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Contemporary analytical techniques reveal the secret composition of a 19th century Jerusalem Balsam

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In 1719, Antonio Menzani di Cuna from the Saint Savior monastery published an alcoholic extract formula made from plant and herb resins under the name Jerusalem Balsam. The Balsam gained high popularity due to its remedial benefits. At the end of the 19th century, Jerusalem Balsam produced by the hermit Johannes Treutler was found to be particularly popular. We analysed a sample of a valuable find coming from the last decade of the 19th century, making it probably the oldest surviving Jerusalem Balsam in the world. The purpose of this work was to investigate the composition of the historical sample and to try to determine the origin of its components. This was achieved by comparing the profile of volatile compounds extracted from the balsam using HS-SPME technique with the profile characteristic for plant resins as classic ingredients of the Johannes Treutler formula. The use of two chromatographic columns of different polarity, as well as the transformation of the polar components of the sample into TMS derivatives, allowed to obtain new information on the historical composition of the Balsam. Also, it can be stated with high probability that plant resins were indeed used in the production of the Balsam as referred to in the original recipe of Johannes Treutler. We also discuss challenges in determining the original composition of the Balsam.

1. Introduction

In 1719, the Franciscan monk Antonio Menzani di Cuna from the Saint Savior monastery in Jerusalem published an alcoholic extract formula under the name Jerusalem Balsam made from herb and plant resins (Moussaieff et al. 2005). According to the current research, Menzani's formula is derived from medieval balsam recipes for wounds known as liquid bandages. For over 200 years, different varieties of Jerusalem Balsam have been used externally, as a reliable remedy for open wounds, burns and various skin diseases, as well as internally, as a kind of panacea. At the end of the 19th century, Jerusalem Balsam produced by the hermit Johannes Treutler was found to be particularly popular. Treutler manufactured it under the name "In Nazareth Aechter Jerusalemer Balsam im goldnen Engel" on Mount Mariańska near the town Kłodzko (currently in Poland). The popularity of the balsam was spread across current territories of Germany, Czechia and Poland. Over the years, the composition of the balsam, its application and therapeutic function have undergone significant modifications (Moussaieff et al. 2005). Nowadays, formulas produced under the name "Jerusalem Balsam", as well as other monastery balsams similar in composition are used orally, mainly in acute and chronic inflammation of the sinuses and upper respiratory tract and as a means of supporting the body's natural immunity. At the beginning of the last decade, in the small town of Skarszewy in Pomerania (Poland), in the attic of a tenement house once occupied by the mayor of this town, an inconspicuous sealed bottle was discovered, whose well-preserved label indicated that it contained the Jerusalem Balsam (Fig. 1). It is estimated that this valuable find was produced in the last decade of the 19th century, making it probably the oldest surviving Jerusalem Balsam in the world. The contents of the bottle were given to one of the co-authors (MJP), whose interest in Jerusalem Balsams has been known for years in monastery and pharmacy circles.

Kurkiewicz et al. (2017) conducted the first study of the historical Balsam using gas chromatography coupled with mass spectrometry (GC/MS) in 2017, comparing the profile of identified ingredients with the profiles of compounds found in monastery balsams produced nowadays. A similar comparison was then made by Łyczko et al. (2020), extending the analytical technique used to include headspace-solid phase microextraction (HS-SPME).

Fig. 1: Historical Jerusalem Balsam (In Nazareth Aechter Jerusalemer Balsam im golden Engel) in the original bottle.

The purpose of this work was to continue research on the composition of the historical sample of the Jerusalem Balsam and to try to determine the origin of its components by comparing the profile of volatile compounds extracted from the balsam using HS-SPME technique with the profile characteristic for plant resins as classic ingredients of the Johannes Treutler formula.

2. Investigations, results and discussion

Table 1 shows all the compounds detected in the historical Jerusalem Balsam using the HS-SPME GC/MS technique. They were identified by comparison of the obtained mass spectra with library spectra and of calculated Kovats retention indices with library indices. The chromatographic separation of volatile

compounds extracted on the fiber was carried out on two columns differing in polarity. In order to determine the source of the identified compounds, their occurrence was checked in plant resins, which most likely were the components of the original balsam formula (Schittny 2015).

Due to the presence of polar compounds, especially those containing hydroxyl and carboxyl groups, an additional GC/MS analysis was performed on the balsam after its derivatization with BSTFA. The results are summarized in Table 2.

