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ORIGINAL PAPER

Back to the roots: dental calculus analysis of the first documented case of coeliac disease

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Abstract
Dental calculus of a Roman woman (late first century–early second century CE), supposed to be the first historical evidence of coeliac disease (CD), was subjected to archaeobotanical investigations for reconstructing diet and phytotherapeutic practices. Light microscopy provided the proof she came in contact with gluten-rich cereals (i.e. Triticeae, Aveninae), which, maybe, were deleterious for a CD genetic predisposed individual like her. Gas-chromatography mass-spectrometry revealed that the young woman ingested, at least once in lifetime, plant foods and several herbs (e.g. Brassicaceae, *Mentha* sp., *Valeriana* sp., Apiaceae, Asteraceae, grapes or wine, honeydew or manna). About the latter, surprisingly, markers of *Curcuma* sp. and *Panax* sp. were detected. The consumption of these rhizomes, already used in the ancient Oriental medicine, supported the existence of cultural contact and exchange with the Eastern Asia. Encouraged by modern knowledge, we hypothesised that the inhumate used these roots as natural remedies to soothe her pathological condition. Our data provided information about the key role of the ethnobotany in Roman Imperial age.

Keywords Gas-chromatography mass-spectrometry · Light microscopy · Starch granules · Plant drugs · Secondary metabolites · Exotic rhizomes

Abbreviations

LM	Light microscopy
GC-MS	Gas-chromatography mass-spectrometry
CD	Coeliac disease
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid

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Introduction

Since time immemorial, plants have played a key role in human health and nutrition. In particular, wild species represented an essential part of the diet and their use was based on the capacity to distinguish edible plants from poisonous ones.

Moreover, ancient communities sought natural drugs for treating ailments (Guarrera and Savo 2016; Petrovska 2012). Indeed, it is well-known that plant organs (e.g. fruit, seed, root, leaf) and their derivatives (e.g. honey, resin, wine) contain both energetic metabolites (e.g. carbohydrates, essential amino acids) and bioactive compounds (e.g. polyphenols, alkaloids, terpenes) (Gurib-Fakim 2006; Guimarães et al. 2009; Christaki et al. 2012; Gismondi et al. 2018a).

In history, medicinal and aromatic plants were applied in ritual activities, food contexts (e.g. flavouring agents, salt substitutes) and medicine (Boi 2012; Inoue et al. 2017; Iskandar and Iskandar 2017). The oldest written sources (5000 years ago) on medicinal plants were found in Asia and mainly derived from Chinese and Ayurveda tradition (Petrovska 2012). These texts strongly influenced knowledge and application of natural drugs until the end of the Middle Ages, passing through ancient Greek pharmacotherapy (Kelly 2009; Gruber and DerMarderosian 2015). For instance, *Hippocratic Collection* provided a list of about 300 plants and relative application (e.g. garlic and celery as diuretics, pomegranate and oak as astringents) (Petrovska 2012; Touwaide and Appetiti 2013). Theophrastus (371–287 BCE), the "father of botany", described about 500 medicinal species in *Historia Plantarium*, while Aulus Cornelius Celsus, the most famous Roman medical writer (25 BCE–50 CE), documented in *De Medicina* the application of natural therapies in clinical cases (Petrovska 2012; Inoue et al. 2017). However, Pedanius Dioscorides, the founder of the pharmacognosy, illustrated and described about 600 plants, including the procedures for the extraction of their bioactive compounds (*De Materia Medica*, first century CE). In this treatise, Fabaceae seeds were cited in dermatology, and Apiaceae and Rosaceae fruits in gastroenterology, while Lamiaceae leaves were recommended for respiratory and gastrointestinal diseases (Staub et al. 2016). Similarly, Pliny the Elder (23–79 CE, in *Historia Naturalis*) and Galen (131–201 CE, in *De Simplicium Medicamentorum Facultatibus*) added new plant drugs to the existing lists (Petrovska 2012; Dikshit et al. 2016). All these works on ancient Mediterranean herbal medicine maintained the highest authority until the Renaissance (Evergetis and Haroutounian 2015; Dikshit et al. 2016; Staub et al. 2016).

