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Study of a more than a hundred years old theriac jar' content: A famous thousand-year-old counter-poison

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ABSTRACT

The purpose of this article is to study the content of a 19th century white porcelain pot from the Pochet-Desroche fabric, offered to the National Order of Pharmacists and probably containing theriac. The aim is to identify the active ingredients of any substances that may still be present and to try to determine the preparation period of the panacea. All the analyzes were carried out according to the reference current methods. Liquid / liquid extractions in a separating funnel, high performance liquid chromatography coupled with three-dimensional diode array molecular absorption spectrophotometry and gas chromatography coupled with mass spectrometry have revealed 218 molecules which may belong to the ingredients of a theriac. 29 of these are clearly still present in the opiate studied. Their comparison with the French pharmacopoeias formulas of 1818 and 1884 pleads for manufacture according to the formula of 1884. The originality of our work lies in the rarity of this type of analysis on very old pharmaceutical samples and in the fact that it concerns a mixture of great complexity.

1. Introduction

On March 18th 2010, a Symposium at the headquarter of the French "Ordre des Pharmaciens", organized by the Research Institute CNRS and the French Society of History of Pharmacy (SHP), was dedicated to the theriac: « Between panacea and pharmaceutical knowledge. From Andromaque's theriac to Moyse Charas ». During this meeting, Mr. Robert Montagut donated a theriac jar to the Pharmacist Order's museum (Fig. 1). Robert Montagut, pharmacist and antiquarian, was a famous specialist in Pharmacy's items. Since 1987, he has produced numerous brochures contained on a main document which is a reference and continues to be updated. This famous colleague died on July 9th, 2017.

This 19th century porcelain white jar has still contained a dry residue that is probably a theriac dating from the jar's origin. Dominique Kassel, in charge of the Historical Collections of the PO's Pharmacy, and Professor Christian Warolin (Pharmacy's historian, SHP's/PHS's Honorary President) gave the mission to our laboratory of verify its hypothesis. Because of the lack of information on the use of this jar for almost 2 centuries and concerning the exact date of production, the success of this work was first compromised.

The jar covered by white porcelain measures 23 cm with a 11 cm diameter. Lid and ornament are doubly ringed by a gold border. The decoration represents a cartouche formed by 2 tied tape flowered palms overcame by Hygie's bowl. The bowl is little bit altered inside its right part and contained in the middle, the inscription: THERIAQ.

On the bottom of the jar the perfectly legible "Pochet Deroche" mark corresponds to the period 1833–1839. The booklet n°11 of the Robert Montagut's gallery indicates that it corresponds to a set of 48 porcelain jars. These jars may have been the last order under Louis-Philippe, customized for the Pochet-Deroche niches in Paris.

This jar is coming from the pharmacy located at Place du Change in Avignon. This pharmacy has a long history going back over the 14th century. Indeed, its woodworks dismantled during 1788's regular clergy reform may be those of the apothecary's Hospice of the order of Saint Antoine, dating from 1308 and near the pharmacy located nearby. The owners are also known since 1786. The last ones, the pharmacists Rouvière, Gras, Gouirand and Bertin up to 1897 carefully preserved the heritage. The arrangement of these jars in the pharmacy has long been devoted to decoration. They have been exposed in a mezzanine, which is

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Fig. 1. Porcelain Theriac jar « Pochet Deroche » (1833-1839) from Robert Montagut's gallery (Photography by Cécile Bui-Boucher, 2017).

a sort of museum, located to the upper level and only eye accessible for customers. However, the alteration of the decor on the jar offered to the "Ordre des Pharmaciens" proves the regularly use of the container, that holds a significative quantity of what could have been a dry opiate.

The objective of this work is to answer at the following questions:

Is it really an old theriac dating back to the time of the jar's acquisition? If yes, some active ingredients are still present?

2. Materials and methods

2.1. Chemical and reageants

The following chemicals: N,O-Bis(trimethylsilyl) trifluoroacetamide/1%Chlorotrimethylsilane, Noscapine hydrochloride hydrate, Papaverine hydrochloride, DL Laudanosine, Morphine hydrochloridetrihydrate, and 7-chloro-1-methyl-5-phenyl-3H-1,4-benzodiazepin-2-one, were purchased from Sigma Aldrich (Merck, France).

Chromatographic grade solvent trichloromethane for HPLC was purchased from Sigma Aldrich (Merck, France). Petroleum ether, diethyl ether, Sodium carbonate (purity >99,5 %) and Sodium hydroxide solution (NaOH) 1 mol l^{-1} (1 N) Titripur were purchased from Sigma Aldrich (Merck, France). For water, double distilled water is used.

2.2. Samples

230 g of a compact blackish mass, very hard, with a slightly aromatic and sour odor has been discovered inside the jar (Fig. 2).

Collect sample from the jar was a delicate operation, not possible without an instrument.

The use of a scalpel was necessary to extract from the mass three compact blocks of 20 g. Each sample was placed in sterile plastic tube, before and after their analysis. Tubes were stored in the dark at room temperature to respect the initial storage conditions.

Before analysis, 1 g was mixed by an automated mechanical grinder to obtain, in a few minutes, mixture of black powder and micro-pellets. A manual grinding with mortar and pilon was unsuccessful; sample was still homogeneous, in one compact and flattened block.

This mixture was then dissolved, at 37 °C, in absolute ethanol with 10 % tartaric acid during 60 h under stirring. Powder is dissolved after only one hour but it takes about 60 h for the total dissolution of the micro-pellets.

After dissolution, the liquid phase at pH 3.4 was treated by the Stas, Otto and Ogier's method adapted to the compact powder of opiate. Filtration of this alcoholic macerate does not leave any significant residue. This filtrate was evaporated at 20 °C under vacuum. After getting a syrupy consistency, absolute alcohol was added under agitation and left at −20 °C for two hours. A second filtration was necessary to remove proteins. This filtration does not leave any significant residue.

This second acidic alcoholic filtrate was then evaporated and the residue was dissolved in distilled water before extraction.