Table 1: Compounds identified in Jerusalem Balsam and in selected resins using the HS-SPME-GC/MS technique

Compound			In the Jerusalem Balsam			Presence in tested resins						
Name	CAS or NIST	Group	RI HP-5	RI NIST	RI DB1701	1	2	3	4	5	6	7
Benzyl alcohol	100-51-6	Al-ol	1037.8	1037.0	1210.3	++						
3-Phenylpropanol	122-97-4	Al-ol	1240.4	1236.0	1408.2							
2-Propen-1-ol, 3-phenyl-, (E)-	4407-36-7	Al-ol	1316.3	1315.0	1509.8							++
Methane, diethoxy-	462-95-3	Acet	673.7	649.0	714.9						+	+
Ethane, 1,1-diethoxy-	105-57-7	Acet	730.0	727.0	768.0						+	++
Benzaldehyde	100-52-7	Ar	964.8	964.0	1089.1	++	+	+	+	+		
Phenol	108-95-2	Ar	980.9	981.0	1217.8	+					+	
Acetophenone	98-86-2	Ar	1072.0	1072.0	1207.7	+					+	+
Benzoic acid, methyl ester	93-58-3	Ar	1099.4	1099.0	1206.3	+						
Cinnamyl ethyl ether	1476-07-9	Ar	1159.7	-	1233.3							
Benzoic acid	65-85-0	Ar	1163.2	1162.0	1406.3	++						+
Benzoic acid, ethyl ester	93-89-0	Ar	1177.4	1177.0	1284.6	++						
1-Butanone, 2-methyl-1-phenyl-	938-87-4	Ar	1263.0	1262.0	1377.5	+						
Cinnamaldehyde	14371-10-9	Ar	1280.2	1277.0	1462.9	+					+	+
Eugenol	97-53-0	Ar	1363.9	1362.0	1518.6	+						++
Ethyl (Z)-cinnamate	4610-69-9	Ar	1382.3	1382.0	1517.9							
p-Vanillin	121-33-5	Ar	1408.4	1409.0	1660.4	++					+	
Ethyl (E)-cinnamate	4192-77-2	Ar	1474.5	1476.0	1616.3	+						
Benzyl benzoate	120-51-4	Ar	1783.1	1784.0	1957.0	++	+	+	+	+		
p-Cymene	99-87-6	MTer	1028.5	1028.0	1076.1	+	+	+	+	+		
D-Limonene	5989-27-5	MTer	1032.9	1033.0	1058.8	+	++	+	++	++	+	+
Camphor	76-22-2	MTer	1153.8	1155.0	1271.2				+	+		
δ-Elemene	20307-84-0	STer	1347.0	1346.0	1372.5	+		+			+	+
β-Bourbonene	5208-59-3	STer	1399.3	1398.0	1429.3							
β-Elemene	515-13-9	STer	1402.7	1400.0	1443.9	+	+					
α-Gurjunene	515-17-3	STer	1417.6	1419.0	1552.1							
α-Santalene	512-61-8	STer	1430.8	1427.0	1456.5							
Germacrene D	23986-74-5	STer	1460.2	1461.0	1498.4			+		+		
α-Bulnesene	NIST#374199	STer	1479.1	-	1511.6							
α-Selinene, (-)-	473-13-2	STer	1490.1	1490.0	1557.0						++	+
(+)-α-Muurolene	483-75-0	STer	1494.2	1495.0	1529.0							
β-Selinene	17066-67-0	STer	1504.9	1509.0	1557.0	+		+		+		
Valencene	10219-75-7	STer	1513.3	1504.0	1545.1				+	+		
γ-Cadinene	39029-41-9	STer	1530.4	1528.0	1571.2	+		+	+	+		
δ-Cadinene	483-76-1	STer	1536.7	1533.0	1578.3	+		+		+		+
cis-Calamenene	72937-55-4	STer	1538.5	1531.4	1600.0				+	+		
α-Cadinene	24406-05-1	STer	1554.1	1546.0	1593.1			+		+		
(4aR,8aS)-4a-Methyl-1-methylene-7-(propan-2-ylidene)decahydronaphthalene	58893-88-2	STer	1561.1	-	1595.4							
Elemol	639-99-6	STer	1563.2	1565.0	-			+				
Epicubenol	19912-67-5	STer	1634.4	1633.0	1731.9							++

