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Chemical profiling and cytotoxic activity of 150-year old original sample of Jerusalem Balsam

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ABSTRACT

Herbal formulations have been used in ethnomedicine and pharmacy around the world for thousands of years. One of them is Jerusalem Balsam (JB), which came into use in the seventeenth century. Today, people still produce and use it regularly as prophylactic supplement.

JB has been widely used in Europe since the nineteenth century, as a remedy possessing antibacterial, antifungal and anti-inflammatory activities. The composition of the product was not known, although possible formulations were reported. In this study the original sample, which dated back to 1870, was investigated for chemical composition and cytotoxic activity. The obtained results were compared with results from more recently produced samples. Several tests were carried out, namely GC-MS, UPLC-PDA-Q-TOF-MS and MTT.

Only the 150-year old sample showed a significant cytotoxic activity on cancer cell lines. At a concentration of 125 μg/mL after 72 h of incubation, the original sample inhibited almost 90% of cell metabolic activity, while contemporary samples showed none or little activity. None of the tested samples showed a significant impact on normal cells. These results may be attributed to the activities of benzoic acid and its derivatives, cinnamic acid derivatives, vanillin, group of sesquiterpenes and cembrene.

1. Introduction

Natural products and their derivatives have been used for ages by civilizations and pharmacists all around the word – from ancient Sumerians, Chinese or Mediterranean civilizations (Kaliora and Kountouri, 2012) up to contemporary civilizations (Peschel, 2016). Some of the natural products used include propolis (Kuropatnicki et al., 2013), oleo-resins (balsams) such as benzoin obtained from the *Styrax* genus trees (Manvi and Sharma, 2017), the balsam of Mataryyia obtained from *Cammiphora* sp. (*C. olibanum* and *C. molmol* Engl.) (Milwright, 2003, 2008) and herbal tinctures like sage one (*Salvia officinalis* L.) (Walch et al., 2012) or more contemporary cannabis (*Cannabis sativa* L.) (Peschel, 2016).

Applying that knowledge of natural products and his own experience, Ostwald Croll, seventeenth-century physician formulated a recipe for the "golden remedy", *Balsamum velnerarium efficacissimum* (1608), and published it in his work, *Basilica Chymica* (1609). It was a mixture of oleo-resins (mastic – *Pistacia lenticus*, olibanum – *Boswellia* sp., styrax balsam – *Liquidambar orientalis* Mill.), herbal extracts (*Hypericum perforatum*, myrrh – *Commiphora myrrha* Engl.) and even additives such as mummy powder (Schnittny, 2015). Then, in 1719, Antonio Menzani di Cuna, a monk from the Franciscan Monastery of Saint Savior in Jerusalem, disclosed the Jerusalem Balsam (JB) recipe (Kaliora and Kountouri, 2012; Perry and Lev, 2003), which turned out to be the same as that formulated by Croll, with a few more compounds – *Aloe* sp., Balsam of Peru (*Balsamum Peruvianum* from *Myroxylon pereirae (Royle),*

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Abbreviations

Å	ångström
CAM	complementary and alternative medicine
DMF	N,N-dimethylformamide
DMSO	dimethyl sulphoxide
ESI-NEG	electrospray negative ionisation mode
FBS	fetal bovine serum
GC-MS	gas chromatography-mass spectrometry
HS-SPME	headspace solid-phase microextraction
IC_{50}	the half maximal inhibitory concentration
JB	Jerusalem Balsam
MTT	thiazolyl blue tetrazolium bromide
PA	pyrrolizidine alkaloids
PBS	phosphate buffered saline
RI	retention indices by Kovats
RT	retention time
SD	standard deviations
SDS	sodium dodecyl sulfate
UPLC-PDA-Q-TOF-MS	ultra-performance liquid chromatography-photodiode detector-quadrupole/time of flight-mass spectrometry

Klotzsch) benzoin (*Styrax* sp.), moschus and ambra. Further, in decades that followed, many variations of this panacea were revealed under different names; however, the main breakthrough came in 1860, when Johannes Treutler – the hermit from Spittelberg (now Mariańska Góra, beside Kłodzko, Poland), acquired the JB recipe and started the production of the panacea and sold it to pilgrims. That was the beginning of JB's eminence in Europe and it has lasted up to this day (Schnittny, 2015).

According to the formula found in the convent's archive, JB was originally composed of four ingredients – *Boswellia* sp., *Aloe* sp., *Comiphora molmol* Engl. and *Pistacia lentiscus* L. (Amar et al., 2005). However, other sources point that the original JB was made with up to forty ingredients (Amar et al., 2005) and, meanwhile, some suggest that JB is only another name for benozin, the resin obtained from *Styrax* genus trees (Manvi and Sharma, 2017). It was used as treatment for a wide range of afflictions such as cardiac disease, headaches, stomach aches, skin disorders and for wound healing, until the development of more modern solutions like patches (Amar and Lev, 2005; Perry and Lev, 2003).