2.2.1. Extraction procedure

A series of liquid/liquid extractions by separating funnel was managed as follow: the acidic aqueous phase, yellow-brown aspect, was first extracted with petroleum ether, second by sulfuric ether, and finally by trichloromethane. Then the aqueous phase was alkalized at pH 9.3 with sodium carbonate, and at pH 10 with sodium hydroxide. New extractions were successively carried out with sulfuric ether and trichloromethane before analysis.

Fig. 2. Appearance of the jar's contents just after opening.

2.3. Liquid chromatography analysis

Each extract was analyzed by a high-performance liquid chromatography (HPLC, Waters 2695 Controller) coupled with three-dimensional diode array molecular absorption spectrophotometer (Alliance DAD Waters 996). HPLC-DAD was selected for the screening of thermo-unstable molecules that cannot be analyzed by gas chromatography. HPLC was equipped with a column C8 Symmetry (25cmx4.6mmx5 µm) heated at 30 °C. 20 µL of each extract were injected. Acquisitions were performed in 40 min. Empower Pro operating software was used for data analysis. Each peak of the UV spectrum was checked manually and automatically to compare their purity and their similarity with the spectrums from database that contains more than 1500 standard spectrums.

2.4. Gas chromatography analysis

A gas chromatography (A HP 6890 series) coupled with a mass spectrometer (HP 5973 series quadrupole) was used for volatile compounds analysis. Gas chromatography was equipped with an HP 5 MS capillary column (30 m x0.25 mm x 0,25 µm). Analysis were carried out with the following parameters: electron impact ionization mode (70 eV), oven temperature was maintained at 140 °C for 3 min, then set up to 290 °C at a rate of 7 °C/min, then hold on during 12 min at 290 °C, temperature of injection and temperature of the transfer line were 280 °C and 300 °C, respectively; helium was used for carrier gas at 1 mL/min and at 13.4 psi. Mass spectrometer conditions were 230 °C for the source temperature and 150 °C for quadrupole. Solvent delay was set at 3.5 min, and acquisition time at 36 min.

1 µL of the three previous extracts dissolved in 1 mL of ethanol, dichloromethane or acetonitrile was injected. 1/5 of each of them was evaporated to dryness and submitted to a tube crimped with a bis trimethylsilyl-trifluoroacetamide/chlorotrimethylsilane (99/1, v/v) silylating agent for 20 min at 80 °C. The tri-methyl-silylated derivatives obtained was injected into the capillary column.

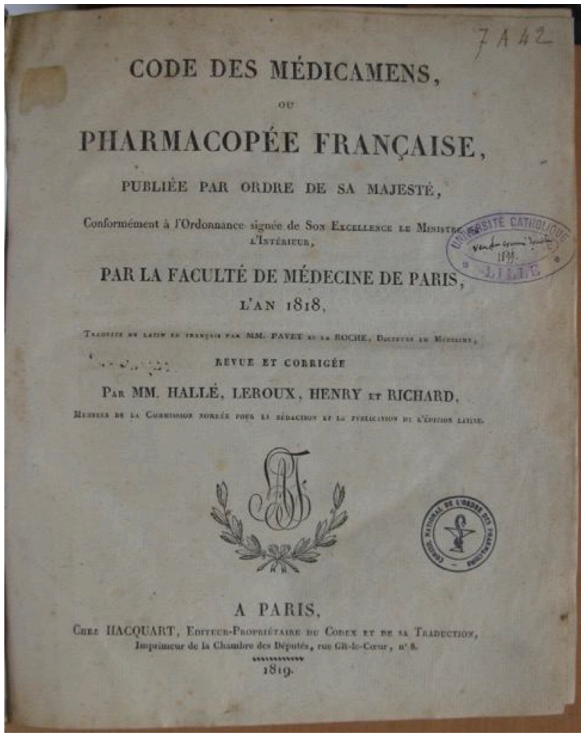
The six chromatograms were collected and the mass spectra of each peak was compared to the available database (PMW TOX2d, Nist 75 K.I, Wiley) that contains approximately 300,000 spectra, supplemented as necessary ("PP Tox Lab" 250 spectra).

2.5. List of possible substances

Study of the literature on ingredients potentially present in theriac was done. This study was realized in order to propose a most exhaustive list of substances characteristic of this electuary mixture. Main goal was: which ingredients are we looking for?

Theriac formula has been changed many times since the first version of Andromache. Comparison of the theriac composition from 1818 codex and the composition from 1884 codex is presented Table 1. Only the contemporary formula of selling the pot and those subsequent to it are only considered here. The search of viper flesh was excluded since nothing specific could be identified in the analyzed mixture. The use of viper flesh has continued until the pharmacopoeia of 1866 but it has been disputed since Moïse Charas' version (1676 Pharmaceutical and Galenic Pharmacopoeia) who still considered it useful but modified its initial preparation. Finally, viper flesh definitively disappeared in the 1884's pharmacopoeia [1]. Few modifications of composition appeared between the 1837 and 1866 pharmacopoeia editions, before its total exclusion from the pharmacopoeia after 1884. That's why this work is first based on the 1818 French pharmacopoeia published in 1819 [2] (Fig. 3) close to the Andromache theriac version, for a possible maximum preservation of around 2 centuries or at least 110 years if we consider only the pharmacopoeia of 1908 [3], first one where the theriac was not listed anymore.

Furthermore, it is known that it was recommended, before its use, to wait for the maturation of the preparation between 7–10 years [4].


CODE DES MÉDICAMENS,

OU

PHARMACOPÉE FRANÇAISE,

PUBLIÉE PAR ORDRE DE SA MAJESTÉ,

Conformément à l'Ordonnance signée de Son Excellence le Ministre de l'Intérieur,

PAR LA FACULTÉ DE MÉDECINE DE PARIS,

L'AN 1818.

REVUE ET CORRIGÉE

PAR MM. HALLÉ, LEROUX, HENRY ET RICHARD,

A PARIS,

CHEZ HACQUART, Editeur-Propriétaire du Codex et de sa Traduction,

Imprimeur de la Chambre des Députés, rue Gît-le-Cœur, n° 8.

1819.

Fig. 3. Pharmacopoeia of 1818.

According to the Middle Ages Arab medicine, the full expression of its properties waited 40 years [5] of storage. It was then interesting to study if, after more than a century, active ingredients have been still present.