Compound			In the Jerusalem Balsam			Presence in tested resins						
τ-Cadinol	5937-11-1	STer	1659.5	1659.0	1770.2					+		
Elemoyl acetate	60031-93-8	STer	1687.2	1675.0	1784.2							
Eudesm-7(11)-en-4-ol	473-04-1	STer	1694.4	1695.0	1749.7							
Cadalene	483-78-3	STer	1695.1	1696.0	1803.6				+	+		
Cembrene	1898-13-1	DiTer	1958.0	1948.0	2014.2					+		
Cembrene A	31570-39-5	DiTer	1988.7	1951.0	2048.4				+	+	+	
Ethyl acetate	141-78-6	SCFA	615.8	616.0	679.6							
Propanoic acid, ethyl ester	105-37-3	SCFA	713.2	714.0	772.7						++	
Propanoic acid, 2-methyl-, ethyl ester	97-62-1	SCFA	758.5	755.7	816.6							
Butanoic acid, ethyl ester	105-54-4	SCFA	802.5	802.0	862.0							
Lactic acid, ethyl ester	97-64-3	SCFA	815.5	815.0	921.5							
Butanoic acid, 2-methyl-, ethyl ester	7452-79-1	SCFA	850.4	850.0	907.6							
Butanoic acid, 3-methyl-, ethyl ester	108-64-5	SCFA	853.5	854.0	912.6							
Butanoic acid, 3-hydroxy-, ethyl ester	5405-41-4	SCFA	936.6	937.0	1006.9						+	
Propanoic acid, 3-ethoxy-, ethyl ester	763-69-9	SCFA	987.2	-	1072.6						++	
Levulinic acid, ethyl ester	539-88-8	SCFA	1062.5	1070.0	1213.7							
Butanedioic acid, diethyl ester	123-25-1	SCFA	1178.8	1182.0	1305.1							
Diethyl 2-methylsuccinate	4676-51-1	SCFA	1205.3	1205.0	1324.3						+	
Pentanedioic acid, diethyl ester	818-38-2	SCFA	1279.2	1279.0	1410.6							
Hexanoic acid, ethyl ester	123-66-0	MCFA	999.2	999.0	1062.7							
Ethyl 5-methylhexanoate	10236-10-9	MCFA	1070.5	1072.0	1135.2							
Octanoic acid, ethyl ester	106-32-1	MCFA	1196.1	1196.0	1263.2							
Octanoic acid, 4-methyl-, ethyl ester	54831-51-5	MCFA	1265.8	1267.0	1333.0						+	
Nonanoic acid	112-05-0	MCFA	1272.9	1273.0	-							
Nonanoic acid, ethyl ester	123-29-5	MCFA	1295.2	1294.0	1363.0						+	
Decanoic acid, ethyl ester	110-38-3	MCFA	1394.5	1394.0	1463.5							++
Dodecanoic acid, ethyl ester	106-33-2	MCFA	1593.8	1593.0	1665.1							
Tetradecanoic acid, ethyl ester	124-06-1	LCFA	1794.0	1794.0	1866.6							
Hexadecanoic acid, ethyl ester	628-97-7	LCFA	1994.2	1994.0	2069.1							
9-Octadecenoic acid (Z)-, ethyl ester	111-62-6	LCFA	2170.4	2171.0	2258.5						+	+
Octadecanoic acid, ethyl ester	111-61-5	LCFA	2194.4	2194.0	2271.4							
Furfural	98-01-1	Fur	835.0	836.0	974.3							
Furfural, 5-methyl-	620-02-0	Fur	966.6	966.0	1114.1							
2-Furancarboxylic acid, ethyl ester	614-99-3	Fur	1054.8	1053.0	1179.8							
5-(Hydroxy-methyl)-furfural	67-47-0	Fur	1229.1	1230.0	1532.8							

Balsamum Peruvianum (1), Mastic (2), Myrrh (3), Olibanum Eritrea (4), Olibanum Indicum (5), Olibanum Oman (6), Styrax Honduras (7). CAS - CAS Registry Number or (if CAS is not available) NIST Library number. RI - Kovats retention index. Acet - acetals, Ar - derivatives of aromatic compounds, MTer - monoterpenes and monoterpenoids, STer - sesquiterpenes and sesquiterpenoids, DTer - diterpenes, SCFA, MCFA and LCFA - short, medium and long chain esters and fatty acids, respectively, Fur - furans. "+" - the compound present on the resin chromatogram. "++" - peak area on the resin chromatogram above 3% of the total peak area.

