits the typical absorbance profile of natural terpenic compounds. It was possible to observe the wide band at 3397 cm^{-1} due to O–H stretching and the stretching vibrations of $–CH_2$ and $–CH_3$ groups at 2927 and 2860 cm^{-1}, related to the resin hydrocarbon skeleton. The peak at 1707 cm^{-1} and the shoulder at 1659 cm^{-1} are attributable to the carbonyl group C=O. Furthermore, the stretching vibrations at 1454 and 1379 cm^{-1} due to aromatic rings were identified as well: these seem to be present also in the triterpenoid resin [16, 25–27]. The peaks between 1250 and 600 cm^{-1} are also present in the Venetian turpentine (a viscous liquid which is collected from the larch, *Larix decidua*, or *L. europaea*) or in any in diterpene resins. Particularly, the peaks between 990 and 600 cm^{-1} are due to the C–H bending out of plane [25–27]. The FT-IR-ATR spectrum of the unknown sample was compared with the spectra in the IRUG (Infrared and Raman Users Group) database [28]: it appears not to be particularly oxidised, most likely because the container was tightly sealed.

Fig. 3 Diffuse light picture of the LS225 sample

GC-MS analysis

According to the preliminary results obtained through FT-IR-ATR, the sample LS225 was subjected to a specific preparation procedure and GC-MS analysis (as described in 2.3). The resulting TIC chromatogram (Fig. 5) suggests a rather complex composition of the unknown pharmaceutical preparation. The assignments of the peaks associated with their mass spectra are reported in Table 1.

The sample LS225 mainly consists of compounds of vegetable origin, precisely:

- saturated medium-short-chain monocarboxylic acids, analysed as methyl esters (with retention times between 8 and 14 min): decanoic (capric) acid and dodecanoic (lauric) acid were identified and their relative MS spectra are reported in the Electronic Supplementary Material (ESM);
- diterpenic compounds (with retention times from 20 to 30 min), namely epimanool, larixol, larixol acetate, isopimaric acid and dehydroabietic acid (whose MS spectra are depicted in the ESM);
- triterpenic compounds (with retention times from 40 to 48 min): betulin, lupeol, lupeol acetate and alpha-amyrin and beta-amyrin were also detected (their relative MS spectra are shown in the ESM).