2.6. Differences between both formulas: 1818 composition and 1884 composition

Comparison of formulas shows that all ingredients of 1884 composition are included in the 72 substances from the 1818 formula except for: catechu and common laurel [2].

2.7. Some differences are noticed concerning the different part of plants

- dry dander instead of pulp for *Scilla maritima*;
- fruits instead of seeds for : *Ammi officinalis*, *Anethum funiculum*, *Seseli tortuosum*, *Pimpinella anisum* and *Atamantha cretensis*;
- seeds instead of fruits for *Elettaria cardamomum*;
- fruits of *Petroselinum crispum* are replace by the seeds of *Bubon macedonicum*;
- tops instead of grass for: *Teucrium polium*, *Marrubium vulgare*, and *Teucrium chamaedrys*

In total, including the two different types of parsley, 18 additional ingredients are present in the 1818 formula. They are all listed, as well as the 54 others, in the theriac described by Galien in *De Antidotis*. Latter considered to be the theriac of Andromache. The same is true of the iris root of Florence, absent even in the three trochisques of the recipe attributed to "Andromachus the father" by Moyse Charas in his 1676 pharmacopoeia [7]. Finally, dosages of the substances are fairly similar considering the different dilution of the ingredients by honey, wine and the ingredients constituents of the trochisques

First, 660 main molecules possibly present in the 56 ingredients of the 1884 French pharmacopeia were selected. Substances that are unusual today are particularly interesting, since their detection would be more meaningful. This is the case of: Castor fiber, *Acorus calamus, Pasticana opopanax, Aristolochia clematitis, Asarum europeum, Aloexylon*

Table 1
Comparison of the theriac composition from 1818 codex and the composition from 1884 codex. Abbreviations for "family" from the codex of 1818: 1 acrid (1ac); 2 bitters (2am); 3 stypical (3st); 4 exotic aromatics (4ar ex); 5 native aromatics (5ar ind); 6 aromatics from umbelliferae (6ar omb); 7 resins and balms (7rb); 8 foul substances (8sf); 9 viral substances (9sv); 10 earthy substances (1ster); 11 gummy substances (11sg); 12 soft and sweet substances (12sds); 13 wine (13v).

	1884		1818	
Rank	Courant name *latine name* (specificity)	Quantity (g)	Rank (family) *difference*	Quantity (g)
01	Ginger *Amomum zingiber* (roots)	60	20 (4 ar ex)	24
02	Iris from Florence *Iris florantina* (roots)	60	41 (5 ar ind)	48
03	Valerian *Valeriana officinalis* (roots)	80	57 (8sf)	20
04	Sweet flag *Acorus calamus* (roots)	30	30 (4 ar ex)	20
05	Rhapontic (rhubarb) *Rheum rhaponticum* (roots)	30	9 (2am)	24
06	Creeping cinquefoil *Potentilla reptans* (roots)	30	15 (3 st)	24
07	(European) birthwort *Aristolochia clematitis* (roots)	10	58 (8sf) Aristol.pistolochia	8
08	European wild ginger *Asarum europeum* (roots)	10	2 (1ac)	2,4
09	Great yellow gentian *Gentiana lutea* (roots)	20	8 (2am)	16
10	Spignel *Meum anthamanticum* (roots)	20	48 (6 ar omb)	16
11	Aloes-wood *Aloexylon agallochum* or *Aloexylum verum*	10	31 (4 ar ex)	2,4
12	Ceylon cinnamon tree *Laurus cinnamomum*	100	18 (4 ar ex)	80
13	Maritime squill *Scilla maritima* (dry dander)	60	1 (acrid) (pulp)	115
14	Cretan dittany *Origanum dictamnus* (leaves)	30	35 (5 ar ind)	24
15	Common laurel *Laurus nobilis* (dry leaves)	30	See *Malabathrum laurus* cas.	24
16	Water germander *Teucrium scordium* (dry leaves)	60	10 (2am)	48
17	Calamintha alpina *Melissa calamintha* (tops)	30	34 (5 ar ind) grass	24
18	White horehound *Marrubium vulgare* (tops)	30	37 (5 ar ind) flowers	24
19	Felty germander *Teucrium polium* (tops)	30	38 (5 ar ind)	16
20	Wall germander *Teucrium chamaedrys* (tops)	20	11 (2am) herb	16
21	Yellow bugle *Teucrium chamaepitys* (tops)	20	12 (2am) herb	16
22	Perforate St John's-wort *Hypericum perforatum* (tops)	20	13 (2am)	16
23	Common centaury *Erythrea centaurium* (tops)	10	7 (2am)	8
24	Gallic rose *Rosa gallica* (petals)	60	14 (3 st)	48
25	Saffron crocus *Crocus sativus* (stigmates)	40	32 (5 ar ind)	24
26	Lavender *Stoechas lavandula* (flowers)	30	36 (5 ar ind)	24
27	Lemon *Citrus medica* (dry bark)	60	33 (5 ar ind)	24
28	Long pepper *Piper longum* (fruits)	120	21 (4 ar ex)	96
29	Peppercorn *Piper nigrum* (fruits)	60	22 (4 ar ex)	24
30	Parsley *Petroselinum crispum* (fruits)	30	See Bubon macedonicum	24
31	Bishop's weed *Ammi officinalis* or majus or *Sison ammi* (fruits)	20	43 (6 ar omb) seeds	16
32	Fennel *Anethum funiculum* (fruits)	20	44 (6 ar omb) seeds	16
33	Anise *Pimpinella anisum* (fruits)	50	45 (6 ar omb) seeds	16
34	Seseli *Seseli tortuosum* (fruits)	20	46 (6 ar omb) seeds	16
35	Candy Carrot *Atamantha cretensis* (fruits)	10	47 (6 ar omb) seeds	8
36	Ervil or bitter vetch *Ervum ervilia**	200	68 (11sg)	75.75
37	Turnip *Brassica rapa* (seeds)	60	4 (1ac)	48
38	True cardamom *Elettaria cardamomum* (seeds)	80	24 (4 ar ex) fruits	16
39	White agaric *Boletus laricinus*	60	3 (1ac)	48
40	Opium poppy or breadseed poppy *Papaver somniferum*	120	63 (9 sv)	96
41	Licorice *Glycyrrhiza glabra* (juice)	60	70 (12sds)	48
42	Catechu *Areca catechu* or *mimosa catechu* (juice)	40	See *mimosa nitolica* bark	16
43	Gum acacia*Acacia senegalensis*	20	65 (11sg)	16
44	Myrrh *Commiphora myrrha* (juice)	40	6 (2am)	32
45	Olibanum-tree *Boswellia sacra* or *Juniperus lycia* (incense)	30	52 (7rb)	24
46	Galbanum *Ferula gummosa* (galbaniflua)	30	59 (8sf)	8
47	Opopanax *Pasticana opopanax* (gum)	10	60 (8sf)	8
48	Gum benjamin tree *Styrax benzoe* or *officinalis* (resin)	20	56 (7rb)	16
49	Castorum *Castor fiber* (resin)	10	62 (8sf)	8
50	Soft bread of dried wheat bread	60	67(11sg)	22.05
51	*Terra sigillata* (Lemnos land	20	64 (10ster)	16
52	Desiccated iron sulfate / red iron peroxide (Colcothar)	20	18 (3 st)	16
53	Judea tar or asphalt	10	55 (7rb)	8
For 1000 g theriacal powder				
54	Turpentine of Chio *Pistacia terebenthinus*	50	53 (7rb)	24
55	White honey (Honey from Narbonne)	3500	71 (12sds)	5250
56	Grenade's wine	250	72 (13v)	1250
Substances only present in 1818 version				
57	Pennycress *Thlaspi arvense* (seeds)	–	5 (1ac)	16
58	Hypocyst *Cytinus hypocistis* (juice)	–	16 (3 st)	16
59	Acacia *Mimosa nilotica* or *vera* (juice)	–	17 (3 st)	16
60	Cassia *Laurus cassia* (bark)	–	19 (4 ar ex)	32
61	Chinese cassia *Laurus cassia* (leaves)	–	25 (4 ar ex)	24
62	Cardamom *Cardamoma racemosum* (fruits)	–	23 (4 ar ex)	32
63	Camel grass *Andropogon schenanthus* (herb)	–	26 (4 ar ex)	56
64	Citronella grass *Andropogon nardus* (roots and stems)	–	27 (4 ar ex)	32
65	Alpine valerian *Valeriana celtica* (roots)	–	28 (4 ar ex)	16
66	Costus *Costus arabicus* (kind of ginger)	–	29 (4 ar ex)	28
67	Cat Thyme *Teucrium marum* (tops)	–	39 (5 ar ind)	2,4
68	Sweet Marjoram *Origanum majorana* (tops)	–	40 (5 ar ind)	2,4
69	Alexanders *Bubon macedonicum* (seeds)	–	42 (6 ar omb)	24