Table 2: Compounds identified in the Jerusalem Balsam after BSTFA derivatization by gas chromatography in combination with mass spectrometry (GC/MS)

Compound	CAS or NIST	Group	RI HP-5	RI NIST	RI DB1701
Ethylene glycol, 2TMS derivative	7381-30-8	Al-ol	988.6	990.0	1013.2
1-Propoxy-2-propanol, TMS derivative	NIST#367032	Al-ol	1005.6	-	1070.2
2,3-Butanediol, 2TMS derivative	53274-85-4	Al-ol	1038.2	1040.0	1048.5
1,3-Dioxane, 2,2-dimethyl-5-trimethylsilyloxy-	NIST#381757	Al-ol	1092.9	-	1185.0
Benzyl alcohol, TMS derivative	14642-79-6	Al-ol	1157.8	1151.0	1223.7
Glycerol, 3TMS derivative	6787-10-6	Al-ol	1282.0	1283.8	1298.1
Cinnamyl alcohol, TMS derivative	NIST#245730	Al-ol	1432.1	-	1520.6
Benzoic acid, ethyl ester	93-89-0	Ar	1176.2	1177.0	1283.5
Benzoic acid, TMS derivative	2078-12-8	Ar	1251.2	1250.0	1342.6
Salicylic acid, TMS derivative	NIST#374326	Ar	1354.9	1354.0	-
4-Hydroxybenzaldehyde, TMS derivative	1012-12-0	Ar	1379.6	1382.0	1532.4
Cinnamic acid, TMS derivative	2078-20-8	Ar	1436.8	-	1552.6
Ethyl (E)-cinnamate	4192-77-2	Ar	1474.5	1476.0	-
Vanillin, TMS derivative	6689-43-6	Ar	1545.5	1545.0	-
Cinnamic acid, (E)-, TMS derivative	55012-82-3	Ar	1551.6	1547.0	1676.1
3-Hydroxybenzoic acid, 2TMS derivative	3782-84-1	Ar	1571.9	1574.0	1669.0
Vanillic acid, 2TMS derivative	2078-15-1	Ar	1774.5	1774.0	1894.0
Benzyl benzoate	120-51-4	Ar	1783.1	1784.0	1957.1
4-Coumaric acid, 2TMS derivative	10517-30-3	Ar	1950.6	1949.0	2089.8
(3-Hydroxy-4-methoxyphenyl)ethylene glycol tris(trimethylsilyl) ether	NIST#72205	Ar	2033.3	-	2081.7
4-Hydroxy-3-methoxyphenylglycol, 3TMS derivative	68595-81-3	Ar	2037.2	-	-
Ethyl glycolate, TMS derivative	80287-79-2	SCFA	1007.5	1000.0	1084.8
Lactic acid, 2TMS derivative	17596-96-2	SCFA	1066.1	1066.0	1116.1
Glycolic acid, 2TMS derivative	33581-77-0	SCFA	1080.0	1080.0	1145.2
Levulinic acid, TMS derivative	55557-12-5	SCFA	1135.8	1130.0	1269.4
Hydracrylic acid, 2TMS derivative	55162-32-8	SCFA	1148.3	1151.0	1205.0
Glyceric acid, 3TMS derivative	38191-87-6	SCFA	1339.8	1335.7	1390.6
Butanoic acid, 3,4-bis[(trimethylsilyl)oxy]-, TMS derivative	55191-53-2	SCFA	1441.1	1439.0	1528.8
L-Threonic acid, tris(trimethylsilyl) ether, TMS derivative	NIST#352533	SCFA	1559.8	-	1594.9
Nonanoic acid, TMS derivative	82326-11-2	MCFA	1361.1	1355.0	1412.2
Palmitic acid, TMS derivative	55520-89-3	LCFA	2047.4	2050.0	2103.9
9,12-Octadecadienoic acid (Z,Z)-, TMS derivative	56259-07-5	LCFA	2516.4		-
2-Furoic acid, TMS derivative	55887-53-1	Fur	1137.5	1131.2	1247.8
3-Methyl-2-furoic acid, TMS derivative	NIST#153538	Fur	1317.0	-	-
2-Furancarboxylic acid, 5-[[(trimethylsilyl)oxy]methyl]-, TMS derivative	55517-40-3	Fur	1556.3	-	1673.4
Ethylphosphonic acid, 2TMS derivative	17882-98-3	Phos	1239.9	-	-
Silanol, trimethyl-, phosphate (3:1)	10497-05-9	Phos	1288.6	1286.0	1391.7
Levoglucosan, 3TMS derivative	7449-14-1	Sacch	1724.5	-	1816.3
NI (105, 147, 75, 175)	NI	NI	1076.5	-	1147.1
NI (73,117, 147, 284, 263, 103)	NI	NI	1297.1	-	1364.5
NI (73, 147, 189, 117, 129, 202, 247, 277)	NI	NI	1385.7	-	1450.2
NI (253, 73, 166, 225)	NI	NI	2022.9	-	2078.0
NI (253, 73, 166, 225)	NI	NI	2026.3	-	2087.5