Scientific investigations aimed at understanding the therapeutic use of natural extracts may confirm the value that plants have had in human evolutionary history. The present archaeobotanical study is a clear example of such type of research. In particular, our work was focused on the analysis of the dental calculus of an 18–20-year-old female individual found in the archaeological site of Cosa (Ansedonia, Grosseto, Tuscany, Italy). Archaeologists dated the burial to late first century–early second century CE, according to tomb architecture ("alla cappuccina") and grave goods. In detail, ceramics and jewellery (two golden earrings, a golden button and three rings with gems) were found with the corpse, suggesting she belonged to a wealthy family (Agricoli et al. 2012).

The skeletal remains showed osteoporosis, osteopenia, *coxa valga*, dental enamel hypoplasia and *cribra orbitalia*, pathological signs linked to bone marrow hypertrophy and anaemic conditions (Gasbarrini et al. 2010, 2012). All this evidence could be evocative of several pathologies, such as coeliac disease (henceforth CD), an immune-mediated disorder characterised by chronic inflammatory condition of the gastrointestinal tract, which determines malabsorption of nutrients (Tursi et al. 2001; Gasbarrini 2008; Gasbarrini et al. 2010; Volta and Villanacci 2011; Ehsani-Ardakani et al. 2013; Pinto-Sánchez et al. 2015; Zanchetta et al. 2016). In detail, CD is caused by a constant exposure to the gluten proteins. These molecules, rich in proline and glutamine aminoacidic residues, are contained in cereals, like wheat (i.e. gliadins, glutenins), rye (i.e. secalins), and barley (i.e. hordeins) (Cervino et al. 2018). Molecular analyses showed that the Roman young woman presented the HLA-DQ 2.5 haplotype, typically associated to high predisposition to CD (Gasbarrini et al. 2012; Amarapurkar et al. 2016), and an isotopic signature (Scorrano et al. 2014) which suggested chronic malnutrition. Thus, CD could have been the cause of her death, along with other complications usually associated to the disease. Therefore, it is possible that this pathology existed at that time. Indeed, Areteus of Cappadocia (second century BCE) used the Greek word "koiliakos", meaning "suffering in their abdomen", to describe the chronic wasting illness associated with diarrhoea, one of the main symptoms ascribable to CD (Rostami et al. 2004; Losowsky 2008; Gasbarrini et al. 2010).

To date, no other archaeological finding related to this illness has been documented. This encouraged us to study the dental calculus of this individual for reconstructing her food habits, since diet influences onset and development of CD.

Dental calculus is the mineralised oral plaque which sticks to tooth surfaces. This matrix is made up of inorganic salts (e.g. hydroxyapatite) and organic compounds, including molecules derived from foods (e.g. starch granules, plant metabolites), oral microbiota (e.g. DNA) and inhaled substances (e.g. pollen grains). As dental calculus preserves well in archaeological contexts, it has been widely used as a powerful instrument for studying paleodiet, ancient lifestyles, paleo oral microbiome and history transitions (Lindhe 1984; Lieverse 1999; Buckley et al. 2014; Power et al. 2015; Warinner et al. 2015; Cristiani et al. 2016; Radini et al. 2017; Arranz et al. 2018; Cristiani et al. 2018; Cummings et al. 2018; Eerkens et al. 2018; Hendy et al. 2018; Power et al. 2018; Bucchi et al. 2019). This investigation is not quantitative, due to the several factors which concur to its development. However, the microremains entrapped in the calculus are indicative of the substances passed through the oral apparatus, at least once in lifetime (Leonard et al. 2015).