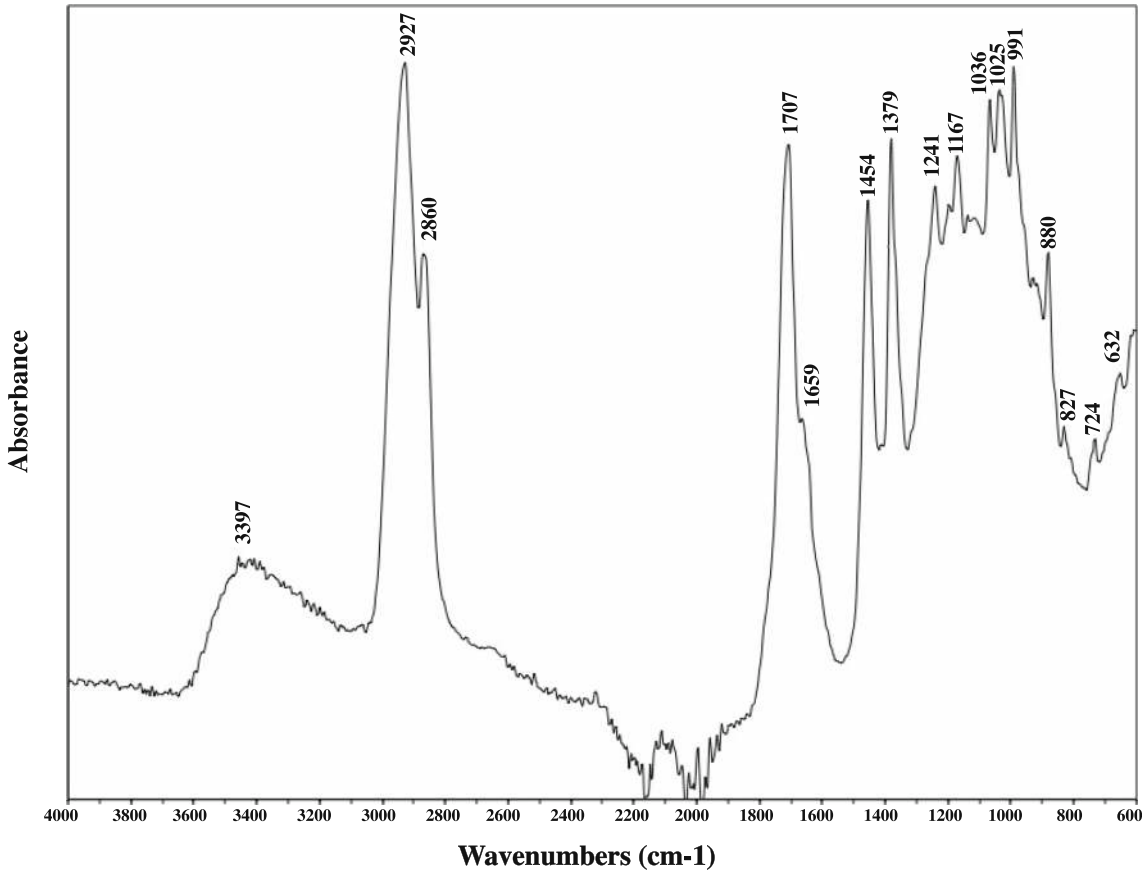

Fig. 4 FT-IR-ATR spectrum of sample LS225

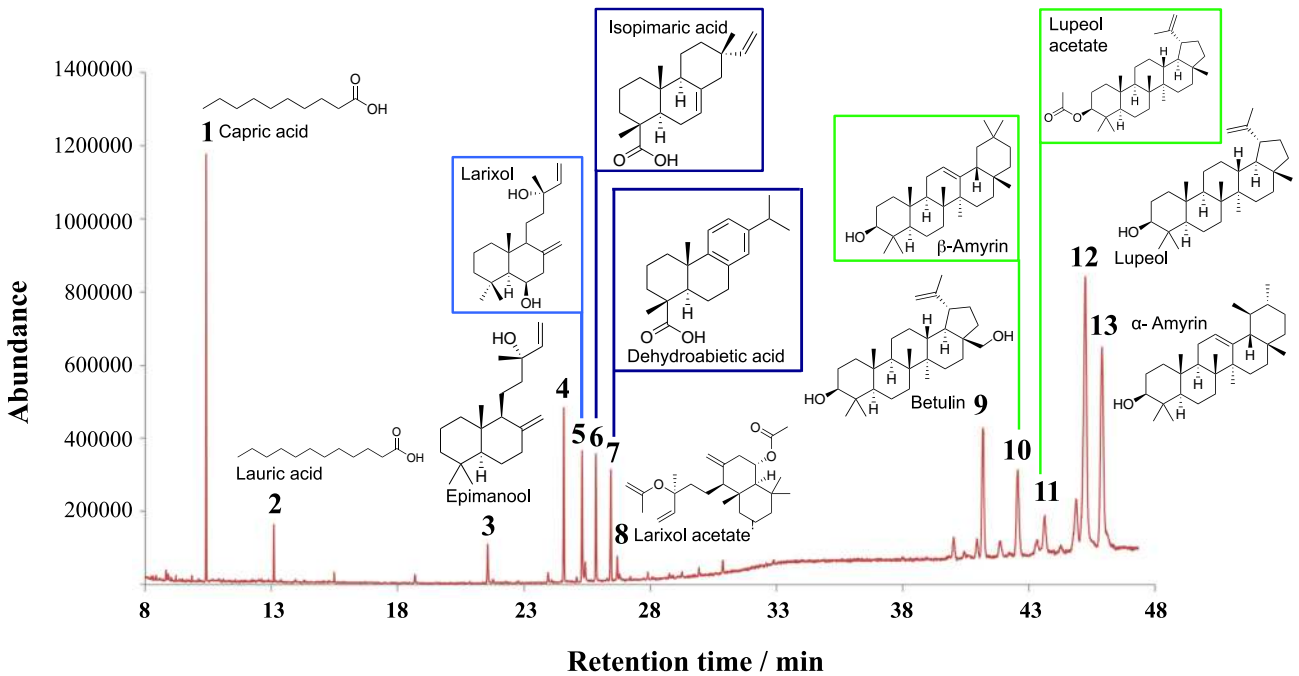

Fig. 5 TIC chromatogram of LS225 sample after transesterification and GC-MS analysis. The assignment of the peaks are reported in Table 1, while the chemical (underivatised) structures of the identified compound are depicted in correspondence of the peaks

Table 1 Assignment of peaks of the chromatogram of Fig. 5

Peak number	RT (min)	Compounds
1	10.41	Decanoic acid ME
2	13.09	Dodecanoic acid ME
3	21.56	Epimanool
4	24.57	Nonadecanoic acid ME (internal standard)
5	25.30	Larixol
6	25.85	Isopimaric acid ME
7	26.44	Dehydroabietic ME
8	26.69	Larixol acetate
9	41.17	Betulin
10	42.54	Beta-amyrin
11	43.63	Lupeol acetate
12	45.24	Lupeol
13	45.86	Alpha-amyrin

ME = methyl ester

NMR analysis

To corroborate the results obtained by GC-MS analysis and the hypothesis of the molecular composition of LS225, NMR experiments were performed. First of all, the presence of the markers of Venetian turpentine (epimanool and larixol) was confirmed. Figure 6 a shows the carbon chemical shifts related to vinyl moiety present in terpenic skeleton of these two molecules. Moreover, inset Fig. 6, two portions of HSQC experiments describe the correlations with the corresponding proton. The chemical shift values indicated into brackets are those referring to the literature data for pure compounds [29].

The direct comparison of H_{16} signals between LS 225 and the Venice turpentine (VT) standard (Fig. 6b) revealed signal splitting, which indicate that both molecules are present. With NMR, in fact, we are able to understand whether the epimanool revealed by GC-MS was really present or whether it was larixol which has lost an •OH due to the high temperatures of the GC-MS analysis, thus becoming epimanool.

The presence of α-amyrin and β-amyrin was confirmed by the chemical shift comparison of vinyl proton as pure compounds. The matching around 5.18 ppm of H_{13} for β-isomer and 5.12 for α-isomer well fits with literature data [30, 31], as well with ^{1}H spectra simulation obtained by MestreNova® software negligible within experimental error (β-isomer 5.22 ± 0.44 ppm, α-isomer 5.43 ± 0.55 ppm) as shown in Fig. 7.

As shown in the chemical structures of Fig. 5, betulin, lupeol and its acetate are structurally similar apart for CH_2–OH. These three structures are confirmed by the literature data for the whole vinyl system which is the same [30–33]. Both HSQC and HMBC experiments allowed to see and confirm the interactions, with cross-peaks between H_{32}-C_{32} (Fig. 8a) and cross-peak long range interactions H_{31}-C_{30}-C_{32} (Fig. 8b).

HMBC experiment allowed finding a carbonyl signal C_{34} characteristic of acetate function at 170.5 ppm (Fig. 8c) with a cross-peak with corresponding methyl H_{35} at 2.04 ppm. These two chemical shifts are, according to the literature data, assigned to lupeol acetate [33]. For clarity in Fig. 8d and e, all of the assignments are summarized in monodimensional spectra ^{1}H NMR and ^{13}C NMR. Whereas a direct connection exist between the $H_{32'}$ and $H_{32''}$ because the protons are in the