Nevertheless, JB is today the subject of various studies and experiments. It is studied as a substance with potential impact on kynurenic acid synthesis (Baran et al., 2017) or as a substance with cytotoxic and antioxidant activity (Sajkowska-Kozielewicz et al., 2016). More recently, the composition of original JB from approximately the middle of nineteenth century was investigated and compared with contemporary ones (Kurkiewicz et al., 2017). This renewed interest in JB may be due to the increased consumer curiosity about prophylactic agents, especially the natural ones. These prophylactic agents of interest include herbal formulations, chiefly containing Chinese and oriental herbs (Leong et al., 2018; Hu et al., 2016; Teng et al., 2016) or propolis products (Virga et al., 2018). The main, recent field of studies are focused on the effect of natural formulations of prophylactics for the treatment diabetes (Khazaei et al., 2018), aging-associated diseases (Leong et al., 2018), neuroprotective activity (Su et al., 2018), respiratory tracts infections (Di et al., 2016) and various anticancer activities (Kamble et al., 2009; Rai et al., 2016a).

The aim of the present work was to identify constituents responsible for potential cytotoxic activity in a JB sample from the mid-nineteenth century, and to compare it to the chemical composition and cytotoxic activity of contemporarily-produced JBs samples. In particular, the volatile profile of examined JBs were studied; however, non-volatile profiles were also examined to consider their influence on cytotoxic activity of examined samples.

2. Materials and methods

2.1. Materials

The original JB, produced approximately in 1870 was provided by Dr. Marcelin Jan Pietryja from Herbary of Saint Francis of Fathers Franciscans in Katowice-Panewniki (Poland) (JB5). Contemporary JBs (produced in 2019) were obtained from pharmacies of Brothers Hospitallers of Saint John of God (O.H.) in Wrocław (JB1), Łódź (JB2) and Cracow (JB3) (Poland) and from pharmacy, Zum Rothen Krebs, in Vienna (Austria) (JB4).

2.2. Methods

2.2.1. Volatile composition analysis

The volatile composition analysis of all JBs was performed by headspace solid-phase microextraction (HS-SPME) and solvent extraction – both coupled with GC-MS technique. Identification of all volatile constituents was based on comparison of experimentally obtained compound's mass spectra with mass spectra available in NIST14 database. Also, the experimentally obtained retention indices (RI) by Kovats was compared with RI available in the NIST WebBook and literature data (Adams, 2012). Shimadzu software GCMS Postrun Analysis (Shimadzu Company, Kyoto, Japan) and ACD/Spectrus Processor (Advanced Chemistry Development, Inc., Toronto, ON, Canada) were used to process the data. The quantification of identified constituents was performed by calculation based on the amount of added internal standard and expressed as a percentage of integrated peaks area.

2.2.2. Headspace solid-phase microextraction (HS-SPME)

HS-SPME (30 min exposure to Supelco 2 cm DVB/CAR/PDMS fiber; analytes desorption at 220 °C for 3 min) was performed on Varian CP-3800/Saturn2000 apparatus (Varian, Wallnut Creek, CA, USA) equipped with Zebron ZB-5 MSI (30 m × 0.25 mm × 0.25 μm) column (Phenomenex, Torrance, CA, USA). Then, 0.2 mL of JB was dissolved in 1.8 mL of 12.5% brine. The sample was put in to headspace vials and kept on magnetic stirrer with magnet at 25 °C. Ten (10) μg of 2-dodecanone (Sigma Aldrich, St. Louis, MO, USA) was added as an internal standard. GC oven temperature was programmed from 50 °C to 130 °C at a rate of 2.0 °C, then to 180 °C at a rate of 10.0 °C, then to 280 °C at a rate of 20.0 °C. Scanning was performed from 35 to 550 *m/z* in electronic impact (EI) at 70 eV and ion source temperature 250 °C. Samples were injected at split ratio 1:10 and gas helium was used as the carrier gas at a flow rate of 1.0 mL/min. Analyses were run in triplicate.