CAS - CAS Registry Number or (if CAS is not available) NIST Library number. RI - Kovats retention index. For unidentified compounds, m/z values with the highest intensity were given. Al-ol - alcohols, Ar - derivatives of aromatic compounds, SCFA, MCFA and LCFA - short, medium and long chain esters and fatty acids, respectively, Fur - furans, Phos - phosphoric acid derivatives, Sacch – saccharides, NI - unidentified compounds.

Taking into account structural features and pharmacological effects, the detected balsam components were divided into the following groups: acetals (Acet), derivatives of aromatic compounds (Ar), monoterpenes and monoterpenoids (MTer), sesquiterpenes and sesquiterpenoids (STer), diterpenes (DTer), esters and short, medium and long chain fatty acids (SCFA, MCFA, LCFA), furans (Fur), alcohols (Al-ol), phosphoric acid derivatives (Phos) and saccharides (Sacch). A group of unidentified compounds (NI) was also distinguished.

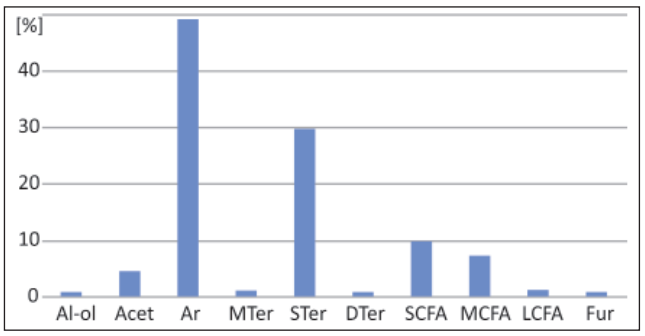

Fig. 2: Percentage of individual groups of compounds identified in the historical Jerusalem Balsam as a result of direct analysis on fiber. Al-ol – alcohols, Acet -acetals, Ar – derivatives of aromatic compounds, MTer- monoterpenes and monoterpenoids, STer – sesquiterpenes and sesquiterpenoids, DTer – diterpenes, SCFA, MCFA and LCFA – short, medium and long chain esters and fatty acids, respectively, Fur – furans. The data are taken from DB-1701 column.

Figure 2 presents the percentage of individual groups of compounds detected in the Jerusalem Balsam as a result of fiber sorption, whereas Fig. 3, after sample derivatization with BSTFA.

Considering the age of the analyzed balsam, it can be assumed that its original composition has undergone significant changes. These include natural aging, compound oxidation, isomerization, polymerization, mutual reactions, as well as degradation from the activity of microorganisms. Adverse conditions of balsam storage may have accelerated these processes (leaky packaging facilitating evaporation of solvent and other volatile components, access of light and oxygen).

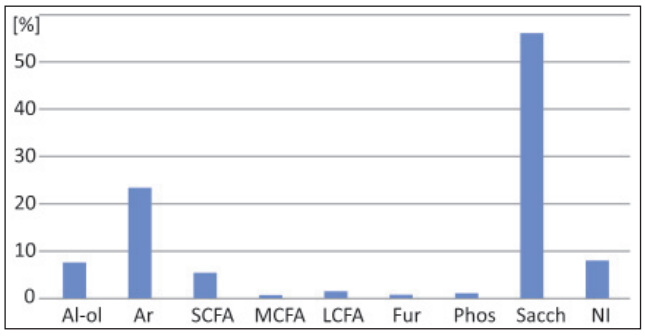

Fig. 3: Percentage of individual groups of compounds identified in the historical Jerusalem Balsam after derivatization with BSTFA. Al-ol – alcohols, Ar – derivatives of aromatic compounds, SCFA, MCFA and LCFA – short, medium and long chain esters and fatty acids, respectively. Fur – furans, Phos – phosphoric acid derivatives, Sacch -saccharides, Nl – unidentified compounds. The data are taken from DB-1701 column.

