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Study of Egyptian mummification balms by FT-IR spectroscopy and GC–MS

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ABSTRACT

Fourier transform infrared spectroscopy (FT-IR) and gas chromatography coupled with mass spectrometry (GC–MS) were used in order to analyse twelve mummification balms from mummy skulls of the *Musée des Confluences* (Lyon, France).

For FT-IR analyses, a simple extraction protocol in dichloromethane and water allowed to separate the materials by their polarity. This study clearly shows that the organic fraction is the main constituent of the Egyptian balms and hides much information in the bulk analyses (made without any extraction). Infrared absorption reveals the presence of (i) several organic materials (proteins, polysaccharides), (ii) inorganic salts ($CaSO_4$, $CaCO_3$ and NH_4Cl) possibly used as natron in ancient time, and (iii) ochre used in order to dye the bandages.

GC–MS analyses were made on the organic fraction of extracted balms, previously trimethylsilylated before injection. Biomarkers and degradation products of oils, fats, resins (with oleanene, lupene, lanostane, masticadiene, and abietane compounds) and beeswax were found. These materials were often used in combination. Many identified byproducts (di and triterpenic molecules, hydroxylated fatty acids, etc) give us the opportunity to discuss the different degradation reactions taking place in such archaeological material. Furthermore, beeswax was identified in numerous samples thanks to the presence of long chain alkanes, long chain fatty acids and palmitate ester. In one balm, the co-occurrence of *brassicaceae* oil chemical markers and cholesterol (and its degradation products) shows the combine use of oil and fat. Finally, a great correlation and complementarity was observed between the two analytical techniques.

1. Introduction

From around −5000 BC to hundreds of years AD, Egyptians preserved corpses in order to insure their eternal life. Thus, in this society, mummification was of major importance. A highly elaborated process divided in numerous steps was developed and evolved throughout the ages [1,2]. Employed techniques were specific of the geographic area, time, body part, social status and maybe age and sex of the dead [3]. All along mummification, an organic balm was applied as antibacterial, water-repellent material, fragrance and maybe for religious symbolism associated with different plants [4].

Balm composition can be elucidated by various analyses [5–12]. To this point, it is known that balms can be composed of mixture of oils, fats, waxes (and especially beeswax), resins, gums, salts, bitumen and various barks and spices [13,14].

Thus, to assess the initial composition of such samples, chemists must deal with a crossover of molecules from various origins and different degrees and ways of degradation.

To overcome such difficulties, a powerful approach is to link techniques that provide general information on the sample to more selective ones. Among the possibilities, Fourier-transform infrared spectroscopy (FT-IR) and gas chromatography with mass spectrometric detection (GC–MS) are two promising and complementary techniques [15,16].

FT-IR allows a non destructive fingerprinting of the sample without any chemical transformation. On the basis of infrared absorption, it is possible to identify different materials present in a sample, as indicated in many works that performed the identification of fresh organic and inorganic materials [17–20]. In archaeological samples, materials are often in complex mixtures of components in various degradation states. For organic substances, such conditions do not allow any extensive identification by a flowchart as usually done in the case of fresh materials [17,18]. Despite that, some identifications of inorganic and organic components are still possible in mummification balms by using FT-IR [21]. Salts and gum are quite insoluble in dichloromethane but soluble in water [22], thus, they can be easily separated by extraction using the proper solvent. Such approach allows the analysis of organic and inorganic fractions and the collection of information about non extractable fraction [23]. Such kind of systematic work has never been done on an archaeological material. In Table 1, different characteristic absorption wavenumbers for some fresh material found in mummification balm are given.

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Table 1
Characteristic infrared wavenumbers of different fresh materials possibly found in mummification balm [17,18,23,24]. All data are in cm^{-1}.

Dichloromethane soluble material Organic material, 3440–3420 (OH), 2950/2850 (νCH), 1700–1720 (C═O)	
Waxes	1460 (C═H bending), 2926 (νCH_2), 2850 (CH_2 Stretch), 1466/1462 and 730/720 (presence of doublets for semi-crystalline structures)
Oils and fats	2926 (νCH_2), 2850 (CH_2 Stretch), 1740, 3440–3420 (OH)
Resins	2926 (νCH_2), 2850 (CH_2 Stretch), 1710–20, 3440–3420 (OH)
Water soluble material	
Gums	1600 (intramolecular bound water & carboxyl group), 1455 (C═H bending), 1415 (CH deformation), shoulder at 1149, 1080 (CO), shoulder at 1035, 780, Poor C═H stretch.
Protein	3295 (bonded N═H stretching), 1650 (C═O absorption of amid I), 1554 (deformation of amide II NH_2), 1449, 1318, 1243, 1169, 1084, 1030 (unassigned bands)
Inorganic salts	cf text

In many cases, FT-IR analyses are not sufficient to allow a precise identification of aged mixtures of natural materials. Thus, GC–MS can be used as a powerful complementary analytical technique.

Thus, GC–MS has been extensively used in order to elucidate archaeological sample composition [25] and more specifically balm composition [5,6,21,26,27]. For this purpose, specific chemical markers give precious information on materials and composition of sample [28–30]. Indeed, for plant materials, the presence of different chemical markers allows to find genus or even species of the original plant [31]. Three types of chemical markers can be pointed out: (i) biomarkers that are present in the original material, (ii) natural degradation markers linked to normal alteration of initial chemical composition and (iii) anthropogenic markers that indicate a specific treatment of the sample. In Table 2, a list of different chemical markers previously found in the archaeological chemistry of Egyptian balms is indicated. However GC–MS is not suitable for inorganic materials, and cannot be used on natural polymers without specific preparation of the sample [32].

In this work, twelve balms were sampled from mummy heads of the *Musée des Confluences* (Lyon, France). These skulls were previously studied by endoscopy, radiography and archaeological data [33]. In order to obtain chemical information from such archaeological material, samples were analysed by FT-IR spectroscopy and GC–MS to allow pertinent identification of organic and inorganic materials. Thus, the aims of this work were: (i) to develop a protocol for balm analysis by FT-IR spectroscopy, (ii) to validate FT-IR results by GC–MS data, and (iii) to bring knowledge to improve the correlation between chemical and archaeological data on Egyptian mummification balms.