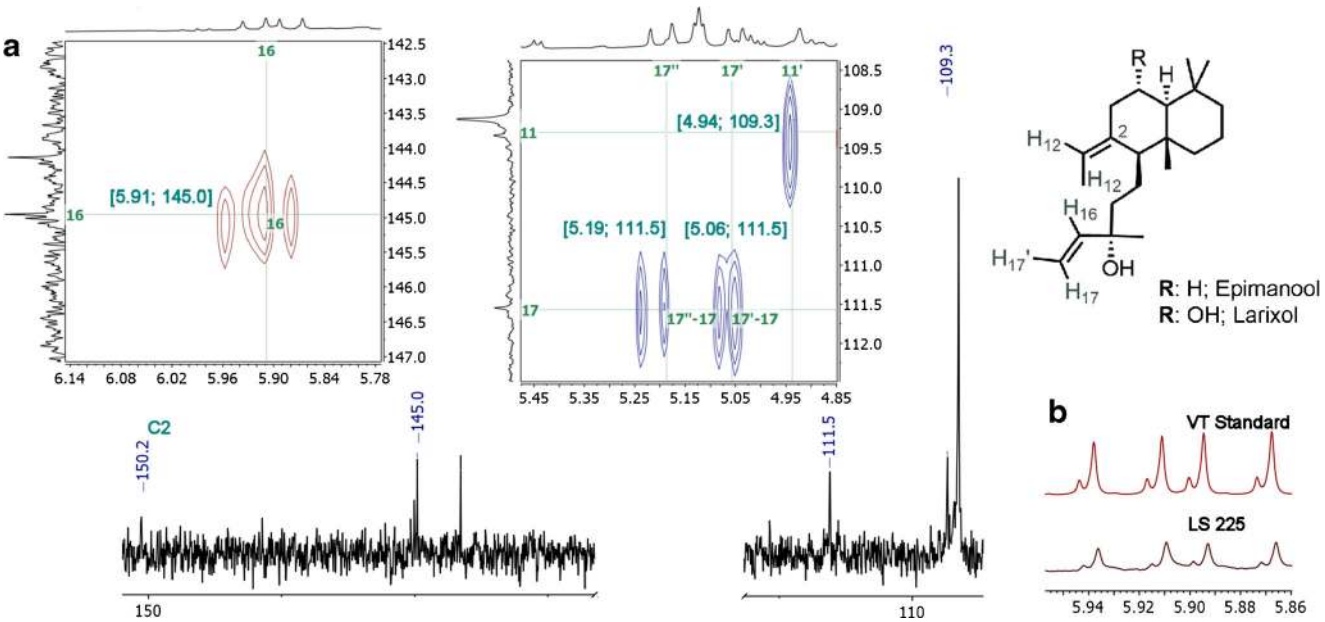

Fig. 6 **a** ^{13}C chemical shift related to vinyl moiety present in terpenic skeleton of epimanool and larixol and inset its HSQC spectrum. **b** Epimanool and larixol peaks in ^{1}H NMR spectra of LS255 and the Venice turpentine (VT) standard

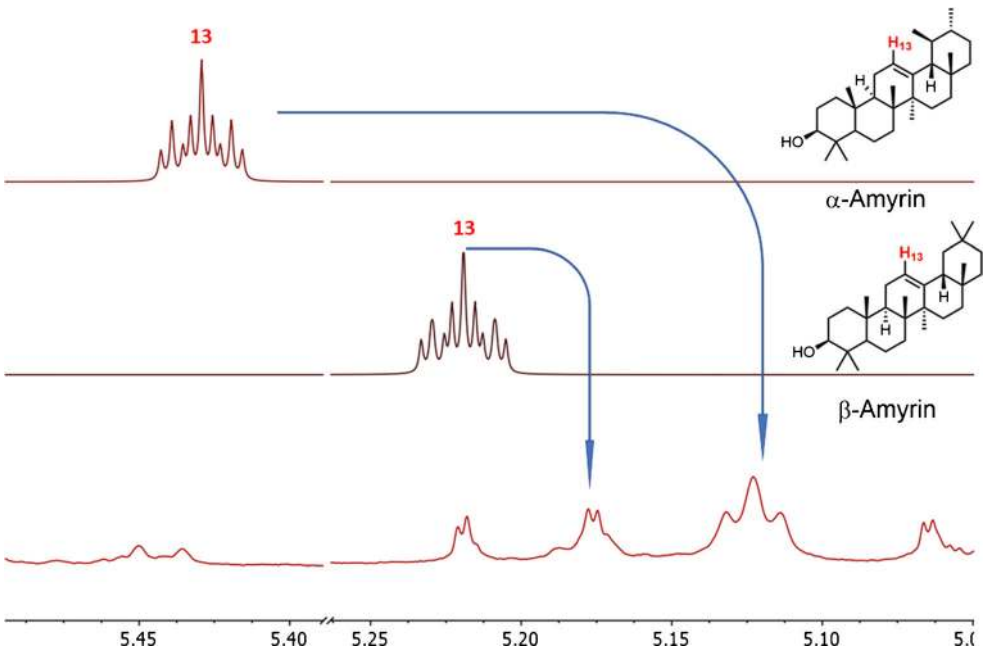

Fig. 7 α- and β-amyrin chemical shifts in ^{1}H spectrum of sample LS 225 confirmed by the simulation of the spectra of pure compounds obtained through the MestreNova® software

opposite part of the molecule, we decide to use NMR DOSY to confirm the presence of lupeol acetate. In Fig. 9 a, the bi-dimensional experiment is shown: it is possible to appreciate how the signals indicate above possess the diffusion coefficient $D = 2.01 \times 10^{-9}$ m^2 sec^{-1}. This result, also confirmed by integration of individual signals using Stejskal-Tanner equation, indicates that H_{32} and H_{35} belong at the same molecule.