(*continued on next page*)

Table 1 (*continued*)

	1884		1818	
Rank	Courant name *latine name* (specificity)	Quantity (g)	Rank (family) *difference*	Quantity (g)
70	Xylobalsamum *Amyris opobalsamum* (wood)	–	49 (7rb)	4
71	Carpobalsamum *Amyris opobalsamum* (fruits)	–	50 (7rb)	16
72	Opobalsamum *Amyris opobalsamum* (resin)	–	51 (7rb)	60
73	Mastic Tree *Pistacia lentiscus* (resin)	–	54 (7rb)	1,2
74	Sagapenum or sepharic gum *Ferula persica* (resin)	–	61 (8sf)	16
75	Avicenna viper *Coluber vipera*	–	69 (11sg)	73

* According to F. V. MERAT and A. J. DE LENS, the Orb flour would come from Ers powder seeds (Ervum ervilia), and not from seeds of species of the genus Orobus. Authors also mention that the seeds of *Orobus vernus* look like those of *Ervum ervilia* [6].

agallochum, Commiphora myrrha, *Boswellia sacra*…

Because of the mass detection limit of 600 AMU for the mass spectrometer detector, high molecular weight molecules were excluded. In fact, none of the expected molecules with a molecular weight (MW) higher than 500 AMU were detected. About fifty compounds are involved. This is particularly the case for the molecules present in the following plants:

1) Squill: scillitoxin (3430), sinistrin (828), glucosinistrin (1008), scillarene A and B, scillaroside (620), scillaphaséoside (547), proscillaridine (531)
2) St John's-wort: hyperforin (537), hypericin (504) skirin (538),
3) Cinquefoil: tormentol (606); leucoanthocyanin (593),
4) Calamintha: stachyone (666), stachyose (667),
5) Licorice: glycyrrhizin (823), lycopene (537), sojasaponins I and II (913),
6) Saffron: zeaxanthin (569), alpha carotene (538),
7) Gentian: gentiopicrin (844), gentioside (553), gentianosis (504)
8) *Florence* iris: isoswertisine (609), iridine (523)
9) Rhubarb: sennoside (863), rutoside (611)

Finally, mineral substance such as: dried iron sulphate, *Terra sigillata*, Judea tar, has not been investigated. 53 ingredients remained to be identify.

3. Results

Our first results were presented at the 43rd International Congress on the History of Pharmacy [8].

Major part of the work was the interpretation of the mass spectra issue from GC–MS analysis.

Only the emetine, not detected by GC–MS, has been found by HPLC. Among all the possible theriac's ingredients, this alkaloid is only present in Asarum roots.

218 molecules have been well identified among the 674 molecules previously selected as potential markers of the presence of 53 components of the theriac. 14 significant additional compounds have been detected, 4 compounds are fusarium or aspegillus-related mycotoxins that can come from the soil and often contaminate cereals (wheat, barley, oats, corn, sorghum), tobacco, dried fruits. These are zearalenone, bikaverine, culmorine, and ent-pimara8,15-diene. Their presence is not surprising in this old preparation. The last 10 compounds could not be linked to an identifiable substance among the 56 listed in the first part of Table 1.

To summarize, 218 significant molecules that correspond to theriac's components have been identified (32,3 %). It's important to notify that for the 53 potential markers, 674 different molecules have been identified, the total of the assigned molecules to each ingredient is 1411.