Therefore, our first step was to verify if she had come into contact with foods containing gluten, searching for evidence of cereal starch granules by light microscopy (henceforth LM). Indeed, the exposure to dietary gluten in a CD genetically predisposed individual may increase small intestine chronic inflammation and induce malnutrition (Bratanic et al. 2010; Ehsani-Ardakani et al. 2013). Then, gas-chromatography mass spectrometry (henceforth GC-MS) analysis allowed us to identify, in qualitative terms, the main plants she used as therapeutic remedies.

Material and methods

Dental calculus sampling

Dental calculus was sampled from the skeleton of the young woman housed at the "Museo Archeologico Nazionale e Area Archeologica di Cosa" (Grosseto, Tuscany, Italy). Once removed, exercising a slight pressure through the use of a fine autoclaved dental curette, small calculus flakes were collected and transported in sterile microcentrifuge tubes to the laboratory of Botany (Dept. of Biology, University of Rome "Tor Vergata") for processing. Sterilisation and decontamination procedures, including relative validation protocols, were performed in laboratories dedicated to the study of ancient biomolecules (where contamination controls were regularly carried out on workspaces, instruments and supplies) (Gismondi et al. 2016; Crowther et al. 2014; Soto et al. 2019; Gismondi et al. 2018c). Dental calculus was treated by UV light for 10 min, immersed in 2% sodium hydroxide for 15 min, washed with sterilised water and dried at 37 °C. No microremain was detected by light microscopy in the samples after decontamination.

LM analysis

To extrapolate micro-debris from calculus, the method of Gismondi et al. (2018c) was applied with some modifications. In detail, hydrochloric acid (0.2 M) was used to dissolve the mineral matrix for 8 h. After three washes with sterilised ultrapure water, the pellet was resuspended in 100 μL of bidistilled water and glycerol (1:1), under a sterile hood (Heraeus HERAsafe HS12 Type), and placed on glass slides to be analysed at optic microscopy. Seventeen milligrams of dental calculus was analysed by LM (Nikon Eclipse E100) under polarised light (Table 1). Each microscopic remain was observed, captured and measured by ProgRes CapturePro 2.9.0.1 software. Starch granules were identified at genus or family level by direct comparison (sizes, structural characteristics and ultrastructural elements) with a modern starch experimental reference (Gismondi et al. 2019). This reference collection was already employed with success by the authors in Gismondi et al. (2018c) and Baldoni et al. (2018). Published data were also consulted for the taxonomic identification of other microremains and pollens (Martin and Harvey 2017; Travaglini et al. 2014).

GC-MS analysis

A qualitative GC-MS analysis was carried out according to Gismondi et al. (2018b) and Baldoni et al. (2018). In brief, 10 mg of dental calculus (collected from upper left canine, upper right 1st premolar and lower right 3rd molar) was solubilised and resuspended in hexane. Then, the sample was derivatised with Methyl-8-Reagent (Thermo Scientific), following manufacturer's guidelines, and loaded into chromatographic instrument at an injection temperature of 280 °C. The analysis, performed in a GC-MS QP2010 system

Table 1 Plant microfossils recovered from calculus flakes of the young woman by LM. Dental calculus locations, weight of the samples, amount and proposed identification of all detected microremains were reported.

Calculus location		Sample weight (g)	Detected microremains	Total	Proposed identification
Teeth*	Surface				
28	Lingual	0.003	Pollen grains	2	*Ligustrum* sp.
			Starch granules *Morphotype II*	8	Aveninae
10	Lingual	0.001	Plant fibres	2	–
16	Buccal	0.001	Plant fibre	1	–
24	Lingual	0.002	Phytolith	1	Poaceae
25	Lingual	0.006	Starch granules *Morphotype I*	41	Triticeae tribe
			Non-diagnostic starch granules	> 70	–
26	Lingual	0.002	Starch granules *Morphotype I*	60	Triticeae tribe
			Non-diagnostic starch granules	> 103	–
32	Lingual	0.002	Starch granules *Morphotype I*	3	Triticeae tribe
			Pollen grain	1	*Ligustrum* sp.