Although identified through GC-MS, betulin was not detected by NMR: in ^{13}C spectrum, in fact, the signal at 60.7 ppm [32] ascribable to –CH_2–OH of betulin cannot be seen, confirmed also by the absence of the corresponding diastereotopic system in ^{1}H-NMR. Probably, it was due to the NMR detection limit compared with GC-MS. In this case sample, LS225 has a very complex matrix and the quantity of

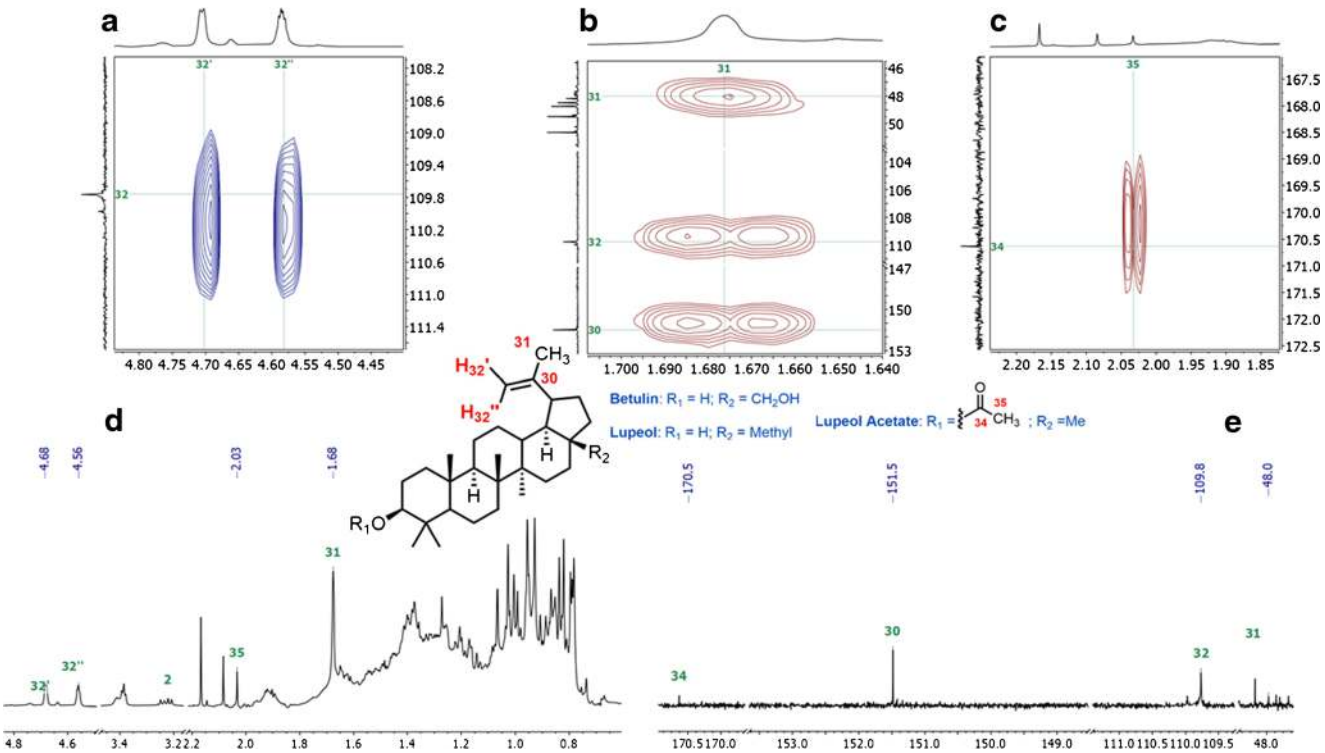

Fig. 8 Lupanic structure triterpene assignments. **a** HSQC of vinyl system $H_{32'}$, $H_{32''}$-C_{32}. **b** HMBC of vinyl system H_{31}-C_{30}-C_{32}. **c** HMBC of acetate structure. **d** ^{1}H NMR summarized signals in LS225 sample. **e** ^{13}C NMR summarized signals in LS225 sample

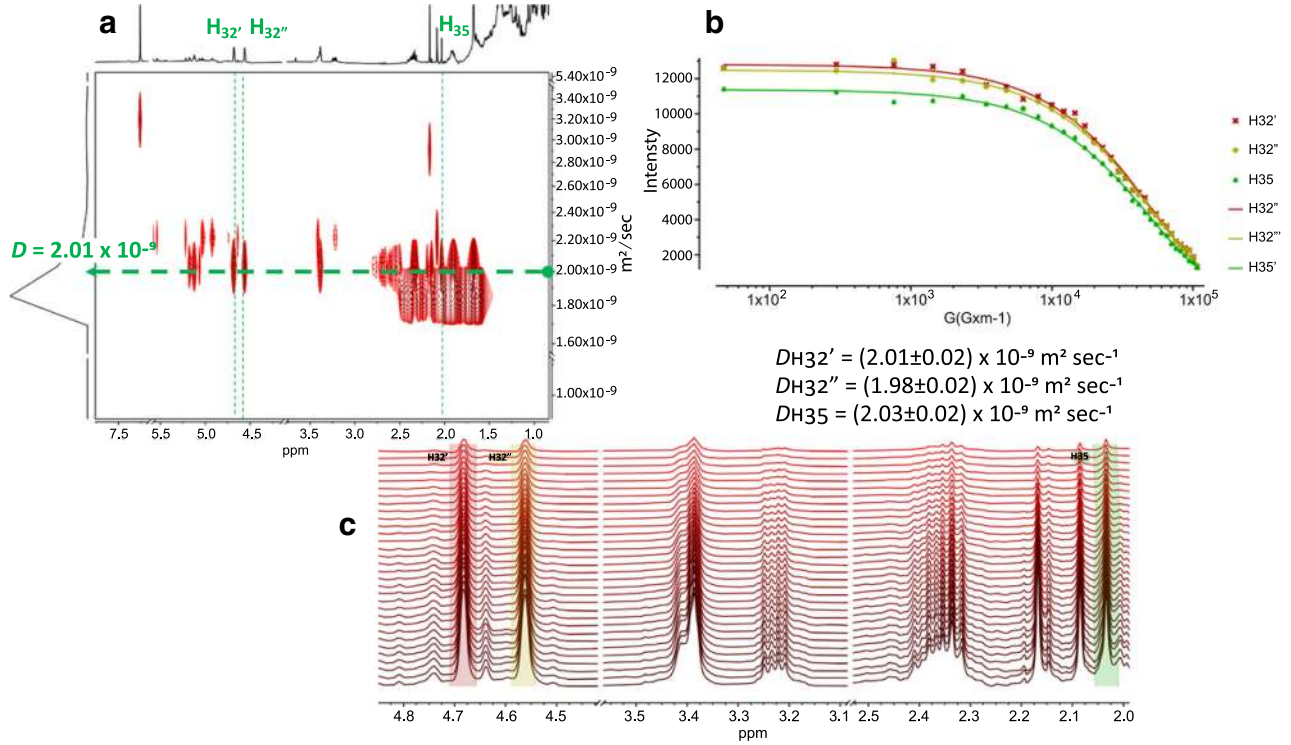

Fig. 9 **a** DOSY bi-dimensional experiment of sample LS225; 400Mz, $CDCl_3$. **b** Diffusion decay of protons $H_{32'}$, $H_{32''}$ and H_{35}. **c** Intensity decay of proton signals for H_{32} and H_{35} in function of gradient applied

betulin present in the sample could be so limited that it cannot be detected by this technique.