2. Experimental section

2.1. Sample description

The analysed samples come from the collection of the *Musée des Confluences* in Lyon [33]. The twelve mummified heads were named B1, B6, B9, B10, B13, B19, B26, B32, B33, B35 and B42. On the B19 skull, the balm was taken from two different spots: (i) from the bandage filling the left eye socket, and (ii) at the lower part of the chin. Samples were all black and amorphous pieces of resin-like material with more or less heterogeneities in the composition (white mineral inclusion, orange-brown sticky material, etc). Samples were collected on the external part of the bandage at different locations. The weight of the samples varied from 23 to 105 mg.

2.2. Materials

All reactants were of the highest purity grade available. Aqueous solutions were prepared with deionised ultrapure water which was purified with ultrapure water (18.3 MΩ·cm) from a Milli Q device (Millipore).

2.3. Microchemical analysis

The presence of sulphate was shown by the reaction of solid balms with $BaCl_2$ [42]. Specific identification of proteins requires the pyrolysis of the sample in the presence of calcium oxide according to Odegaard et al. [43]. Iron(II) detection was done by reaction with thiocyanate (KSCN, 160 $g \cdot L^{-1}$) in acidic medium according to classic protocol [42]. Iron(III) was confirmed by reaction with potassium ferricyanure ($K_3[Fe(CN)_6]$, 100 $g \cdot L^{-1}$) in acidic medium following [44]. All microchemical analyses, except protein test, were done under a binocular microscope.

2.4. FT-IR analysis

Preparation of samples was made according to the protocol described in Fig. 1. The solvents, dichloromethane and water, were chosen according to Sarmiento et al. [23]. Organic fraction was split into two in order to perform both FT-IR and GC–MS analyses.

Table 2
Characteristic chemical markers of some fresh and aged materials found in mummification balms [25,34–41].

	Botanical or animal origin	Chemical markers
Oils/fats	*Oils*: castor, balanos, safflower, horseradish, linseed, sesame, olive, almond, radish, colocynth, lettuce, poppy, cinnamon, tiger nut, rape *Fats*: beef and mutton's tallow, pig, duck and goose fat, cow, goat and sheep's milk, hen's eggs	*Biomarkers*: fatty Acid (FA), Sterol, Tri- and Di-acylglycrols (DAG & TAG). For more precise identification see [26,35]. ↙ *Oxidation of unsaturated FA*: dicarboxylic acid, ↙ *Hydroxylation of FA*: hydroxyl or dihydroxy FA, ↙ *Heating treatment*: long chain lactones, long chain ketones
Beeswax	Bees	*Biomarkers*: Palmitic acid ester (W), n-alcane (AL), FA, n-Alcool ↙ *Hydroxylation of W*: hydroxylated hexadecanoic acid esters
Mastic resins	Anacardiaceae (species *Pistacia lenticus*)	Oleanonic, Moronic, 11-hydroxyoleanolic, masticadienoic acids ↙ *Degradation products*: 28-norolean-17-ene-3-one
Olibanum resins	Burseraceae (*Boswellia*)	Boswellic, O-acetyl boswellic, 24-lupeolic, 3-O-acetyl-lupeolic, 11-ceto-β-boswellic, 3α-cetyl-11-ceto-β-boswellic acids ↙ *Heating treatment*: 24-noroleana-3,12-diene, norursa-3,12-diene, 24-norlupa-3,20(29)-diene
Conifer resins	Pinaceae (*Abies*, *Pinus*, *Cedrus*) Cupressaceae (*Cupressus*, *Juniperus*, *Tetraclinis*)	*Biomarkers*: Δ8-isopimaric, pimaric, sandaracopimaric, isopimaric, levopimaric, palustric, abietic, neoabietic acids, larixyle acetate ↙ *Natural oxidation products*: dehydroabietic (DHA), 15-DH-DHA, 7-oxo-DHA, 15-OH-7-oxo-DHA, 7,15-diOH-7-oxo-DHA ↙ *Heating treatment*: 18 and 19 nor-abietatriene, tetrahydroretene, retene, 15-OH-DHA, methyl-dehydroabietate, 7-methyl-retene, 18-nor-7-oxo abietane
Bitumen	Transformation of ancient organisms and algae	Hopanes, steranes, polycyclic aromatic hydrocarbon, AL

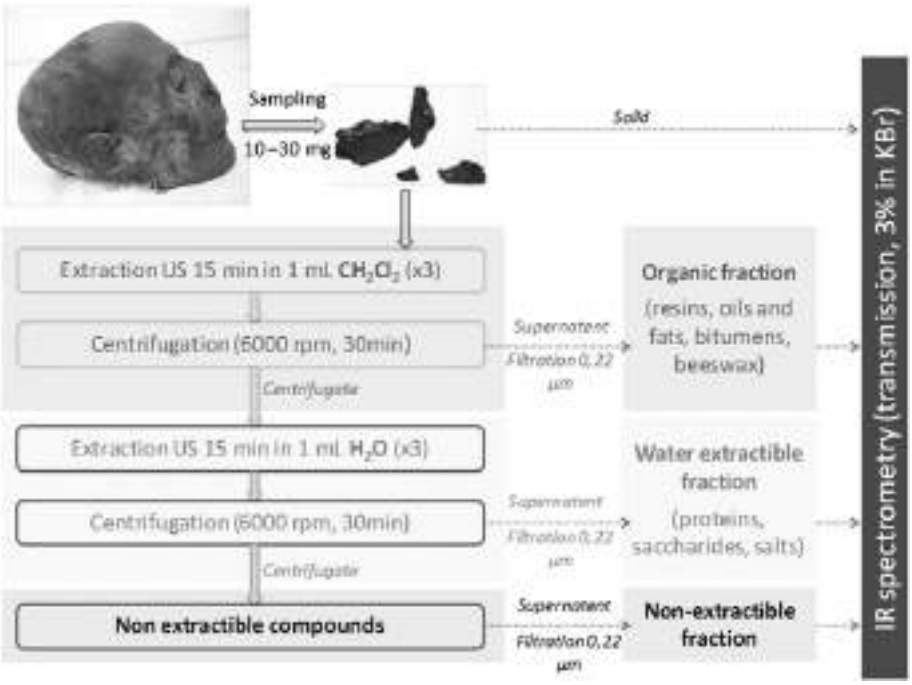

Fig. 1. Extraction protocol and preparation of the samples. (US = Ultrasound).

Each fraction was mixed with 200 mg of KBr, homogenized and pressed under 10 T/cm^{-2} in order to form a thick KBr pellet. These samples were directly analysed by infrared spectroscopy.