Aromatic compounds, sesquiterpenes and aliphatic organic acids dominate among the volatile components in the headspace gas of the tested balsam (Fig. 2). However, after derivatization to trimethylsilyl (TMS) derivatives, the dominant group of compounds were saccharides, aromatic compounds and alcohols (Fig. 3). The aromatic compounds identified by the HS-SPME GC/MS technique are dominated by benzoic acid ethyl ester (25.8% of the total peak area), benzoic acid (17.3%), benzyl benzoate (1.7%) and ethyl (E)-cinnamate (1.5%).

A detailed analysis of the chromatograms indicates Balsamum Peruvianum (1) and Styrax Honduras (7) as the most likely sources of aromatic compounds in the historical balsam. It should be noted that a large amount of benzoic acid ethyl ester may be due to the possible esterification of benzoic acid in the ethyl alcohol medium which is a constituent of the balsam formula. Much less cinnamaldehyde was found in the historical balsam compared to the analysed resins. Our own observations show that this aldehyde can be oxidized to an acid that easily reacts with ethanol given long-term storage of its alcoholic solutions with the exposure to atmospheric oxygen. This progression is suggested by the detection of ethyl (E)-cinnamate (1.49%), ethyl (Z)-cinnamate (0.27%) and in small amounts of cinnamyl ethyl ether.

Terpenes and terpenoids are the main components of essential oils and are commonly found in plant extracts and resins. The Jerusalem Balsam is dominated by isoprene derivatives with fifteen carbon atoms, such as isomeric elemenes, isomeric selinenes and valencene, γ-cadinene, τ-cadinol and cembrene (Table 1). The small amount of monoterpenes and monoterpenoids (0.55%) compared to the tested resins may result from the high volatility of these compounds and their tendency to polymerize. The presence of polymerization products of isoprenoid derivatives in the historical balsam may be indicated by its dark colour and visible sediment. However, it should be noted that the dark colour of the balsam is primarily due to its recipe ingredients, especially *Balsamum Peruvianum* and St. John's wort (Hypericum) extract.

Sesquiterpenes and sesquiterpenoids are commonly found in mastic (2), myrrh (3) and olibanum (4-6) resins (Fernandez et al. 2005; Cao et al. 2019). Olibanum is a natural oleo-gum-resin obtained from the trees belonging to the *Boswellia* genus of the Burseraceae family which grow in semidesert areas mainly in Arabia, on the east coast of Africa and India. The resin obtained from *Boswellia* is a complex mixture of about 65-85% alcohol-soluble resins (diterpenes, triterpenes), other water-soluble gums (polysaccharides) and 5-9% aromatic essential oil consisting mainly of mono- and sesquiterpenes (Hamm et al. 2005). Hamm et al. also show that even in the case of archaeological samples over 2,000 years old, analysis of olibanum volatiles can be helpful in determining the origin of this resin. By studying the historical balsam, its components were compared with volatile compounds extracted from Olibanum Eritrea (4), Indicum (5) and Oman (6). It was found that Olibanum Oman (6) has the greatest resemblance to the balsam in terms of the type of aromatic compounds identified, but those, as suggested earlier, may also come from other resins. On the other hand, Olibanum Indicum (5) shares the highest amount of similarities with the Jerusalem Balsam in terms of compounds from the terpenes and terpenoids group, so it can be assumed that it was the resin from the Indian peninsula that was used as a raw material for the production of the tested Jerusalem Balsam. However, Musiał (2011) believes that olibanum imported to Europe during Treutler times came from the lands of Africa or South Arabia.