An average of 2.1 molecules are common to each substance (from 0 common molecules to 23). On the 674 different molecules 434 have been cited only once and 103 (23.7 %) of them have been detected. The 240 other compounds have been cited from 2 to 23 times among the 53 potential markers according to the following diagram (Fig. 4). 115 of these compounds have been detected (47,9 %). Lower the relevance of the presence of the substances which contain these compounds more their frequency is important. The detection of linalool and linalool oxide, present respectively in 23 and 11 of the simples contained in theriac, are of little significance. It is the same for the presence of alphacopaene, delta cadinene, terpinolene, borneol, stigmasterol, palmitic acid, beta-elemene or alpha terpineol, beta-caryophyllene, (of respective frequencies n = 13,13,12,10,10,9,8,8, and 6), as well as the absence of alpha or beta-pinene, eugenol, limonene, beta-myrcene, sabinene, para-cymol or germacrene D (respectively n = 18,15,15, 14,12,10, 8 and 8) (Fig. 5).

Nevertheless, the proportion of the detected compounds for each ingredient is a probability criterion for the significant presence of the ingredient. It suggests, despite the age of the preparation, that 29 of the expected substances out of 53 sought (55 %) have a high probability of presence considering the weight of the more specific molecules (110 mentioned only once and 61 mentioned 2 times among 674). For example, chavicin present in long pepper and black pepper or oroselone present in valerian and white horehound or beta-amyrone present in lemon peel and olibanum resin. In particular, all the opium alkaloids are present.

Table 2 shows the percentage of detected compounds for each ingredient. Any actives molecules have been identified for 2 ingredients: cinquefoil and catechu.

For 4 ingredients, only 10–16.7% of the active ingredients or molecules usually present have been identified (gentian root, pennyroyal, saffron, squill). Even if they were present in the preparation, this result was expected because of their molecular weight.

For 25 substances, from 22 to 50 % of the active ingredients or molecules usually present have been identified: their presences are not proven but possible. It is more likely for those whose more significant molecules have been detected. This is the case for 7 substances: valerian (8), soft bread of dried wheat bread (8), castorum (7), myrrh (6), parsley (6), seseli from Marseille (6) and common laurel (5).

The last 22 ingredients, classified in Table 2 from 1 to 22, have from 50 % to 84 % of detection of active ingredients or molecules usually present in the ingredient, which makes their presence probable.

In total, 29 substances common to the two formulations of theriac of the 1818 and 1884 pharmacopoeias, are still present today in the jar offered to the "Ordre des Pharmaciens". Among these 29 substances, there are those listed above as more significant for this kind of preparation: sweet flag, cinnamon, aloes-wood, long pepper, black pepper, bitter vetch, myrrh, olibanum-tree, opopanax, benzoin, castorum…

Three examples are presented in Table 3:

- Opium: 84 % of the expected molecules are present, including 100 % of alkaloids.
- Olibanum-tree: 52.9 % of the selected composition has been detected that represents 13 of the main characteristic molecules.
- Castoreum: despite the small initial quantity, less than 4.6 g in 4 800 g of preparation (0.1 %), 40 % of the expected molecules have been indentified, including 7 of the most significant ones, such as nupharidine derivatives and castoramine.

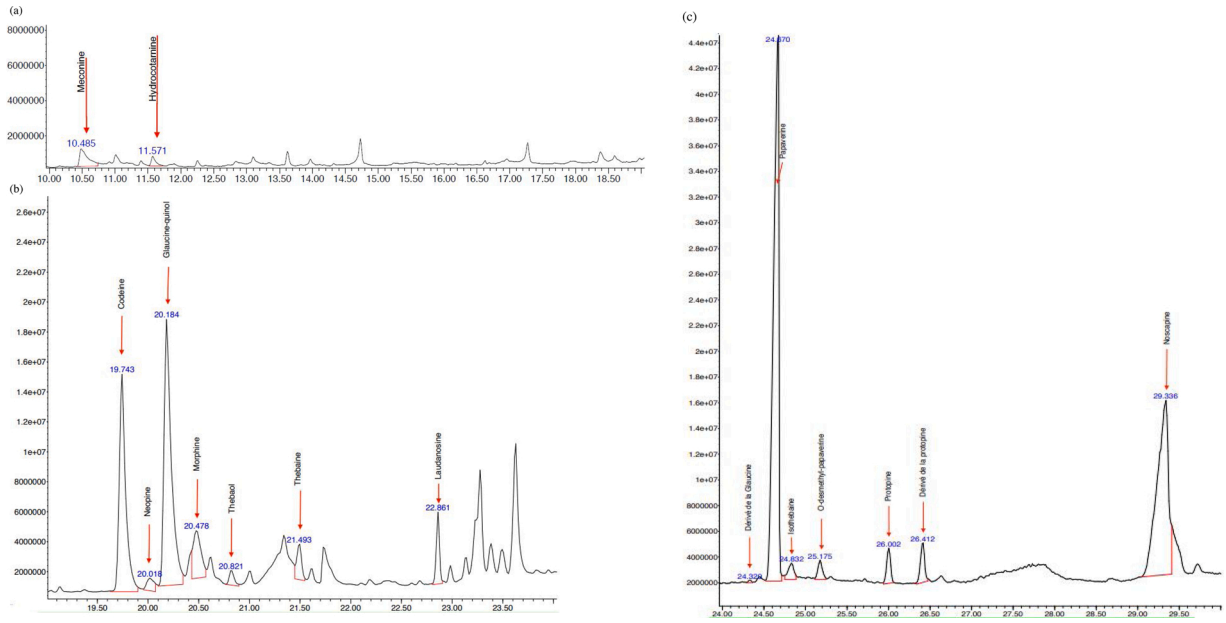


Fig. 4. GC–MS chromatogram of the extract in diethyl ether / alkaline (EI mode, full scan detection): (a) detection from 10 to 19 min, (b) detection from 19 to 24 min, (c) detection from 24 to 30 min.