The FT-IR spectroscopy analyses were made with a Thermo-Nicolet AVATAR 360 FT-IR spectrometer in transmission mode with OMNIC software. All FT-IR spectra were collected in the middle infrared (400 to 4000 cm^{-1}) recording 64 scans.

2.5. Preparation of the samples

All organic fractions were analysed by GC–MS. These fractions were previously derivatised by trimethylsilylation. For this purpose the solutions were evaporated to dryness and mixed with 500 µL of pyridine, 450 µL of hexamethyldisilazane (Sigma Aldrich, St Louis, USA) and 300 µL of trimethylchlorosilane (Sigma Aldrich, St Louis, USA) for 30 min. The trimethylsilylated extract was dried under a stream of nitrogen, dissolved in 0.5 to 1 mL of hexane, filtered on 0.22 µm filters (Sartorius Stedim Biotech, Goettingen, Germany). 1 µL of this solution was injected in the GC–MS apparatus in triplicate.

2.6. GC–MS conditions

GC–MS analyses were performed using a Varian Saturn 3900 gas chromatograph equipped with a Varian 1177 injector and coupled with a Varian 2100 T ion trap mass spectrometer (Varian, Walnut Creek, CA, USA). The GC column was a fused silica capillary column Varian CP-Sil 8 CB low bleed/MS (30 m length × 0.25 mm i.d. × 0.25 µm film thickness). Molecular components were eluted using helium as carrier gas at a constant flow of 1 mL·min^{-1} with the following temperature programme of the oven: 50 °C for 2 min, 50–250 °C at 8 °C·min^{-1}, 250–350 °C at 3 °C·min^{-1}, and let at 350 °C during 20 min. 1 µL of each sample was injected with a splitting ratio of 1:20 and injector temperature was set at 250 °C. Mass spectra were recorded in electron impact (EI) mode with an electron ionization voltage of 70 eV, an ionization time of 25,000 µs and a mass range of 40–650 m/z. Transfer line, ion trap and manifold temperatures were respectively set at 300 °C, 200 °C and 50 °C.

2.7. Identifications

Identifications were done with the help of our laboratory mass spectrum databank (from fresh and artificially aged materials like conifer resins, mastic resin, beeswax, oils and from commercial standards), NIST 2008 databank and comparison of mass spectrum with published ones [25,29,34,37,45–47].

3. Results and discussion

3.1. FT-IR results

3.1.1. Global spectra

In the global spectra, all samples exhibited similar global spectra showing methylene (2926 and 2850 cm^{-1}), hydroxyl (3430 cm^{-1}), and carbonyl (1690–1740 cm^{-1}) groups. This confirmed that all samples contain some organic material [21]. Global infrared spectra are often very complex and dominated by organic material absorption. Thus, without preparation, this technique does not give maximum information about the sample. Therefore, the analyses were carried out after extractions in dichloromethane (organic compounds), and water (salts, saccharides and proteinous material), and for the non-extractable fraction (inorganic and non-extracted organic contents). Identifications were done by comparison with commercial standards and data from the specialized literature. All given wavenumbers may differ slightly from sample to sample depending on the chemical environment.

3.1.2. Organic fraction

In the organic fraction, very similar spectra were obtained for all the samples as shown on Fig. 2. From the absorption bands, main organic components were present in all balms (O═H stretching around 3400, CH_2 and CH_3 stretching between 2800 and 2950, C═O stretching between 1690 and 1750). Furthermore, all spectra show fat or oil infrared signal notably with absorption bands centred at 720 and 1166 cm^{-1}.

In samples B9, B10, B13, B26 and B32, the specific shape of infrared spectrum shows a high C–O absorption band centred on 1243 cm^{-1}. Such absorbance can be due to highly oxidized chemical contents as

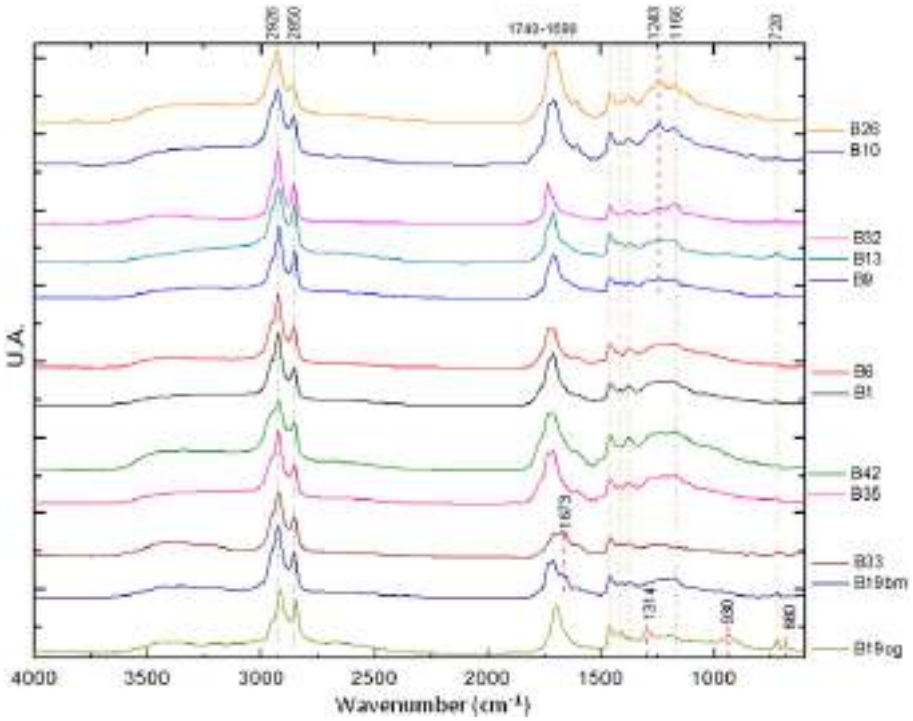

Fig. 2. Infrared spectrum of organic fractions of the different balms.

found in thermally aged conifer resin [48]. B33 and $B19_{bm}$ have a specific absorption band centred on 1673 cm^{-1}. $B19_{og}$ is very different from the other spectra with unidentified absorption bands at 680, 930 and 1314 cm^{-1}. For C═O absorption band, fresh material analysis shows that resins present a maximum between 1690 and 1705 cm^{-1} depending on terpenic resin type, and around 1735–1740 for fats, oils and beeswax (data not shown), which is well correlated with other studies [17,18]. However, this band shifts during the degradation process. Thus, in complicated aged mixtures, as balms, this band is not suitable for identifications. For the organic fraction, the results have been completed and validated by GC–MS experiments.