*Universal Teeth Numbering System

(Shimadzu, Japan; column DB-5 Phenomenex; helium as gas carrier) by a splitless injection mode, was repeated ten times. All chromatographic profiles were similar and showed no significant differences. Each compound was identified by direct comparison of its mass spectrum with those registered in the NIST (National Institute of Standards and Technology) Library 14 loaded into the detection software. Similarity values were considered acceptable only if higher than 85%. Food categories or specific plants ingested by the young woman, at least once during her lifetime, were extrapolated by studying and associating of the detected metabolites through scientific food databases (FoodDB 2013; TGSC 2015) and published data.

Results and discussion

Dietary habits and medical culture of the young woman found in the archaeological site of Cosa were reconstructed by archaeobotanical investigations on her dental calculus (Fig. 1).

Microscopic evidence of gluten-rich food consumption

By LM analysis (Table 1), we detected the presence of three *Ligustrum* sp. (Oleaceae) pollen grains, demonstrating the existence of this plant genus in the studied area. This approach also revealed three fragments of plant fibres, one Poaceae phytolith and 112 starch granules (Fig. 2), not including the small non-diagnostic grains (about 173 units) which were not easily countable (being included into aggregates) and did not show the distinct features for the taxonomical identification. Two different starch morphotypes were classified and described below, using the International Code for Starch Nomenclature (ICSN 2011). Both groups were assigned to the Poaceae, according to the morphological and morphometric parameters reported in our experimental collection (Gismondi et al. 2019).

Morphotype I This first class of starches showed a bimodal distribution (co-presence of small and large grains) of simple granules. All of them revealed morphological features typical of Triticeae tribe (e.g. barley, wheat). In particular, both large and small granules were oval to circular in shape and the size of the largest ones ranged from 10 to 37 μm in length to 6–27 μm in width. This morphology and dimension fit with those of the Triticeae granules described in our reference collection (Gismondi et al. 2019). Fifty-one starches were potentially attributable to *Triticum* sp., showing a centric-indistinct *hilum* and concentric-complete-distinct *lamellae*. Three other granules might be ascribable to *Hordeum* sp., for the presence of centric-indistinct *hilum*, eccentric-complete-indistinct *lamellae* and a longitudinal fissure. The remaining ones (forty-nine) were not classified at genus level because diagnostic characteristics were not distinguishable. Among them, sixteen starches have been probably subjected to modification events, such as grinding process, cooking procedure in water and/or exposure to alfa-amylase, according to their altered shape, as suggested by Copeland and Hardy (2018).

Morphotype II Dimension (ranging from 3 to 8 μm in length to 2–8 μm in width) and shape (polyhedral with centric and indistinct *hilum*) of this second type of granules was consistent with Aveninae starches. All these granules were detected as an aggregate of 8 units.

This finding suggested consumption, or handling, of cereals which were commonly used in many main dishes (e.g. polenta, gruels and soups). Indeed, similarly to ancient Greeks, Romans based their diet on cereals, olive oil and wine, the so-called Mediterranean triad. In particular, barley (*Hordeum* sp.) was mainly used by poor people, while wheat was preferred by the upper classes (Keenleyside et al. 2009; Borstad et al. 2018). The aetiology of CD is linked to several factors, both genetic and environmental (e.g. infections), which can trigger intolerance to ingestion of gluten synthesised in several common cereals, such as Triticeae (Green and Cellier 2007; Di Sabatino and Corazza 2009). The caryopses of these species, containing immuno-peptides (e.g. gliadin in