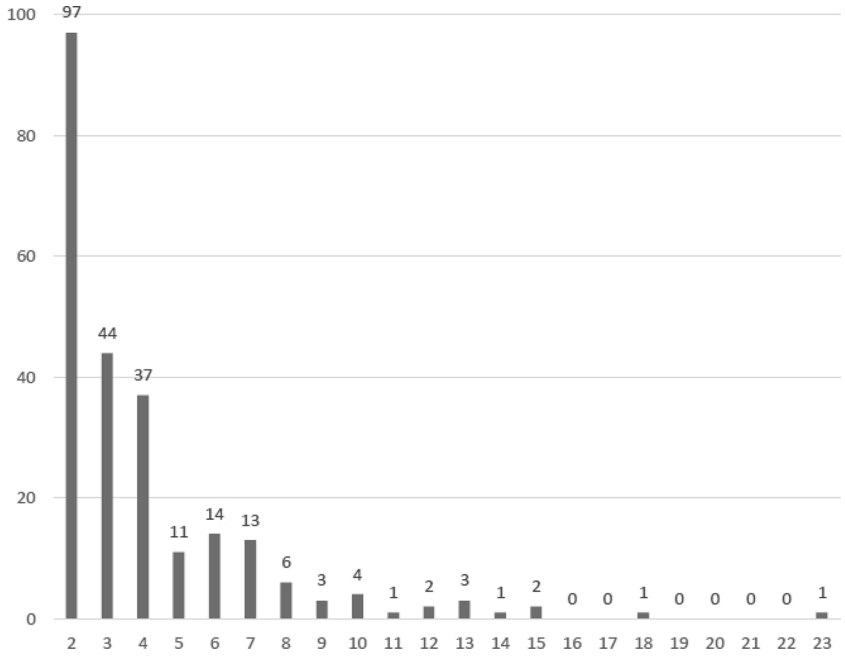


Fig. 5. Frequency of repetition.

The question arises: Is it possible to specify the date of production of this theriac?

The acquisition date of the jar is between 1833 and 1839. Instructions of the 1818 pharmacopoeia have to be observed during this period. The last French pharmacopoeia containing theriac formulation is those established in 1884. By logical deduction and by the observation of the loss of the decoration indicating a regular use, the fabrication of the jar content was made before 1908, which is the first pharmacopeia where theriac was not included. All the ingredient of the 1884 edition are presents except the cachou, which probably replace the juice of pods of mimosa nitolica (like mimosa catechu), and *laurus nobilis*, which probably replace the leaves of *laurus cassia malabathrum*, and finally the parsley fruits *petroselinum crispum* substituted by seeds oat Macedonia parsley *bubon macedonicum*.

By the same previous method, the 18 ingredients concerned, numbered from 57 to 74 in Table 1, were researched. Results led to the inventory of 340 molecules including 135 new ones not listed for the 56 initial ingredients. The results are shown in Table 4.

According to the results, three substituted ingredients in the 1818 formula (3, 10, 13) are not present. Less than 22.2 % of the expected molecules have been detected for 12 of the additional ingredients in the 1818 formula and none of their characteristic molecules were identifiable. That is not the case for the *Valeriana celtica*, the three forms of *Amyris opobalsamum* and for *Andropogon sch.*

For *Valeriana celtica*, a valerianaceae, 18.8 % of the expected molecules have been detected. The (10-isopropyl-2,2,6-trimethyl-

Table 2
Percentage of detected compounds for each ingredient.

	Ingredients	Number of expected molecules (potential markers)	Number of detected molecules	Number of not detected molecules	Positive detection (%)	Number of identified molecules
1	Officinal opium	25	21	4	84	16
2	Bitter vetch	10	8	2	80	2
3	Grenade's wine	15	11	4	73,3	0
4	Aloes-wood	6	4	2	66,7	4
5	Long pepper	21	14	7	66,7	8
6	White honey	34	22	12	64,7	3
7	Lavender	34	22	12	64,7	1
8	Dry bark lemon	28	18	10	64,3	8
9	Sweet flag (Calamus)	41	26	15	63,4	7
10	Birthwort	24	15	9	62,5	5
11	Turpentine of Chio	18	11	7	61,1	3
12	Gum acacia	10	6	4	60	4
13	Benzoin	17	10	7	58,8	6
14	White horehound	19	11	8	57,9	2
15	Common centaury	28	16	12	57,1	2
16	Cinnamon	37	21	16	56,8	2
17	Black pepper	64	36	28	56,3	8
18	Mountain Spignel	11	6	5	54,5	3
19	Olibanum-tree	34	18	16	52,9	13
20	Ginger	42	22	20	52,4	4
21	Bullwort	10	5	5	50	2
22	Opopanax	26	13	13	50	1
23	Valerian	67	32	35	47,8	8
24	Dessicated wheat bread	49	23	26	46,9	8
25	Myrrh	28	13	15	46,4	6
26	Wild turnip	11	5	6	45,5	1
27	Common laurel	43	19	24	44,2	5
28	White agaric	16	7	9	43,8	3
29	Cretan dittany	32	14	18	43,8	1
30	Parsley	32	14	18	43,8	6
31	Water germander	12	5	7	41,7	0
32	Castorum	35	14	21	40	7
33	Spignel	23	9	14	39,1	1
34	Red rose petals	18	7	11	38,9	0
35	Galbanum	21	8	13	38,1	2
36	Seseli	37	14	23	37,8	5
37	True cardamom	35	13	22	37,1	1
38	Cretan dittany	38	14	24	36,8	2
39	Yellow bugle	29	10	19	34,5	1
40	Florentine iris	44	15	29	34,1	1
41	Wild ginger	12	4	8	33,3	3
42	Calamint	54	18	36	33,3	0
43	Rhapontic rhubarb	20	6	14	30	2
44	St John's-wort	34	10	24	29,4	1
45	Anise	14	4	10	28,6	1
46	Wall germander	12	3	9	25	0
47	Liquorice	59	13	46	22	1
48	Pennyroyal	6	1	5	16,7	0
49	Saffron	10	1	9	10	0
50	Gentian	19	1	18	5,3	1
51	Maritime squill	26	1	25	3,8	0
52	Cinquefoil	8	0	8	0	0
53	Catechu	6	0	6	0	0
54	Sigillated land	Not investigated				
55	Desiccated iron sulfate / red iron peroxide	Not investigated				
56	Bitumen of Judea	Not investigated				

2,3,4,5tetrahydronaphtha [1,8-b] oxocin-5,11-diol) is one of the 10 molecules detected which could not have been assigned to one of the 56 initial ingredients. It is not impossible that the root of *valeriana officinalis* also contains it, but no scientific article that has been available to us does mention it. However, because of the 47.8 % of the predicted composition of the *valeriana officinalis* compared to 18.8 % for the *Valeriana celtica*, only officinal valerian seems to be present.