3.1.3. Water extractible fraction

In water extractible fraction (Fig. 3), hydrophilic materials, such as soluble proteins, saccharides (gums, etc) and inorganic salts, were found. All materials were identified according to the presence of the specific absorption bands listed in Table 1 for organic materials.

First of all, the specific signal of SO_4^{2-}, with absorption band centred on 620 and 1130 cm^{-1} is present in all samples in agreement with literature [19,24]. The presence of sulphate was confirmed by microchemical test for specific sulphate detection (reaction with $BaCl_2$, [42]). Sulphate may come from the natron salt commonly used by the embalmers in order to desiccate the dead body [49]. In fact, natron is composed of

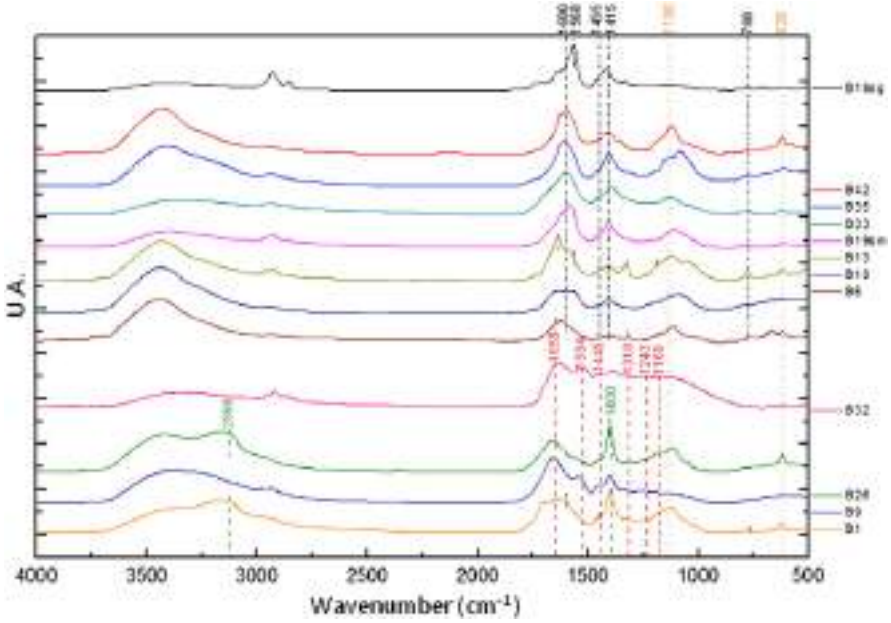

Fig. 3. Infrared spectrum of water extractible fractions of the different balms.

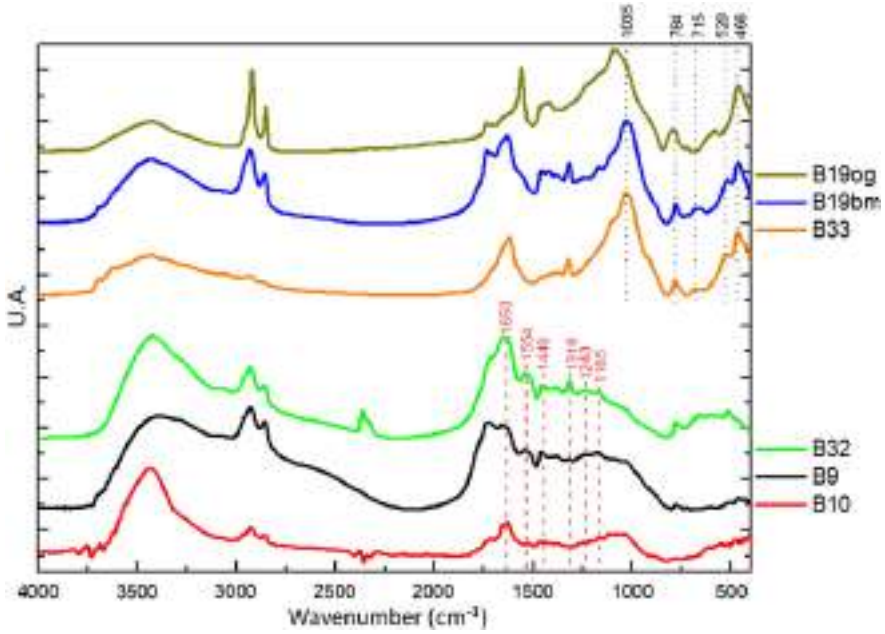

Fig. 4. Infrared spectrum of non-extractible fractions of the different balms.

sodium carbonate, sodium sulphate, traces of sodium bicarbonate, sodium chloride and gypse ($CaSO_4$) in varying proportions [50,51]. Lucas [13] quantified the proportion of sodium sulphate in natron from traces to 70% depending on the sample and the localization of the deposit (Wadi, El Kab). In this way, calcium carbonate signal (with low 710, 880 and 1430 cm^{-1} absorption bands [21]) is weak but present in samples B10, B13, B19og, B19bm and B35. The band at 1430 cm^{-1} is often partly or completely hidden by gum and protein C–H deformation or ammonium signal. Besides, characteristic bands of sodium carbonate may be masked by those of calcite as already mentioned in the literature [21]. In samples B1 and B26, the signal of ammonium can be seen with a fine and intense absorption band at 1400 cm^{-1} confirmed by a broad band between 3030 and 3300 cm^{-1} [23,24]. These absorptions most probably refer to NH_4Cl salt or Amun salt. Such material was mined and traded in ancient Egypt notably in the oasis of Siwa where is located the temple of Amun [52]. Forbes [53] wrote that the salt of Amun could be used as a sort of natron in ancient Egypt. Another hypothesis is that ammonium comes from the hydrolysis of proteins present in these two samples, during the extraction or during the natural ageing of the balm.