In Fig. 10, the ^{1}H NMR summarized all signals assigned using all of experiments above described.

Discussion

The interpretation of the results obtained by FT-IR-ATR, GC-MS and NMR was combined to a tailored bibliographic

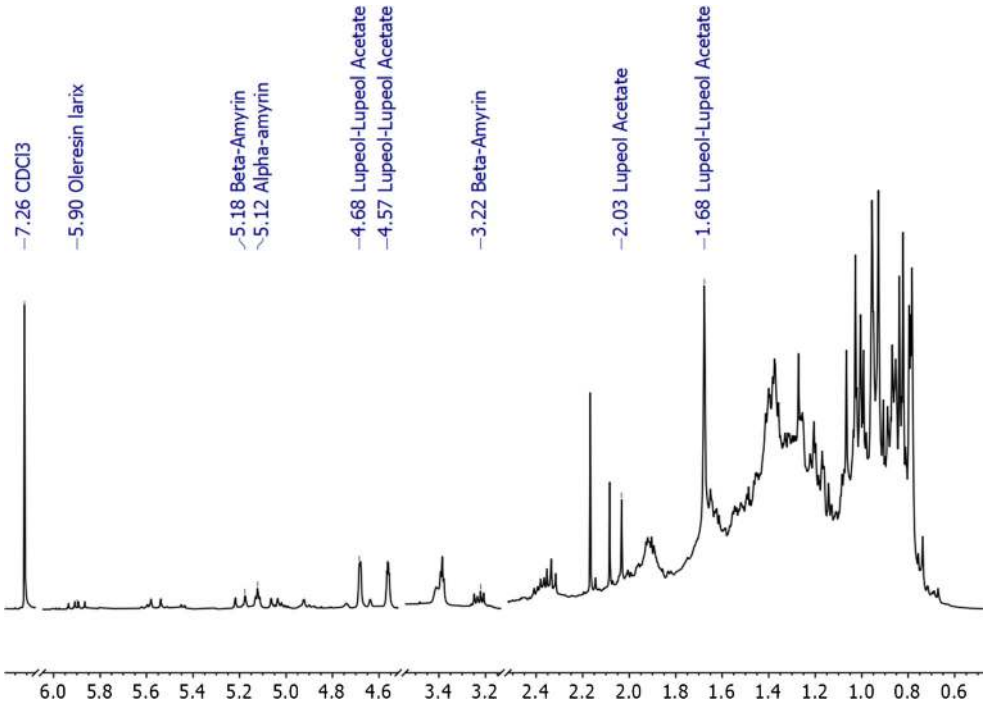

Fig. 10 LS225 ^{1}H NMR spectrum; 400 Hz, $CDCl_3$

Table 2 Pentacyclic triterpenoids identified in the sample LS 225 and in different plants from literature data

Compounds					Plants
Beta-amyrin	Lupeol	Alpha-amyrin	Lupeol acetate	Betulin	
x	x (lupeol)	x	x		*Dorstenia arifolia (Moraceae)* (Brazil) [37]
	x (lupeol)				*Dorstenia harmsiana (Moraceae)* (Africa) [39]
	x (lupenone and lupeol)				*Dorstenia convexa (Moraceae)* (Africa) [40]
x	x (lupeol)	x	x		*Pseudobrickellia brasiliensis (Asteraceae)* (Brazil) [38]
x	**x**	**x**			*Bursera spp. and Protium spp. (Burseraceae)* (Mexico) [41–43]
x	x (lupeol)		x		*Viscum album (Santalaceae)* [44]
	x (lupenone and lupeol)	x			*Deertongue* *Scolopendrium officinale* *C. odoratissimus* [45]
x	x (lupenone and lupeol)				*M. caesalpinifolia* (Brazil) [46]
x	x (lupenone and lupeol)	x		x	*Phoradendron brachystachyum (Viscaceae)* [47]
x	x (lupenone and lupeol)	x		x	*Terminalia brasiliensis (Combretaceae)* (Brazil) [48]
x	x (lupenone and lupeol)	x		x (in crude extract)	*Byrsonima microphylla (Malpighiaceae)* (Brazil) [49]
x	x (lupeol)	x		x (in crude extract)	*Roupala montana (Proteaceae)* (Brazil) [50]
x	x (lupeol)	x	x	x (in crude extract)	*Struthanthus syringifolius (Mart.) (Loranthaceae)* (Brazil) [50]
	x (lupenone and lupeol)				*Schefflera vinosa (Araliaceae)* (Brazil) [50]
x	x (lupenone and lupeol)	x			*Anadenanthera colubrina (Mimosaceae)* (South America) [51]

research on Reaxys Database. This method, similar to an archaeobotanical approach [33], was especially adopted to find out the source of the identified terpenic structures.

Indeed, the presence of saturated fatty acids (capric and lauric acids) can be more easily explained by the use of fatty substances as carriers to take the remedy and/or to reduce the viscosity of balsams, the basis for mixing more "exotic" ingredients.

The presence of characteristic biomarkers of diterpenes allowed considering the occurrence of conifer resin. Epimanool, larixol and larixol acetate (whose structures are shown in Fig. 8 a) were detected by GC-MS. These neutral labdanic compounds are considered biomarkers for the identification of Venetian turpentine, a semi-liquid resin extracted from *Larix Decidua Miller* (also called larch turpentine) [34]. Venetian turpentine could eventually also be used as a diluent in the drug formulation.