For lemongrass, 35 % of the expected molecules have been detected, with the presence of one of the 6 specific molecules. However lemon grass seems not to be use for the preparation because of the absence of: piperitone, delta-2carene, geraniol or geranial, normally abundant in the extract of this plant.

Finally, *Amyris opobalsamum* as *boswellia sacra*, produces *olibanum* resin and is part of *burseraceae* family. They both contains ursanes, oleanes and pimaranes derivatives (alpha- and beta-amyrine, beta-amyrone and sandaracopimara-8 (14) 15-diene). They respectively belong genus *commiphora* and genus *boswellia*, distinguish by some differences in composition. Presence of alpha- and beta-boswellic acids as well as 11-keto-beta-boswellic acetate which is not found in *balsamum* and the absence of cryptopimaric acid, brine, friedeline, mearnsetine, from malinadiol, more specific to *balsamum*, tend to exclude the presence of the 3 forms: wood, fruit and resin, in the preparation and to confirm the presence of olibanum resin.

It therefore seems more likely, according to our results, to consider that the opiate analyzed was prepared according to the 1884 pharmacopoeia rather than the 1818 edition. In any case, it is more than a

Table 3
Three examples, det : detected molecule, yes (+), no (-).

Olibanid resin	det	Officinal opium	det	Castoreum	det
Thunbergol	+	Morphine	+	(-)-1 epi, 7-epi-desoxynupharidine	+
Ac 11-keto-beta-Boswellic	+	Carvacrol	–	(-)-7-Demethyl-desoxynupharidin	–
20(S)-protopanaxadiol	–	Codeine	+	1-epi-desoxynupharidine	–
2-alpha, 3alpha-dihydroxy-urs-12-en-24-oic acid	+	Glaucine	+	4-ethoxy-phenol or 4-Ethoxyphenol	–
3-alpha-hydroxy-urs-9,12-diene-24-oic acid	–	Hydrocotarnine	+	4-methoxyacetophenone	–
3-alpha-hydroxytirucall-7,24-dien-21-oic acid	–	Laudanosine	+	4-methyl-1,2-dihydroxybenzene	–
3-alpha-hydroxytirucall-8,24-dien-21-oic acid	–	Linoleic acid	+	4,6-dimethyl-1-heptanol	–
3-alpha-O-acetyl-tirucall-8,24-dien-21-oic acid	–	Linolenic acid	–	5-methoxysalicylic acid	+
3-beta-acetoxy-16 (S), 20R dihydroxydammar-24-ene	–	Meconin	+	6-methyl-1-heptanol	–
3-Beta-hydroxytirucall-8,24-dien-21-oic acid	–	Narcotin	+	7-epi-deoxynupharidine	+
3-beta, 20 (S) -dihydroxydammar-24-ene	–	Néopine (beta codeine)	+	Acetophenone	–
3-keto-tirucall-8,24-dien-21-oic acid	–	Oleic acid	+	Benzoic acid	+
Alpha-amyrin	+	Palmitic acid	+	Benzylic alcohol	–
Alpha Boswellic acid	+	Papaverine	+	Rhododendrol	–
Beta-amyrin	+	Papaverine-M (O-desmethyl)	+	Borneol or iso-borneol	+
Beta-Amyrone	+	Papavéroline	+	Castoramine	+
Beta-Boswellic acid	+	Protopine	+	Cinnamic acid	+
Beta-cadinene	+	Protopine-M(Isomer-1AC)	+	Desoxynupharidine	+
Cembrene C and A	+	Stearic acid	+	4,4'-ellagic di-hydroxy acid (Pigment II)	–
Cembrenol	–	Thebaine	+	Dicrotyle (Sulfure)	–
Dehydroabietane	+	Thebaol	+	Didihydroxy-4,4'-dibenzo-alphapyrone (= Pigment I)	–
Dehydroabietic acid	+	Vitamin B	–	Dionone	–
Incensole	–	Vitamin PP	–	Gentisic acid	–
Incensole oxide	–	Cholesterol chloroformate	+	Hydroxy-benzoic acid (para and meta)	+
Isocembrene	–	1,2,3,4-tetrahydro isoquinoline, 6,7-dimethoxy-1-methyl-2-phenmethanol	+	Isocastoramine	–
Isoincensole	–			Isopinocamphone	–
Longifolene	+			Linalool oxide	+
Lupeol	–			Mannitol	+
Lupeolic acid	–			4-(4-methoxyphenyl)butan-2-ol	–
Octadec-9Z-enol	+			Oxo-5-cis-tetrahydro-ionone	+
Deoxy- podophyllotoxin	+			Para-hydroxyacetophenone (Piceol)	–
Stigmasta-3,5-diene-7-one	+			Phenylpropionic acid	+
Rhamnol	+			Hydroxyphenyl propionic acid	+
Urs-12-en-3alpha,24-diol	+			Pyrocathechol	–
Verticilla-4(20),7,11-triene	–			Acid or Salicylic Aldehyde	–

Table 4
Additional ingredients from 1818.