B1, B6, B9, B13, B26 and B32 samples showed infrared fingerprints of water soluble proteins with absorption bands at 3295 cm^{-1} (bonded NH stretching), 1650 cm^{-1} (C=O absorption of amid I), 1554 cm^{-1} (deformation of amide II), 1449, 1318, 1243 and 1169 cm^{-1} (unassigned bands). Moreover, great similarities can be found between B9 and albumin infrared spectra. The presence of protein was confirmed by specific microchemical tests (pyrolysis in the presence of $CaO_{(s)}$, [43]). Furthermore, different absorption bands around 1600, 1455, 1415 and 780 cm^{-1}, and shoulders at 1149 and 1035 cm^{-1}, are present on B1, B6, B10, B13, B19bm, B33, B35 and B42 infrared spectra. Such fingerprint is specific of a saccharide material. Polysaccharides may come from different sources including wax, different sorts of gums, etc.

The B19og infrared spectrum is very specific and shows two intense bands centred on 1410 and 1568 cm^{-1}, characteristics of carboxylate function. Such compounds could be formed by a saponification reaction between a fatty acid and an unknown cation. Such conclusion is coherent with organic fraction analyses, which showed the presence of oil or fat.

Fig. 5. Picture of the B33 and B19 skulls.

Table 3
Overview of different material identifications in the balms. The table is based on GC–MS results for the organic fraction and FT-IR results for both water extractible and non extractible fractions.

	Organic fraction	Water extractible fraction	Non extractable fraction
B1	Fat, beeswax, mastic resin	Na_2SO_4, NH_4Cl, polysaccharides proteins	Unknown organic material
B6	Oil or fat, beeswax	Na_2SO_4, polysaccharides, proteins	–
B9	Fat, *brassicaceae* oil, beeswax, conifer resin	Na_2SO_4, Albumin	Insoluble proteins
B10	Oil or fat, conifer resin	Na_2SO_4, $CaCO_3$, polysaccharides	–
B13	Fat, beeswax, conifer resin	Na_2SO_4, $CaCO_3$, proteins, polysaccharides	Unknown organic material
B19bm	Oil or fat	Na_2SO_4, $CaCO_3$, polysaccharides	Ochre
B19og	Oil or fat	Na_2SO_4, $CaCO_3$, saponified carboxylate acid	Ochre
B26	Oil or fat, conifer resin	Na_2SO_4, NH_4Cl, proteins (probably albumin)	Unknown organic material
B32	Fat, beeswax, conifer resin	Na_2SO_4, proteins	Insoluble proteins
B33	Oil or fat	Na_2SO_4, polysaccharides	Ochre
B35	Oil or fat, beeswax	Na_2SO_4, $CaCO_3$, polysaccharides	Unknown organic material
B42	Oil or fat, beeswax	Na_2SO_4, polysaccharides	Unknown organic material

Table 4
Chemical markers of oils and fats found in the different balms (G = Glycerol, FA = Fatty Acid, DA = Dicarboxyllic Acid.* Presence of threo and erythro isomers. All molecules (excepted cholesta-3,5-dien-7-one) were detected in trimethylsilylated form.

	G	Saturated FA	Other FA	DA	Sterol
B1	x	14, 15, 16,17, 18, 20, 24	16:1Δ9, 16 OH9,10*, 18 OH9,10*	8, 9	Cholesterol, cholestanedienol
B6	x	9, 14, 15, 16, 17, 18, 20, 24	16:1Δ9 16 OH9,10*, 18 OH9,10*	4, 5, 7, 8, 9	
B9	x	9, 10, 12, 13, 14, 15, 16, 17, 18, 20, 22, 24	16:1Δ9, 20:1Δ11, 16 OH9,10*, 18 OH9,10*, 22 OH12,13*	7, 8, 9, 10	Cholesterol, cholestadienol, cholesta-3,5-dien-7-one
B10	x	8, 9, 10, 14, 16, 18	16:1Δ9	9	
B13	x	8, 9, 12, 13, 14, 15, 16, 18	14:1Δ9, 16:1Δ9	8, 9	Cholesterol
$B19_{bm}$	x	8, 9, 10, 14, 15, 16, 17, 18	18:1Δ6, 16 OH9,10*, 18 OH9,10*	6, 7, 8, 9	
$B19_{og}$	x	8, 9, 10, 13, 14, 15, 16, 17, 18, 20, 22, 24	16 OH9,10*, 18 OH9,10*	4, 5, 6, 7, 8, 8 OH2, 9, 10	
B26	x	8, 9, 10, 12, 14, 15, 16, 18		7, 8, 9	
B32	x	14, 15, 16, 18	16:1Δ9		Cholesterol, cholestanedienol
B33	x	8, 9, 10, 12, 13, 14, 16, 18	18:0 OH7	8	
B35	x	14, 16, 18		8, 9	
B42	x	8, 9, 10, 12, 14, 16, 17, 18, 20, 22, 24, 26		4, 5, 9	

3.1.4. Non-extractible fraction

Different samples (B6, B10) did not present relevant quantities of the non-extractable fraction and others (B1, B13, B26, B35, B42) exhibit weak signal of unknown residues of non-extracted or polymeric organic material. All other spectra are given in Fig. 4. From these data it can be distinguished that there are two types of materials: insoluble proteins and minerals. In B9 and B32, infrared signal shows specific fingerprint of proteins (cf Table 1). Such results can probably be due to the presence of hairs, and thus keratin, in the balm. B33, B19og and B19bm analyses presented different absorption bands: (i) from iron oxide at 466 and 520 cm^{-1}, (ii) from silicate at 1035 cm^{-1}, and (iii) from quartz at 784 and 715 cm^{-1}. The presence of Fe^{2+} and Fe^{3+} anions was confirmed by specific microchemical tests (reaction with potassium ferricyanure and thiocyanate, [42,44]). Thus, in these three samples, there was at least iron oxide and clays, which entails the use of ochre in the mummification process. Such material was used by the Egyptians in order to dye the linen since the earliest dynasties [14]. In addition, the two mummy heads have an orange-red dyed bandage filling and covering the cranium (cf Fig. 5). Ochre may come from the dying of the linen bandage used in the mummification process.

3.1.5. GC–MS results

In order to validate FT-IR results and to have a more precise identification of organic components in balms, GC–MS analyses were carried out. We found the presence of 3 types of materials in the different balms: fat or oils, waxes and resins.

3.1.6. Fats and oils

Fat and oil chemical compositions are given in Table 4. All the samples contain biomarkers of aged oils and fats. The association of Glycerol (G), fatty acids (FA), dicarboxylic acids (DA) and sterol is characteristic of the presence of oils and fats in the samples [22].