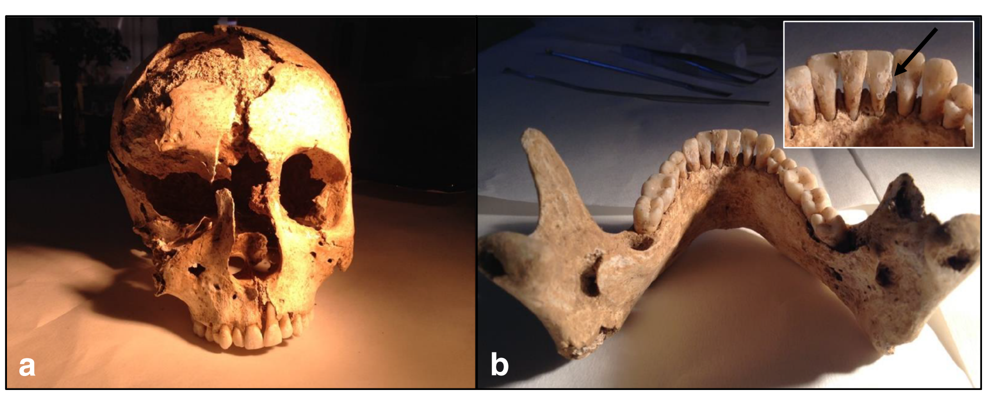

Fig. 1 The inhumate found in Cosa (Ansedonia). Skull (**a**); mandible, the arrow in the upper box indicates the dental calculus preserved on these teeth (**b**)