An intriguing group of ingredients in the historical balsam are compounds containing a furan ring, mainly furfural derivatives and furoic acid derivatives. Such compounds were also detected in contemporary monastery balsams analyzed with GC/MS technique, but with 10 to 30 times smaller amounts (Kurkiewicz et al. 2017). At the same time, small amounts of amino acids were found in modern balsams that were not detected in the historical sample. It seems likely that in the presence of high concentrations of reducing sugars they could be used as substrates in the Maillard reaction. Furans are one of the products of this reaction, which can already take place at room temperature (Limacher et al. 2008). Furans can also be formed in the absence of amino acids through the degradation of sugars and in 20 °C during longer storage of food, especially in impervious packaging. Another source of furans may be reactions related to oxidation and degradation of fatty acids or triglycerides, especially polyunsaturated ones (Kettlitz et al. 2019). The factor favoring furan formation is high temperature (Vranova and Ciesarova 2009). According to the original formula (Schittny 2015), the production of the Jerusalem Balsam required many hours (twice for 24 hours) of heating the ingredients on the stove.

The dominant group among TMS derivatives identified in the Jerusalem Balsam were saccharides (51.3%). The presence of sugars in a balsam intended for external application for wounds is unanticipated. Among five of the different formulas found in the archives of the Saint Savior monastery in Jerusalem, all referred to as "The Jerusalem Balsam", only one (dated 1904) contains

sugar. This suggests that the formula was designed for internal rather than for topical use (Moussaieff et al. 2005). According to historical sources (Schittny 2015), around 1887 the pharmacist Johannes Schittny suggested Johannes Treutler to modify the Jerusalem Balsam recipe so that it could be taken orally. The modification consisted of diluting the lotion to reduce the alcohol concentration (from 70 to 30%). However, there is no information about the change in the composition of the mixture by sweetening it. Nonetheless, it cannot be dismissed that such a modification could have already taken place in order to increase profits from the sale of the product after Treutler's death, when other pharmacists began to use the original recipe on a larger scale. Some of the sugars could originate from one of the essential ingredients of the Balsam formula, commonly known as "aloe suc". Leaves, juice or gel coming from aloe species are rich in polysaccharides, which can easily depolymerize, especially at lower pH. It remains an open question whether such depolymerization with the formation of simple sugars could occur under the conditions of preparation or – more likely – storage of the Balsam for more than 100 years.

After derivatization of Jerusalem Balsam with BSTFA, the chromatogram showed the presence of about 50 peaks, which can be classified into the sugar group based on the characteristic look of the mass spectrum. Such a large number of compounds from this group may be a result of processes related to aging of the sample, including oxidation, isomerization, non-enzymatic transformations (e.g. Maillard reaction). Due to the large similarities in the spectra of various structural isomers, precise determination of their identity requires the use of standards. These were used for levoglucosan identification. This compound is considered to be a molecular marker of biomass combustion, because it is formed during wood pyrolysis at temperatures higher than 300 °C (Zhang et al. 2008). The presence of levoglucosan suggests that the analyzed Jerusalem Balsam may have been in contact with smoke from wood burning, most likely during the heating of the prepared mixture on/in the oven recommended in the original Menzani recipe from 1719 (Schittny 2015). The high temperature could also partially explain the dark colour of the historical Balsam, which may result from caramelization processes, during which polymers with 24 to 125 carbon atoms are formed (caramelans, caramelens, and caramelins). By lowering the pH, the caramelization temperature can be significantly reduced (Suárez-Pereira et al. 2010).

Another group of compounds identified in the Jerusalem Balsam are fatty acids, among which short chain acids predominate. In a direct analysis of the volatile components extracted on the fiber, their ethyl esters (9.31%) were identified, which may have been formed by esterification in an ethanol environment. Ethyl acetate, 3-methylbutanoic acid ethyl ester, butanedioic acid diethyl ester and propanoic acid ethyl ester were found in the largest quantities. Free fatty acids were also present in the historical balsam, which were only detected after being converted to trimethylsilyl derivatives (4.7%). In turn, this group was dominated by glycolic acid, lactic acid, hydracrylic acid, and ethyl glycolate. Similar acids were detected in contemporary balsams (Kurkiewicz et al. 2017), but up to dozen times less. Such a large amount of short chain fatty acids is probably a result of metabolising sugars by bacteria and fungi. The effect of such activity of microorganisms could possibly cause a decrease in the pH of the Balsam, which in turn could promote the process of caramelization of sugars at the storage temperature of the historical balsam. The presence of medium and long fatty acids was also found in the analysed balsam, with a significant predominance of saturated acids. Their most probable source are plant extracts that are also included in the balsam formula along resins. Long chain fatty acids have also been found in modern balsams however 3 to 10 times more than in the sample of the original balsam, the most dominant being linoleic acid (Kurkiewicz et al. 2017). This acid is easily degraded, hence the historical sample contains much less of it.