In order to identify if it is whether oil or fat, a common method is to quantify the C16:0/C18:0 ratio [22,47,54]. However, in the case of mummification balms, different factors do not allow the use of this ratio: (i) lipid thermal degradation leading to changes in saturated fatty acid proportion [22,55], (ii) oxidized beeswax containing large amount of palmitic acid coming from the hydrolysis of palmitate esters [56], and (iii) embalmers could have used a mixture of different oils or fats.

In B1, B6, B9, $B19_{bm}$ and $B19_{og}$, the presence of azelaic acid (D9) and dihydroxy fatty acids (FA16 OH9,10 and/or FA18 OH9,10) indicates that an oxidation in position 9 of the alkyl chain has occurred on a Δ9 unsaturated fatty acid (oleic or palmitoleic acid), as illustrated in Fig. 6. Such compounds produce a 317 Da mass fragment.

As mentioned by Colombini et al. [46] and Copley et al. [47], *brassicaceae* oils (radish, turnip or mustard) contain a high amount of oleic acid (FA18:1Δ9) leading to high amount of azelaic acid. Furthermore, such oils have high concentrations of gondoic (FA20:1Δ11) and erudic (FA22:1Δ13) acids [46]. Such unsaturated carboxylic acids undergo thermal degradation in the dihydroxyl derivates (as FA22 OH12,13 threo and erythro) [46]. All this compounds (DA9:0, FA20:1Δ11, FA18 OH9,10 and FA22 OH12,13 threo and erythro) were found in B9 as indicated in Fig. 7. Moreover, in this sample cholesterol and its degradation

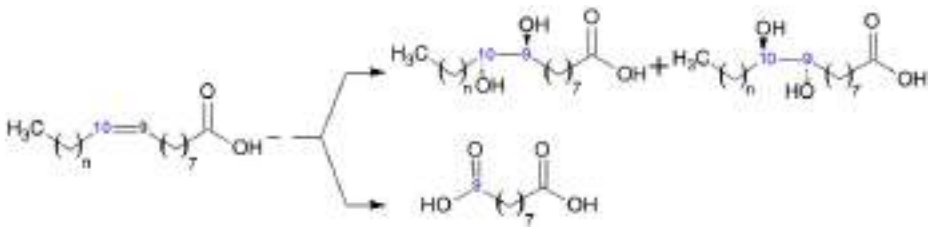

Fig. 6. Major oxidation products of oleic and palmitoleic acid found in mummified balms.

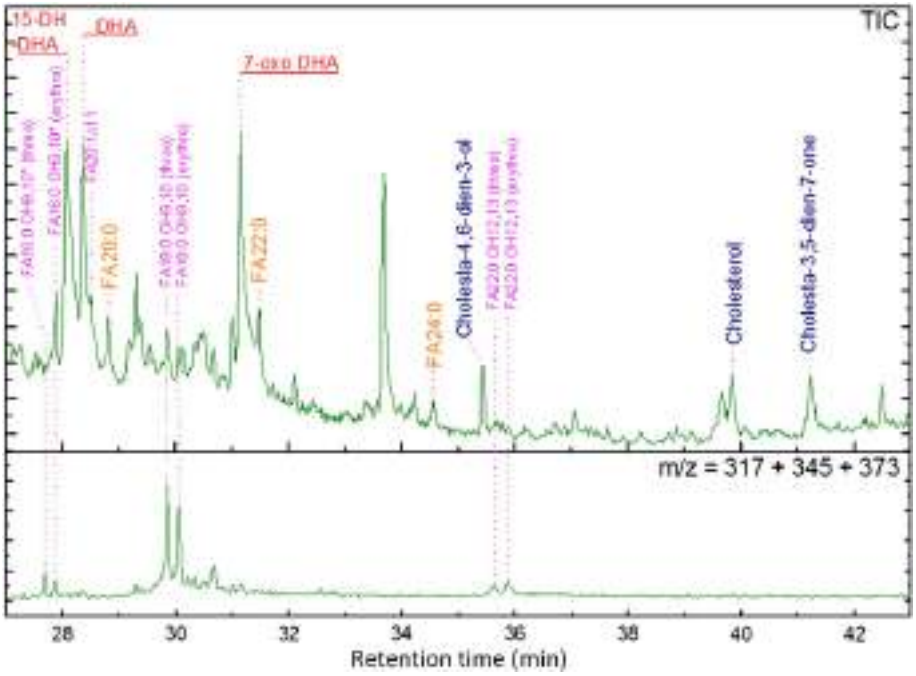

Fig. 7. Partial chromatogram of B9 samples with TIC signal and extracted signal of addition of m/z 317 + 345 + 373. m/z 317 is the base peaks of FA16 OH9,10 and FA18 OH9,10, m/z 345 of FA20 OH12,13, m/z 373 of FA22 OH12,13. All molecules (excepted cholesta-3,5-dien-7-one) were detected in trimethylsilylated form.

products were found which indicates the possible co-occurrence of animal fat and *brassicaceae* oil.

The presence of cholesterol and its degradation products, characteristic of the presence of fat in the mixture, was found in B1, B9, B13 and B32. It is important to note that human tissue can be a source of pollution that leads to false interpretation of fat in archaeological samples. However, those samples were collected from balms over the bandage, highly limiting the possibility of contamination from the body.

To conclude, all samples were made with oil and/or fat, showing their importance as bases for mixing the other ingredients.

3.1.7. Beeswax

Fresh beeswax is composed of different molecules [37,57,58], including: (i) linear alkanes (Al) and fatty acids, widely distributed in natural products' chemical compositions, and (ii) different esters including notably palmitate or wax esters (W). Palmitate esters have very specific mass spectrum with a high 257 fragment [37].

Nevertheless, Regert et al. [36] have pointed out the possibility of the loss of different compounds during the natural ageing of the sample, especially linear alkanes. Association of wax ester and linear alkanes has been reported as highly characteristic of the presence of beeswax. All chemical markers found in the different balms are shown in Table 5.

Table 5
Chemical markers of beeswax found in the different balms.

	Wax ester (W)	Linear alkanes (Al)
B1	40, 42, 44, 46, 48	27, 29, 31
B6	40, 42, 44, 46, 48	25, 27, 29, 30,31, 33
B9	40, 42, 44, 46	29
B10	–	–
B13	40, 42, 44	27, 29, 31
B19bm	–	–
B19og	–	25, 27, 29
B26	–	–
B32	40, 42, 44, 46, 48	25, 27, 29, 31
B33	–	25, 27, 29, 31
B35	40, 42, 44	27, 31, 33
B42	40, 42, 44, 46, 48	27, 29, 31, 33, 35

As mentioned in Fig. 8, the chromatogram of the organic extract of B42 shows characteristic pool of chemical markers of beeswax. In this way, beeswax was identified in several samples (B1, B6, B9, B13, B32, B35, and B42).