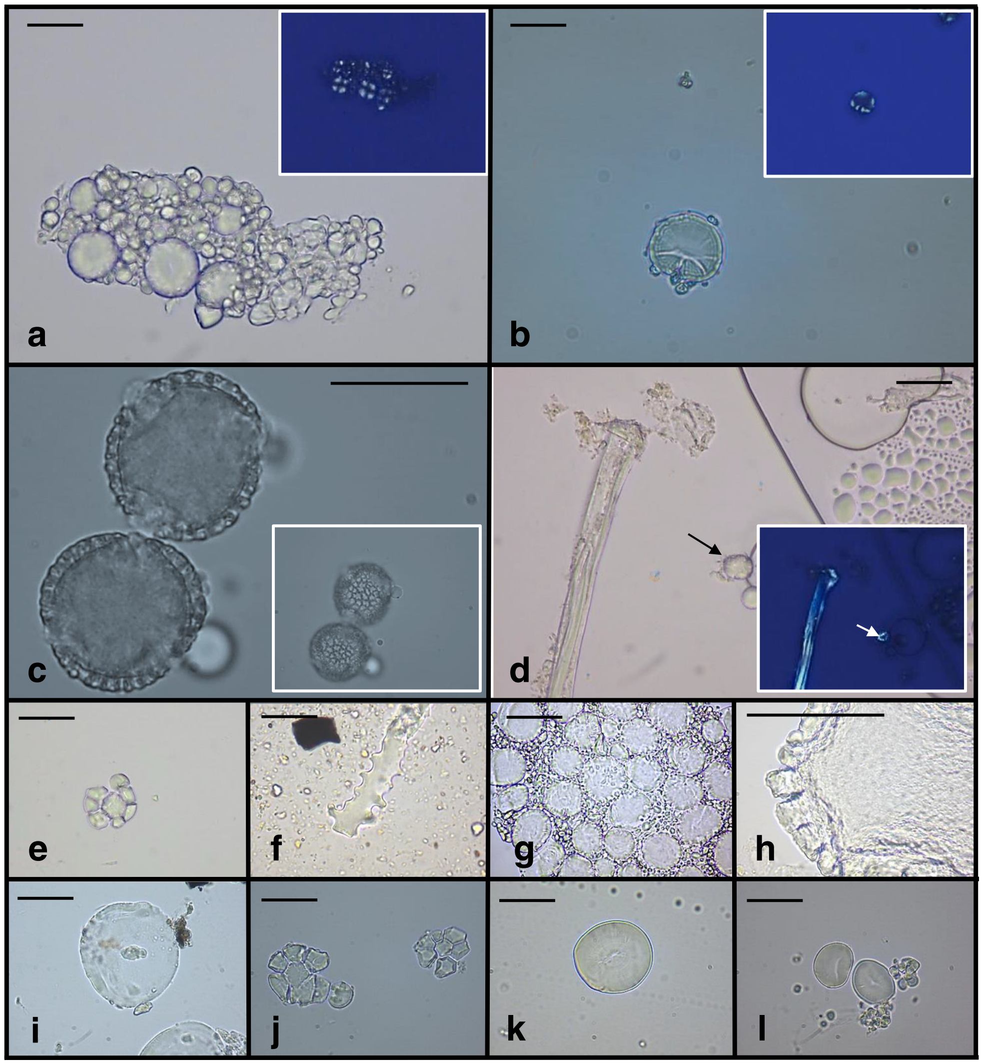

Fig. 2 Starch granules and other microremains at light microscopy. Representative images of microfossils found in dental calculus: aggregate of Triticeae starch granules and relative polarised image (**a**); *Hordeum* sp. starch granule and relative polarised image (**b**); *Ligustrum* sp. (Oleaceae) pollen grains (**c**); plant fibre and one indeterminate starch granule (indicated by an arrow) and relative polarised image (**d**); aggregate of Aveninae starches (**e**); Poaceae phytolith (**f**); aggregate of *Triticum* sp. starch granules (**g**); indeterminate starch granules (**h** and **i**). Representative images from modern starch reference collection: *Avena sativa* L. (**j**); *Triticum dicoccon* L. (**k**); *Hordeum vulgare* L. (**l**). The scale bar indicates 20 μm

wheat), could have caused in the young woman the typical CD inflammatory response, that is an abnormal CD4^{+}T cell-triggered immune reaction, and consequent effects. Indeed, considering the supposed prosperity of her family, the possibility she came in contact with gluten-rich foods increased (Bellini et al. 2008; Gasbarrini et al. 2010; Agricoli et al. 2012). Dioscorides recommended the use of *Triticum* sp. as a remedy for stomach aches (Staub et al. 2016), but if this medical indication had been adopted by the Roman woman, it would have been deleterious.

Use of endemic and exotic plants for medicinal and dietary purposes

GC-MS analysis revealed several types of organic compounds. In all chromatograms, *n*-alkanes and *n*-alkenes (C_4-C_{35}) were the most abundant molecules; according to Eglinton et al. (1962) and Buckley et al. (1999), they probably represented decomposition elements of organic material (oral microbiota, plant and animal microremains). The molecules identified in dental calculus (excluding *n*-alkanes and *n*-alkenes) were listed and clustered in biochemical groups (Table 2).

The detection of several saturated fatty acids (e.g. pentadecanoic, exadecanoic and octadecanoic acids), along with unsaturated ones (e.g. 9-octadecenoic acid), might indicate intake of animal fats, plant oils, oil-rich seeds and fruits (Kanthilatha et al. 2014; Gismondi et al. 2018c).

The presence of lactose and galactopyranose might testify ingestion of milk and/or dairy products, widely used as animal protein source at Roman time (Borstad et al. 2018).

Identification of derivatives of EPA and DHA ω-3-polyunsaturated fatty acids in calculus flakes supported consumption of dried fruits (e.g. walnuts, hemp seeds) or aquatic protein foodstuffs (e.g. algae, blue fish, fish-oil and molluscs) (Swanson et al. 2012).

Glucosinolate metabolites (e.g. isothiocyanatoacetaldehyde and isothiocyanic acid), dihydrobrassicasterol and 13-eicosenoic acid (erucic acid) were also recognised (Bell and Wagstaff 2017), suggesting the use of Brassicaceae (e.g.