As reported in the literature [34], di- and triterpenic compounds are never present simultaneously in the same natural resin. This supports the hypothesis that the unknown drug formulation is a mixture of different terpenic compounds.

Furthermore, other compounds derived from conifer resins have been used in the preparation of this unknown remedy: the simultaneous presence of isopimaric acid and dehydroabietic acid emphasizes that the material contains a resin obtained from the distillation of a piece of wood of the *Pinaceae* family, namely rosin or colophony [16, 35].

Dehydroabietic acid can be present in fresh diterpene resins, but also in historical/aged ones: while the volatile fraction of this type of resins decreases, its concentration tends to increase over time, in fact, it can be present as a degradation product of abietadiene acids, easily degradable due to a conjugated double bond [36].

The triterpenic fraction detected is composed by pentacyclic compounds, in particular, ursanic and oleanic compounds, which differ only by the position of the methyl group. Triterpene represent an important class of natural compounds and their simultaneous presence (in particular amyrins and lupeol) seems to be associated to a species of South American plant: for instance, the *Dorstenia arifolia*, a herbaceous genus of the *Moraceae* family [37], the *Pseudobrickellia brasiliensis* (*Asteraceae*) [38], or other plants from Brazil (see Table 2). Moreover, these triterpenes

Table 3 Uses and provenance of the compounds identified in the sample LS225

Compounds	Plant(s)/family	Country/ region of origin	Use in drug formulations
Saturated fatty acids (capric and lauric acids)	–	n.d.	Generally used as carriers and/or to reduce the viscosity of balsams or other viscous materials
Diterpenoids from a pine resin (isopimaric and dehydroabietic acids)	*Pinaceae* family	Europe (?)	Used in medicinal, antiseptic or ritual balms
Diterpenoids from Venetian turpentine (epimanool, larixol and larixol acetate)	*Larix decidua Miller*	Europe	Ingredient in many ointments, liniments and lotions for the treatment of pains and colds. In traditional medicine, mainly used in the treatment of rheumatism, muscle pain, stiff joints, toothache, blisters and sores due to its rubefacient and anti-irritant properties. Used as an antimicrobial. Used as a diluent in the drug formulation as well.
Pentacyclic triterpenoids (betulin, lupeol, lupeol acetate, alpha and beta-amyrins)	*See* Table 3	South America	Employed for their anti-inflammatory properties: in particular, betulin and its acid (betulinic acid) used for their antimalarial, antimicrobial and spasmogenic activities; used as gastroprotectives as well. Used as incense in ceremonies (as main constituents of copal).

with oleanic, ursanic and lupanic structure are also the main constituents of the non-volatile fraction of copal, a resin from the *Burseraceae* family originating in Mexico. In particular, as reported in the literature, alpha- and beta-amyrins are dominant in *Protium* spp. while *Bursera* spp. contain also lupane compounds (e.g. lupeol) [41–43]. Moreover, several studies report that betulin, lupeol and lupenone are typical constituents of birch bark and its related exudate [52–55].

Table 2 compares the pentacyclic triterpenoids identified in the sample LS 225 and in different types of plants from literature data. Although it was not possible to establish with certainty which is the *genus* from which the triterpene resin(s) derived, some hypotheses were formulated and presented in Table 2.

A study carried out by Zapata-Sudo and co-workers [56] shows that *Dorstenia arifolia* was used in folk medicine as an antipyretic, expectorant and anti-inflammatory, and this could further confirm our hypothesis. Meanwhile, copals were historically used as incense for rituals, but it was also used as a binder mixed with pigments, for the decoration of murals [41].

From a historical and documentarist point of view, this hypothesis is strengthened by the fact that the Carmelite friars controlled the trade with the Western Indies [3, 4].

The pentacyclic triterpenoids detected by GC-MS are characterised by particular steroid structures which determine the anti-inflammatory properties and gastroprotective activities [57, 58].

Lupane-pentacyclic triterpenes, such as betulin detected in the unknown drug formulation, have different medicinal properties. The antitumor property of triterpene plant extracts derived from lupane has been demonstrated in the past 25 years for their cytostatic activity on various in vivo cancer model systems. Betulin and its acid (betulin acid) are part of this class of triterpene pentacyclic and have anti-cancer properties. Both compounds have also been shown to possess an anti-inflammatory activity that has been attributed to the inhibition of non-pathway neurogenic [59].

For the reconstruction of the "recipe" of the LS225 sample, it is also helpful to refer to Pliny, who in his treatise *Naturalis Historia* reported the use of mixtures consisting of resins and oils for medical use. In addition, he described the composition of perfumed ointments, whose ingredients are several resins and oils, together with spices and perfumed essences [60]. And there were earlier treatises that referred to these substances and their therapeutic uses, such as the *Edwin Smith Papyrus*, which is attributed to the architect and physician Imhotep (3rd Dynasty, Old Kingdom), and the *Papyrus Ebers*, dated in the New Kingdom, in around 1500 BC, both Egyptians. In fact, from Antiquity to the present day, a lot of authors such as Theophrastus (371–287 BC), Dioscorides (about 40–90 AD), the aforementioned Pliny (first century AD), Galen (about 129–201/215 AD), Mesue the Younger (1013), Avicenna (978–1036), John Serapion (1070), Bartholomeus Anglicus (1220), Hieronymus Cardanus (1501–1576), Pietro Andrea Mattioli (1501–1577), Pierre Belon (1517–1564), Alvaro Alonso Barba (1569–1662), Luis de Oviedo (1581), Giuseppe Donzelli (1596–1670), Moyse Charas (1618–1698), Michael Bernhard Valentini (1657–1229), Samuel Hentschel (1658) and Jaume Bech (2010, 2016) have devoted some of their writings or theses to the origin, classification, quality and uses of these organic substances with therapeutic properties.

According to the information gathered and based on the historical and archaeobotanical research, the studied unknown

drug formulation is likely a complex mixture that could have been used as an ointment with an anti-inflammatory purpose.