The presence of compounds from the acetal and fusel alcohol group in the historical balm is not unforeseen. These are well-known and well-described compounds that have arisen as a result of the activity of microorganisms during the production of wine. They are responsible for its "aromatic bouquet". The amount of these compounds increases with the maturing processes of alcoholic beverages (Rapp 1988).

To summarize, the presence of many compounds detected in the Jerusalem Balsam by Łyczko et al. (2020) was confirmed using the same HS-SPME GC/MS technique. However, the use of different fiber for microextraction and two chromatographic columns of different polarity, as well as the transformation of the polar components of the sample into TMS derivatives, allowed to obtain new information on the historical composition of the balsam. Also, it can be stated with high probability that plant resins were indeed used in the production of the balsam as referred to in the original recipe of Johannes Treutler.

The examined sample of Jerusalem Balsam is very valuable, not only because of its historical nature. As demonstrated by Łyczko et al. (2020), due to its unique chemical composition, it showed cytotoxicity towards cancer cells, significantly affecting the metabolism and viability of CLBL-1 and CLB70 cell lines. It should be emphasized that determining the full composition of such a complicated sample is difficult. The use of the GC/MS technique is limited to testing compounds with sufficient volatility and stability at the analysed temperature (up to 350 °C). Information on the composition of the historical Jerusalem Balsam sample could undoubtedly be enriched by other analytical techniques such as e.g. high performance liquid chromatography in combination with mass spectrometry (HPLC / MS) or for the analysis of high-molecular substances MALDI-TOF MS. It is not clear, however, whether there is enough of the historical material to learn everything we would like to know.

3. Experimental

The HS-SPME GC/MS technique was used to analyse a sample of the historical Jerusalem Balsam and the following plant resins: Balsamum Peruvianum (1), mastic (2), myrrh (3), Olibanum Eritrea (4), Olibanum Indicum (5), Olibanum Oman (6) and Styrax Honduras (7). Samples of 50 mg were placed in screw-cap glass vials with a capacity of 4 mL each. Those were conditioned in 50 °C for 10 min, which allowed for the volatile ingredients to move towards the gas phase. For the next 30 min, the PDMS/DVB 65 µm fiber (Agilent Technologies) was inserted to all the vials, and left in contact with the headspace. Subsequently, it was introduced into the split-splitless injector of the Agilent Technologies 7890A gas chromatograph where the desorption of extracted compounds was carried out for 1 min in 250 °C. The GC separations were performed on a HP-5MS (5% diphenyl, 95% dimethyl polysiloxane) and DB-1701 (14% cyanopropylphenyl, 86% dimethyl polysiloxane) fused-silica capillary columns (Agilent Technologies, 60 m 0.25 mm id, 0.25 µm film thickness). A split ratio 1:20 and helium was used as the carrier gas at a flow rate of 1.6 mL/min. The GC oven temperature was programmed from 35 °C (isothermal for 1 min) to 260 °C at a rate of 5 °C/min. The final temperature was held for 5 min. The GC column outlet was connected directly to the ion source of the Agilent Technologies 7000 GC/MS Triple Quad mass spectrometer. The GC/MS interface, the ion source, and the quadrupoles were kept at 270, 230, and 150 °C, respectively. The ionization energy was 70 eV. The mass spectrometer was operated in a full scan mode (m/z 29–450). The software used for data collection and mass spectra processing was MassHunter GC/MS Acquisition B.07.05 and MassHunter Workstation B.07.00 (Agilent Technologies). Analysed compounds were identified by comparison of their mass spectra with the library standards (the NIST/EPA/NIH Mass Spectral Library 2014 and the Wiley Registry of Mass Spectral Data 10th Edition) and by comparison of the Kovats retention indices (RI) calculated on HP-5MS and DB-1701 column with the tabulated values (NIST).

In order to broaden the range of identified compounds, an additional analysis of the Jerusalem Balsam was carried out after its derivatization to trimethylsilyl (TMS) derivatives. 20 mg of the Balsam was evaporated to dryness under a stream of argon, and the residue was silylated with 500 µl mixture N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and 3% pyridine. The reaction mixture was heated at 60 °C for 1 h and after cooling was analyzed by GC/MS under the conditions described above.

Conflicts of interest: None declared.

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