Table 2 Chemical compounds identified in dental calculus by GC-MS. The molecules identified in all chromatographic profiles, excluding *n*-alkanes and *n*-alkenes, were listed and clustered in biochemical classes

Molecules detected in dental calculus by GC-MS			
Sugars			alpha-Galactopyranose; lactose; melezitose
Amino acids			Alanine; serine
Fatty acids	Saturated		Pentanoic acid; tridecanoic acid; tetradecanoic acid; pentadecanoic acid; hexadecanoic acid; octadecanoic acid; nonadecanoic acid; heneicosanoic acid; docosanoic acid; tetracosanoic acid; heptacosanoic acid; octacosanoic acid; triacontanoic acid
	Unsaturated	Omega-3	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene; docosa-2,6,10,14,18-pentaen-22-al, 2,6,10,15,18-pentamethyl
		Omega-6	9,12-Octadecadienoic acid; 8,11-eicosadienoic acid; 8,11,14-eicosatrienoic acid
		Omega-7	13-Eicosenoic acid
		Omega-9	9-Octadecenoic acid; 9-octadecenoic acid; 13-docosenoic acid
		Omega-12	6-Octadecenoic acid
Alcohols			1-Heptanol; 1-decanol; 1-hexadecanol; 2-dodecenol; heptacosanol; octacosanol
Terpens and terpenoids	Monoterpenes and derivatives		2-Bornanone; citronellol, dihydro-; artemiseole; dihydrocarvone; limonene; eucalyptol; menthol; linalool; terpinen-4-ol
	Sesquiterpenes and derivatives		beta-Elemenone; alpha-bergamotene; curcumenol; delta-selinene; dihydroartemisinin; gamma-selinene; ginsenol; ginsinsene; juniperol; panaginsene; cadinol; ylangenal; zederone
	Diterpenes and derivatives		Thunbergol; valtrate
Steroidal compounds			Sitosterol; dihydrobrassicasterol; cholesterol; cholesta-3,5-diene; stigmasterol
Phenolic compounds and derivatives			Pyrogallol; cumene; coumarin, 3,4-dihydro-4,5,7-trimethyl-; 3,4-dihydrocoumarin, 4,4,7,8-tetramethyl-; phenol, 2,2′-methylenebis[6-(1,1-dimethylethyl)-4-ethyl-; Phenol, 3,5-bis(1,1-dimethylethyl)
Other plant markers			alpha-Isolupanine; betaine; tartaric acid; isothiocyanatoacetaldehyde; isothiocyanic acid

cabbages) in diet (Ermolli et al. 2014). The consumption of these vegetables was also recommended for their diuretic properties (Touwaide et al. 1977; Staub et al. 2016).

The ancient calculus also revealed tartaric acid and pyrogallol, usually associated in literature with ingestion of grapes and/or wine (Badler et al. 1990; Guasch-Jané et al. 2004; Salvini et al. 2008). It has been documented that wine was employed both as a beverage and for its digestive, sleep-inducing, and appetite-stimulating effects (Riddle 1986; Norrie 2003).

Melezitose is a sugar contained in phytophagous insects' secretions which are collected by bees for honeydew production (Di Marco et al. 2017). Moreover, this trisaccharide is also the main component of manna, a white, sticky and crystalline exudate of some plant species (e.g. *Larix* sp., *Fraxinus* sp.) (Donkin 2013), which has been used, since ancient times, for its weakly laxative properties (Harrison 1950; Adams 2013). Therefore, the finding of this molecule led us thinking that, probably, the young woman ate honeydew or manna.

The detection of several derivatives of terpens and terpenoids (e.g. eucalyptol, cadinol, ylangenal, citronellol, bornanone) supported the usage of Lamiaceae herbs, endemic to Mediterranean latitudes. Among these compounds, dihydrocarvone, limonene, terpinenol, linalool and menthol might be attributed all together to *Mentha* sp. (Golparvar et al. 2015). Indeed, this genus has been largely used in the past in cosmetics (*M. viridis* L.), cooking activities (*M. suaveolens* Ehrh.) and traditional medicine (*M. piperita* L.). Mint leaf extracts or essential oils were usually employed as anti-inflammatory, diuretic and carminative substances in cases of anorexia, stomach ache, migraine and ulcerative colitis (Petrovska 2012; Nagara and Saour 2015; Staub et al. 2016; Ahmed et al. 2017). For this reason, we hypothesised that the young Roman woman consumed these herbs, to quell her putative gastro-intestinal ailments.