This is in good accordance with literature on mummification process. Indeed, Plinus [59] and Lucas [13] indicated the use of beeswax to cover incisions and body apertures, mainly localized on the head (nose, eyes, mouth and ears).

3.1.8. Terpenic material

Different chemical markers of two different natural resins (Table 6) were also found. First, samples B9, B10, B13, B26, and B32 contain diterpenic compounds, specific of resins exuded from conifer trees. In fact, all identified compounds come from abietic acid, a molecule present in fresh conifer resins [22,25,31,34]. This molecule undergoes degradation reactions through time [25] leading to dehydroabietic acid (DHA). DHA can also undergo: (i) different reactions of oxidation leading to 7-oxo-DHA and 15-OH-7-oxo-DHA and (ii) dehydrogenation leading to 15-DH-DHA. Such compounds are widely cited in the literature and all mass spectra are already published [25,34]. All these degradation markers were found in the balms mentioned above, as shown in Fig. 9 for the chromatogram of the B26 sample. In this sample, triterpenic molecules are non specific and do not give any information.

In B1 and B6, the second type of resin was identified by the presence of different triterpenic molecules with oleanane, lupene, lanostane and masticadiene chains. The presence of such a pool of chemical markers is characteristic of mastic resin exuded from *Pistacia* trees. In this way, Fig. 10 shows the partial chromatogram of the B6 sample. More precisely, oleanonic acid and especially moronic acid are biomarkers present in fresh resin, and are relatively stable. These two compounds have a very characteristic fragmentation (526, 511, 409/408, 391, 306, **203**, 189, 119, 105, 91, 73). These two isomers can be easily distinguished based on their retention times and their fragmentation, with an abundant fragment at m/z 408 specific of oleanonic acid shifted at m/z 409 for moronic acid. 28 Nor-17(18) oleanen-3-one was previously shown to be produced during the heating of mastic resin [60]. However, as such compound is naturally present in mastic resin in low proportion, anything can be concluded about the heating treatment of the sample. 28

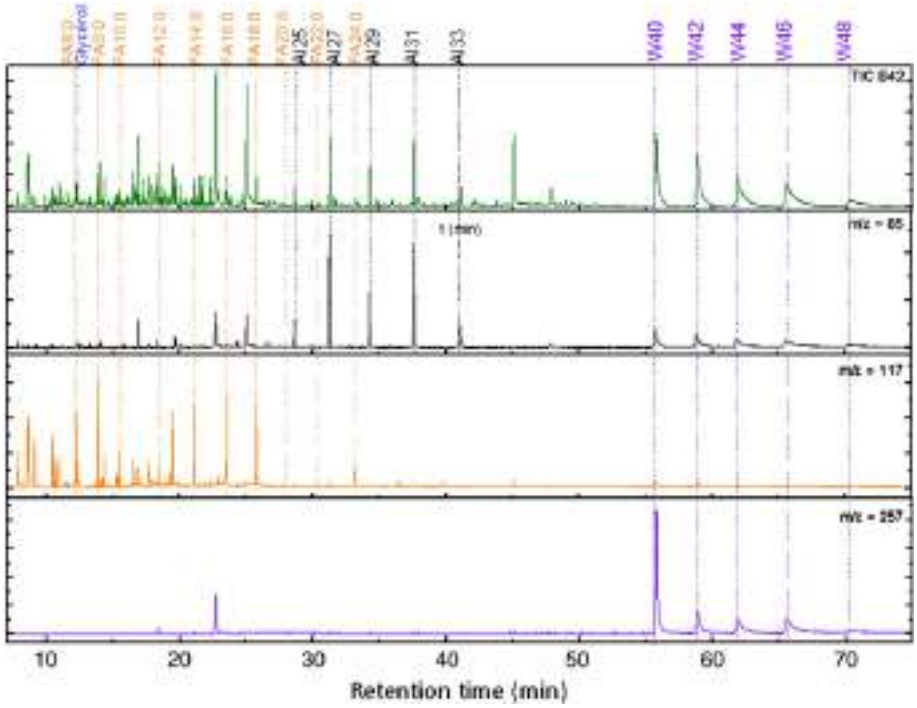

Fig. 8. Chromatogram of B42 samples with TIC signal and extracted signals of m/z 85, 117, and 257. m/z 85 is intense in linear alkanes, m/z 117 is the base peak of main saturated fatty acids, m/z 257 is intense in palmitate esters. FA and G molecules were detected in their trimethylsilylated form.

Nor-17(18) oleanen-3-one has a very specific fragmentation with an abundant fragment at m/z 163 and 191 due to a retro Diels–Alder reaction in cycle C specific of the presence of a double bond between carbons 17 and 18 [45,61]. Occotillone-type molecules are characteristic of previously oxidized dammarane compounds [62]. They have a mass spectrum with a prominent base peak at m/z 143 [60,62]. Different components, noted U1, with characteristic fragmentation (183, **198**, 216), were not identified in this study. Unknown oleanene compounds were named U2.

These results are really coherent in the archaeological context, as conifer resin was cheap and abundant in ancient Egypt [14,21,63,64], and mastic was previously found in mummification balms [5].

3.1.9. Correlation between FT-IR spectroscopy and GC–MS

Many results were well correlated by the two analytical techniques. In fact, all samples that included fat, identified by GC–MS, in their composition also contain some proteins, identified on the basis of infrared spectrum, sometimes very close to albumin. This is pertinent because fats usually contain high amounts of animal proteins [14]. Moreover, all samples with infrared absorption bands at 1243 cm^{-1} (C–O bond) display abietane compounds and thus, conifer resin in their composition. This result is well correlated with literature [65]. Finally all materials show characteristic infrared signals (with absorption bands centred on 720 and 1166 cm^{-1}) and chemical marker signals (FA, DA, G, sterols) of oils and fats.

Table 6
Chemical markers of diterpenic and triterpenic resins found in the different balms, DHA = Dehydroabietic acid (DHA), DH = Dehydro. All molecules, excepted 28 nor-17(18) oleanen-3-one and lup-20(29)-en-3-one, were detected in trimethylsilylated form.