A detailed description is illustrated in Table 3.

n.d., not defined

Conclusions

The combined use of different analytical techniques (FT-IR-ATR, GC-MS and NMR) allowed studying the chemical composition of an unknown historical drug formulation. The complementarity of the techniques proved to be of fundamental importance: a complete set of data was collected and the results were corroborated.

The experimental evidences associated with the archaeobotanical and historical studies carried out permitted to formulate a compositional hypothesis. The unknown drug appears to be a complex mixture used as an ointment with an anti-inflammatory purpose.

The main ingredients of its "recipe" were found to be:

- Venetian turpentine, a diterpene oleoresin identified thanks to its characteristic markers (epimanool, larixol and larixol acetate);
- a pine resin (namely rosin or colophony) from the *Pinaceae* family;
- an exudate of a plant from South America, whose identified components are triterpenic compounds such as alpha- and beta-amyrins, betulin, lupeol and lupeol acetate;
- saturated fatty acids which act as carriers and/or to reduce the viscosity of abovementioned exudates and resins.

The study of historical drugs is important not only in order to know the practices handed down by the *speziali* in the past and to learn more about past medicines and the technology in their manufacture, but also to formulate new remedies based on the reconstruction of historical recipes. In this sense, numerous laboratories currently support research lines based on the study of old substances and pharmaceutical formulations that can inspire new dermatological, cosmetic, hygienic and current healing products. The Laboratory of Aboca Coop. Agricola (Sansepolcro, Italy), the main source of funding for the research project in Santa Maria della Scala (Rome), is one of them.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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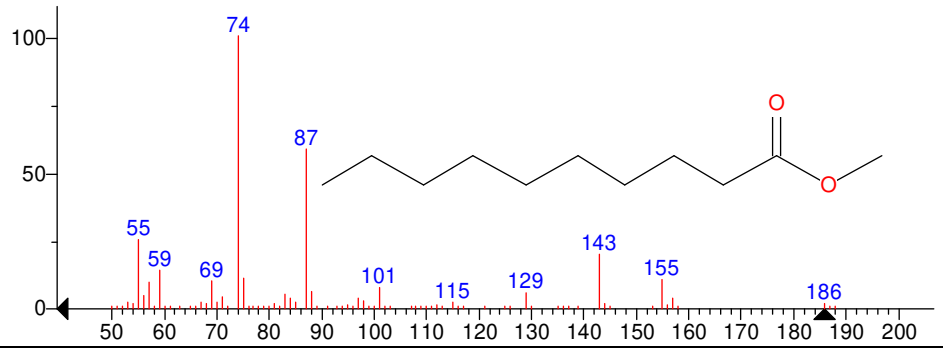
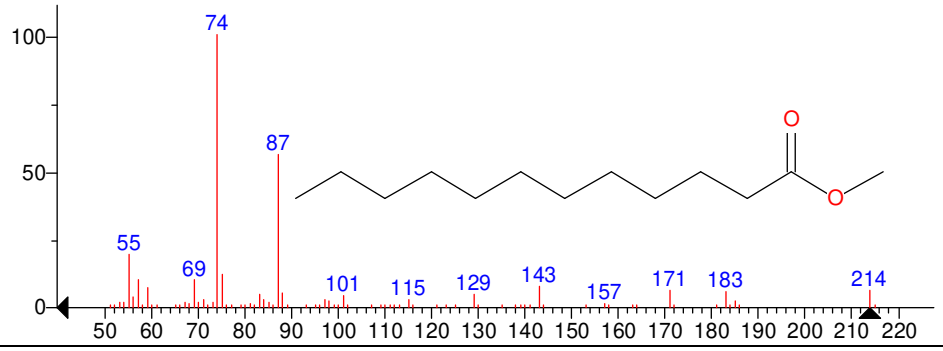
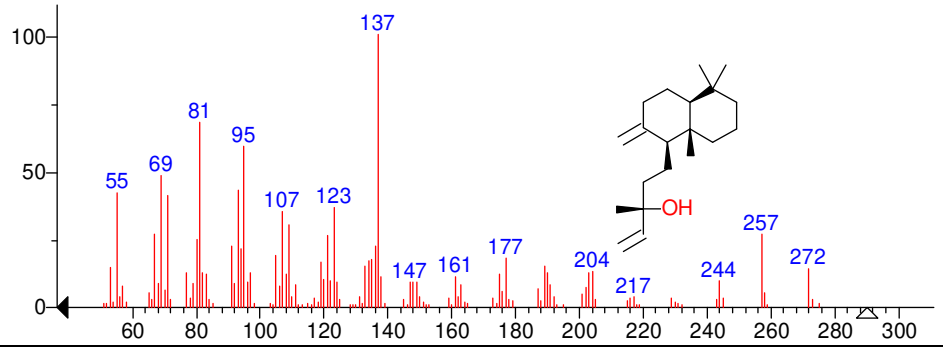
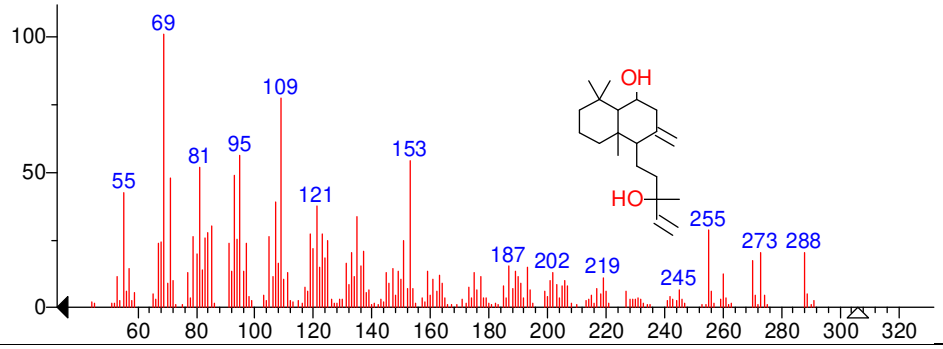
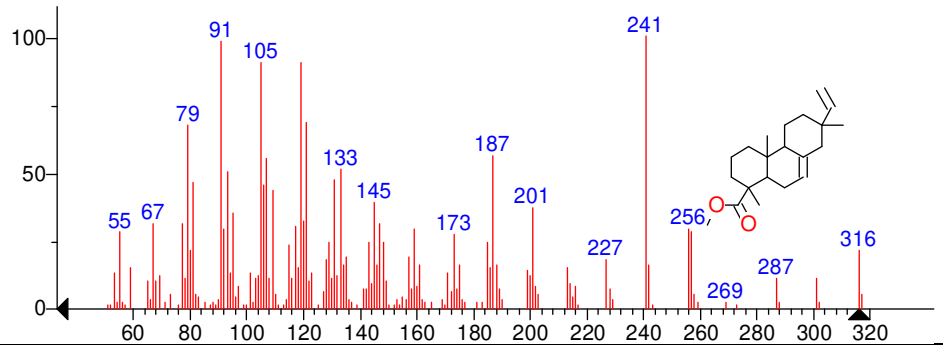
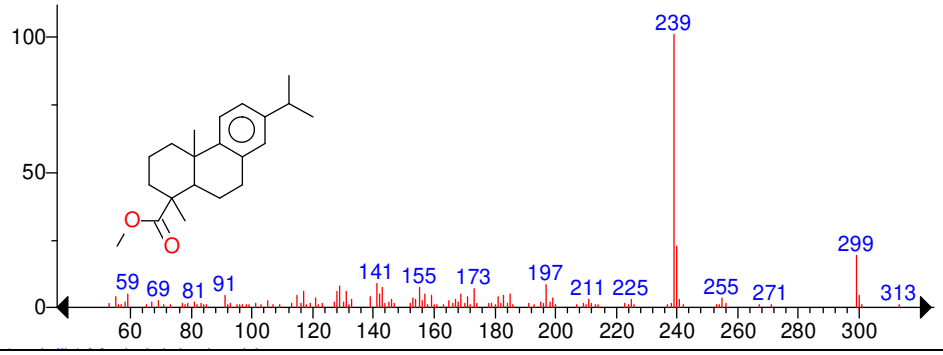
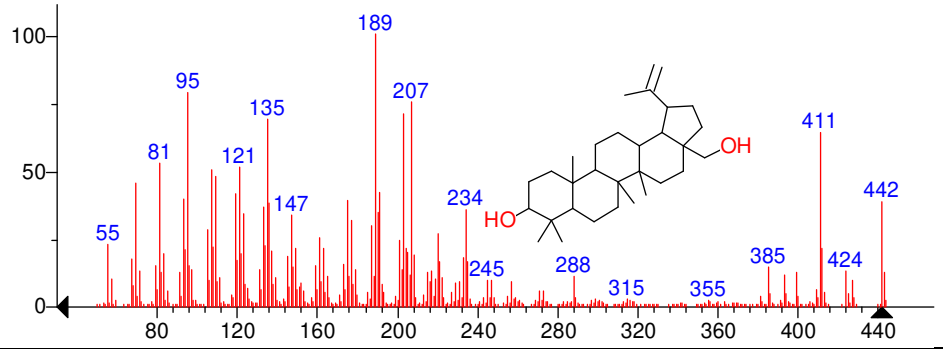
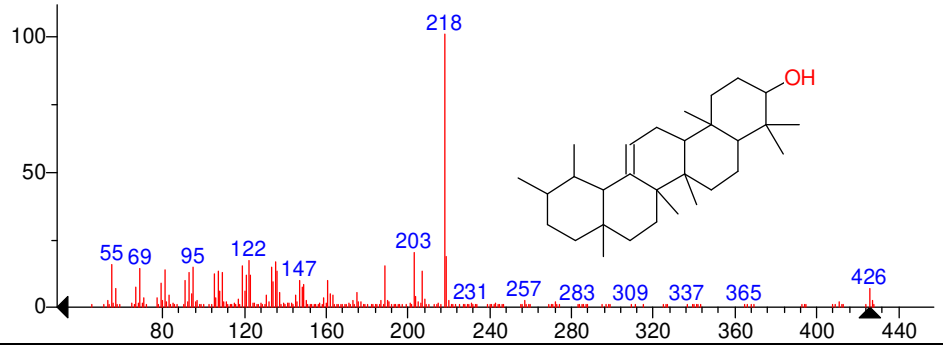
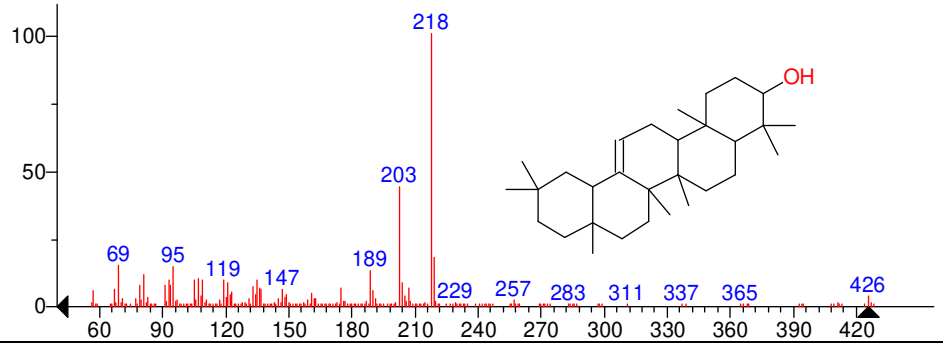
Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Disclosing the composition of unknown historical drug formulations: an emblematic case from the *Spezieria* of St. Maria della Scala in Rome

Giulia Carolina Lodi, Giuseppe Borsato, Maria Luisa Vázquez de Ágredos Pascual, Francesca Caterina Izzo

	Mass spectrum of Capric acid, methyl ester
	Mass spectrum of Lauric acid, methyl ester
	Mass spectrum of Epimanool
	Mass spectrum of Larixol

	Mass spectrum of Isopimaric acid, methyl ester
	Mass spectrum of Dehydroabietic acid, methyl ester
	Mass spectrum of Betulin
	Mass spectrum of Alpha-Amyrin
	Mass spectrum of Beta-Amyrin