Dental calculus also contained a typical marker of *Valeriana* sp., the valtrate, which could indicate consumption of this sedative, sleep-aid, antispasmodic and diuretic plants. Historically, dried roots of these species were considered by Greeks and Romans as phytotherapeutic agents for the treatment of digestive problems, heart palpitations, high blood pressure and urinary tract infections (Touwaide et al. 1977; Nagara and Saour 2015; Staub et al. 2016).

Specific metabolites, such as dihydroartemisinin, artemiseole, selinene and coumarin derivatives, that we found in the samples might be attributed to usage of plants belonging to Apiaceae and Asteraceae family, which include both food (e.g. *Daucus carota* L., *Lactuca sativa* L.) and medicinal (e.g.

Ferula sp., *Artemisia annua* L.) species. For instance, *Artemisia* sp. was widely known for their antimalarial, anxiolytic, analgesic, narcotic, diuretic and carminative properties (Touwaide et al. 1977; Evergetis and Haroutounian 2015; Staub et al. 2016).

The most surprising finding of our research was the detection in dental calculus of markers for *Curcuma* sp. and *Panax* sp., two plant genera endemic of Eastern Asia (Richter et al. 2005; Kumar et al. 2011). The ethnopharmacological relevance of the underground districts of these plants dates to ancient civilizations (Choi 2008; Kumar et al. 2011). In particular, the detection of curcumenol, zederone and elemenone suggested that the Roman woman could probably have ingested turmeric, considering its bronchodilator, antispasmodic, antimicrobial, hepatoprotective and anti-inflammatory activity (Deng et al. 2007; Rawal et al. 2015; Liu et al. 2016; Kocaadam and Şanlier 2017). Moreover, the identification of sesquiterpen compounds typical of *Panax* genus, such as panaginsene, ginsinsene and ginsenol (Richter et al. 2005), assumed the consumption of these Asian rhizomes. Finally, the presence of several steroidal compounds, which can derive from the same biochemical pathway of ginsenosides (other specific metabolites of *Panax* species) (Liang et al. 2009), strengthens the previous observation. Ginseng extract, improving muscle activity and stimulating the endocrine and immune systems, is a good energiser (Abd El-Aty et al. 2008; Choi 2008; Deng et al. 2007; Chung et al. 2016; Kim et al. 2016), which could have been applied as a healthy remedy for her ailments.

Conclusions

Plants have always represented a key factor in human culture and diet, since ancient times. Indeed, they strongly influenced human evolution by providing energy (e.g. carbohydrates) and bioactive compounds (e.g. polyunsaturated fatty acids, polyphenols). To date, dental calculus surely constitutes one of the most promising resources for investigating such types of relationships. The application of microscopy and chromatographic techniques on this matrix allowed us to identify food and medicinal habits of a Roman female individual, probably affected by CD. The present research clarified the complex role of the plants, in negative and positive terms, in the life of the inhumate. Indeed, our archaeobotanical data provided evidence that she came in contact with gluten-rich cereals (e.g. barley, wheat, oat). This result, associated with the indication of a genetic predisposition to CD and a metabolic stress observed by stable isotope analysis, could justify the individual's premature death and all degenerative signs on her skeleton. The detection of specific molecular markers, embedded in tartar flakes, supported the potential use and/or consumption of several herbal products (e.g. *Mentha* sp., *Valeriana* sp., Apiaceae, Asteraceae) and other plant derivatives (e.g. grapes or wine, honeydew or manna). Surprisingly, we also found secondary metabolites typical of exotic rhizomes (*Curcuma* sp. and *Panax* sp.), nowadays known for their energising, anti-inflammatory and immunomodulatory properties, which could have been probably used by the young woman as natural drugs to treat her precarious health condition. These latter data can be considered scientific evidence supporting historical information about the existence of trades between Mediterranean area and Eastern Asia at Imperial Roman time. Moreover, they confirm that ethnopharmacological traditions, based on these plant species and reported in ancient Oriental texts, were also applied in Roman medicine.

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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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