	Diterpenes	Triterpenes
B1	–	28 nor-17(18) oleanen-3-one, lanosterol, occotillone molecules, moronic and masticadienoic acids
B6	–	Lanosterol, 28 nor-17(18) oleanen-3-one, lanosterol, lup-20(29)-en-3-one, occotillone molecules, 3-oxoolean-12, 15-dien-28-oic, moronic, oleanonic, masticadienoic and isomasticadienoic acids
B9	15-DH-DHA, 15-OH-7-oxo-DHA, 7-oxo-DHA, DHA	–
B10	15-DH-DHA, 15-OH-7-oxo-DHA, 7-oxo-DHA, DHA	–
B13	DHA	–
B26	15-DH-DHA, 7-oxo-DHA, 15-OH-7-oxo-DHA, DHA	Epi lupeol, α-amyrine, β-amyrine
B32	15-DH-DHA, 15-OH-7-oxo-DHA, 7-oxo-DHA, DHA	

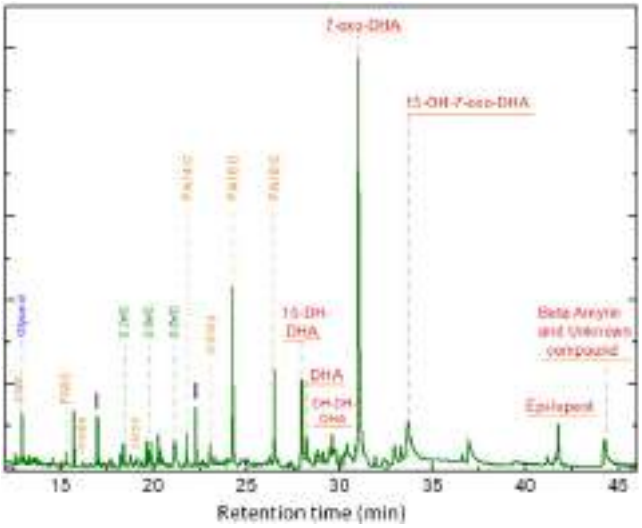

Fig. 9. GC–MS Chromatogram of B26 samples (TIC signal). All molecules were detected in trimethylsilylated form.

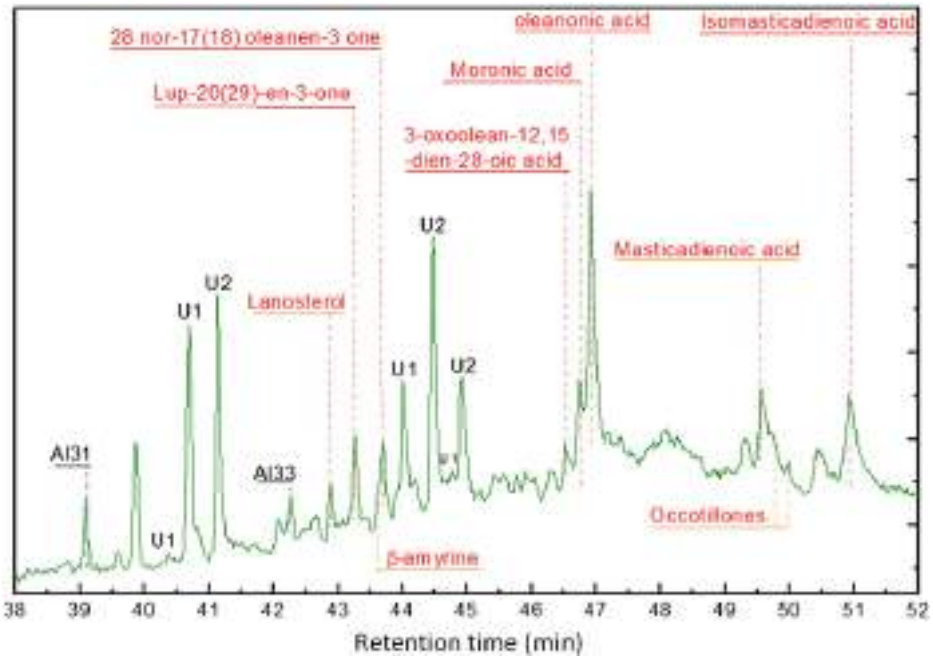

Fig. 10. GC–MS Chromatogram of B6 samples (TIC signal). U1: unknown compounds with characteristic fragmentation (183, **198**, 216), study. U2: unknown oleanene molecules. All molecules, excepted linear alkanes, 28 nor-17(18) oleanen-3-one and lup-20(29)-en-3-one, were detected in trimethylsilylated form.

4. Conclusion

This study aimed for the identification of the composition of several Egyptian balms from the *Musée des Confluences* (Lyon, France). It was found that those balms were made of complex organic and inorganic mixtures (oils, fats, beeswax, resins, proteins, polysaccharides and minerals). This work clearly shows the high variability of formulation changing from sample to sample. All identifications are given in Table 3. The combine use of FT-IR spectroscopy and GC–MS reveals the great complementarity of those two analytical tools.

For FT-IR, after applying a simple extraction protocol, the analysis allowed the identification of: (i) inorganic salts (carbonate, sulphate and ammonium salts) possibly used as natron in the ancient time, (ii) proteins and polysaccharides, and (iii) ochre used in order to dye the bandages. The FT-IR analyses pointed out the occurrence of a saponification reaction, probably between fatty acids and a cation.

GC–MS is a destructive technique for the analyses of the archaeological materials. However, it enabled a more precise identification of chemical components. GC–MS analyses showed the common use of oils, fats and waxes for the making of a balm that covered mummy heads. These materials were often used in combination or mixed with conifer or mastic resins. Many identified byproducts indicated degradation reactions taking place in such archaeological material. For instance, the oxidation products of many saturated fatty acids (dihydroxylated fatty acids and dicarboxylic acids) and the degradation products of abietane (dehydroabietic acid, 15-dehydro-dehydroabietic acid, 15-hydroxy-7oxo-dehydroabietic acid, 7-oxo-dehydroabietic acid), dammarane (occotillone type molecules) and oleanene (28 nor-17(18) oleanen-3-one) molecules were found in the samples. These results show that (i) oil and/or fat were used as base for mixing other materials, (ii) beeswax was often used in head mummification, and (iii) the most common resin employed was the conifer resin.

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