Rank	Additional ingredients from 1818	New molecules	Total number of molecules	Detected molecules	Specific molecules	Specific detected molecules	Total of detected molecules (%)
7	Lemon grass *Andropogon sch.* (grass)	5	40	14	6	1	35,0
8	Indian nard *A.nardus* (roots and stems)	5	27	6	4	0	22,2
16 15 14	*Amyris opobalsamum* Xylo (wood); carpo (fruit) and opo (resin) Balsamum	13	28	6	21	1	21,4
4	Cassia lignea *Laurus cassia* (bark)	5	14	3	3	0	21,4
6	Cardamom (fruit)	0	19	4	1	0	21,1
10	Arabic costus *Costus arabicus*	11	15	3	9	0	20,0
9	Celtic nard *Valeriana celtica* (roots)	10	16	3	10	2	18,8
5	Malabathrum *Laurus cas.* (leaves)	8	33	6	6	0	18,2
3	Acacia *Mimosa nilotica* (juice)	7	18	2	5	0	11,1
12	Marjoram *O.majorana* (buds)	13	32	3	12	0	9,4
17	Sagapenum	34	50	4	21	0	8,0
13	Macedonian parsley (seeds)	5	16	0	5	0	0,0
11	Marum *Teucrium marum* (buds)	6	11	0	6	0	0,0
2	Hypocist *Cystinus hypocistis* (juice)	7	10	0	7	0	0,0
1	Thlaspi *Thlaspi arvense* (seeds)	6	11	0	5	0	0,0

hundred years old formula. Detecting so many chemical substances in a so old preparation is an enigma when you know that an injectable ampoule of morphine hydrochloride that turns brown and deteriorates relatively quickly.

Quantification of a few opium alkaloids to estimate the level of preservation of the main active ingredient has been considered. Main difficulty was the constitution of an electuary matrix standard control.

A standard for calibration was prepared as follow: honey was spoked 1 and 2 mg of narcotine, morphine, papaverine and laudanosine and 1 mg of diazepam as internal standard. Same treatments that for opiate

were applied.

1 mg of internal standard was added to the collected opiate extracts and mixtures were analyzed by GC–MS. The same procedure was done for the control mixture. Results gives an idea of the residual quantities of alkaloids.

In the 1884 pharmacopoeia formula, the quantity of opium is 120 g. 1,000 g of theriacal powder contains 54.3 g or 1.13 % of the fresh preparation. The proposition of alkaloids in opium varies according to the origin and to the method of preparation, but it contains (mean value): 0.5–1.5 g/100 g of papaverine, 2–10 g % of narcotine, 10–20 g % of morphine and 0.05 to 0.1 g % of laudanosine. This would correspond for 1 g of fresh opiate to: morphine, 1.1–2.2 mg g^{-1}; papaverine 0.06 to 0.16 mg g^{-1}; narcotine, 0.23–1.1 mg g^{-1}; laudanosine, 0.006 to 0.013 mg g^{-1}.

The compact mass analyzed is the result of a long, slow and significates dehydration which concentrated the constituents. The measured quantities are: morphine, 0.8 +/- 0.4 mg g^{-1}; papaverine, 0.08 +/- 0.06 mg g^{-1}; narcotine, 0.6 +/- 0.5 mg g^{-1}; laudanosine, 0.017 +/- 0.017 mg g^{-1}.

These results show an astonishing conservation since they are part of the possible ranges calculated for fresh substance. Dehydration greatly contributes to rise the finding concentrations, but they still very significant. Assuming a loss of water equal to 50 % of the fresh preparation weight (hypothesis not proven) during the century of preservation (minimum estimated period), the loss of alkaloids from opium would be of the same order.

4. Discussion and conclusion

Results have been commented as their description progress in order to better understand their significance given the difficulty of considering all the parameters that may affect their relevance. However, their meaning must be based on the past of this panacea which has animated debates of so many scholars throughout all over the world since antiquity; whether the composition of simples and other ingredients which constitute the mixture which has given rise innumerable variants or the numerous medical indications supposed to find a benefit in their use.

To be focus on the contemporary period and to understand the evolution of the concept until the fortuitous discovery of this jar of theriac « Pochet Deroche », surprisingly spared by the years, some works brings answers to many questions. This is the case of the work finalized for the colloquium mentioned in introduction, « La Thériaque d'Andromaque à Moyse Charas »; published in its full version in the journal "Histoire de la pharmacie" [9].

After ten years of hard work, some authors, who were present during the colloquium and other, have published in 2020 « La thériaque Histoire d'un remède millénaire » [10] under the direction of professors Françoise Micheau and Véronique Boudon Millot. This publication develops all the pharmacological and historical aspects from the small theriacs to the great theriac of Andromaque, also the transmission to the Byzantine, Syriac and Arab worlds, and finally the theriac's apogee in the Western world up to the contemporary period.

Another study from Persian medical literature is focus on the evolution of theriacs. This study provide some understanding [11] as well as the one published by Tsatsakis AM et al. more focus on dose-response aspect of ancient medicals integrating theriacs [12].

Our analysis cannot confirm the validity of the ancient practices that made theriacs famous [13]. However, it seems to confirm the long shelf life of the active compounds of this preparation.

This study does not make it possible to state with certainty that this watch jar did indeed contain a theriac, since all the ingredients participating in the formula of the pharmacopoeias of the time of its acquisition or afterwards have not been identified. However, undetected products are not necessarily absent, they may be either degraded or in trace amounts not detectable by the means of implemented. On the other hand, two thirds of the components, including the most significant ones, are recognized and do not correspond to any other kind of electuary listed in the pharmacopoeias.

In spite of the incomplete nature of the formula reconstituted by the described analysis above, the most plausible hypothesis seems to fit well with that of a theriac executed according to the pharmacopoeia of 1884. If the preparation being analyzed actually dates from the assumed period, it can be deduces that honey, apart from having its own pharmacological properties, is also an excellent preservative of the substances it dilutes. Approximately 60 % of them are still detectable more than a century after their preparation.

Little work is published on the composition of ancient specimens. The most often these concern resinous materials of archaeological origin, coming for example from mummies [14] or archaeological excavations [15].

The originality of our work is due to the rarity of this type of analysis on very old pharmaceutical samples and to the fact that it concerns a mixture of great complexity. Our results are an encouragement to carry out such research and the use of mass spectrometry using high-resolution analyzers such as TOF (time of flight) or the Orbitrap, by accessing the exact molecular mass of the analytes, should to enable them to be optimized

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Conflict of Interest

The authors declare no conflict of interest.

Declaration of Competing Interest

The authors report no declarations of interest.

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