2.2.3. Solvent extraction of volatiles

For solvent extraction, hexane:ethyl acetate mixture 85:15 [v/v] (both POCh, Gliwice, Poland) was prepared and the extraction was carried out according to Jiang et al. (2016). The volume of 1.0 mL of JB with 1 mg of dodecanone (Sigma Aldrich, St. Louis, MO, USA) as an internal standard was washed 3 times with 0.5 mL solvent mixture portions. Crude extract was dried with magnesium(II) sulfate(VI) (PoCH, Gliwice, Poland) and filtered. The analysis was performed on Shimadzu GCMS-QP 2020 (Shimadzu, Kyoto, Japan) equipped with Zebron ZB-5 (30 m × 0.25 mm × 0.25 μm) column (Phenomenex, Torrance, CA, USA). GC oven temperature was programmed from 50 °C, kept for 2 min, later to 130 °C at a rate of 4.0 °C, then to 270 °C at a rate of 10.0 °C and kept for 5 min, and finally to 290 °C at a rate of 10.0 °C

and kept for 5 min. Scanning was performed from 50 to 400 *m/z* in electronic impact (EI) at 70 eV and ion source temperature 250 °C. Helium was used as carrier gas with a flow rate of 3.0 mL/min and at a split ratio was 1:50. Analyses were run in triplicate.

2.2.4. UPLC-PDA- Q-TOF-MS analysis

Pure JBs were diluted in methanol and their composition was analysed using Waters Acquity UPLC system (Waters, Milford, CT, USA) equipped with PDA 200–500 nm, mass spectrometer Xevo-Q-TOF (Waters, Milford, CT, USA) and column BEH C18 130 Å, (1.7 μm, 2.1 mm × 150 mm) (Waters, Milford, CT, USA). Column temperature was set to 30 °C and elution system flow was 0.3 mL/min. The elution system consisted of acetonitrile (solvent 1) and 0.1% solution formic acid in water (solvent 2). The gradient elution program was: 1–12 min, 20–30% MeCN, 12–15 min, 30–100% MeCN, 15–17 min, 100-20% MeCN. Photodiode array data was obtained in the range of 200–500 nm. Electrospray negative mode (ESI-NEG) was used for ionisation. Parameters of ESI-NEG were set at capillary voltage of 2.80 kV, sampling cone of 66 kV and extraction cone of 4.0 kV. Collision energy was set at 0, 20, 20–30, 30 and 30–50 kV. Data were processed using Masslynx 2.0 (Waters, Milford, CT, USA) and MestreNova 9.0 (trial version, Mestrelab, Research, Santiago de Compostela, Spain). Single components were identified by comparison of experimental mass, UV absorption spectra and retention time to standards and literature data.

2.2.5. Cytotoxic activity

2.2.5.1. Cell lines. J774E.1 (murine macrophages) and NIH/3T3 (murine fibroblasts) cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). CLBL-1 (B-cell lymphoma cell line) were obtained from Barbara C. Ruetgen, Department of Pathobiology, Institute of Immunology at the University of Veterinary Medicine in Vienna (Kleiter et al., 2010) and CLB70 cell line was established by one of the co-authors of the manuscript (Pawlak et al., 2016). All cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin and 100 mg/mL streptomycin and L-glutamine in the concentration of 2 mM. Cells were cultured in 25 cm^2 cell culture flasks (Corning) and sub-cultivated every other day to keep optimal density. The culture was maintained in the atmosphere of 5% CO_2 and 95% humidified air at 37 °C.

2.2.5.2. Chemicals and reagents. Fetal bovine serum (FBS), L-glutamine, penicillin and streptomycin solution, thiazolyl blue tetrazolium bromide (MTT), sodium dodecyl sulfate (SDS), N, N-dimethylformamide (DMF) and doxorubicin hydrochloride were purchased from Sigma-Aldrich (Steinheim, Germany). Dimethyl sulphoxide (DMSO) was bought from POCh (Gliwice, Poland). Phosphate buffered saline (PBS) and RPMI 1640 culture medium were obtained from the Institute of Immunology and Experimental Therapy, Wrocław, Poland. Stock solutions of the tested compounds were freshly prepared for each experiment. The culture media were used as solvents for obtaining further solutions. The compounds were tested at the final concentration range of 15.6–125 μg/mL (15.6 μg/mL, 31.25 μg/mL, 62.5 μg/mL, 125 μg/mL). In the case of doxorubicin hydrochloride, the concentration range was 1–0.05 μg/mL (1 μg/mL, 0.5 μg/mL, 0.1 μg/mL, 0.05 μg/mL).

2.2.5.3. Determination of cell metabolic activity. To determine cell metabolic activity, 1×10^4 cells per well were seeded in a 96-well-plate (TPP, Trasadingen, Switzerland). The compounds were prepared at several dilutions in the culture medium and added to the wells containing the cells. The cells were incubated in the medium alone or in the medium containing increasing concentrations of the tested substances for 72 h. After incubation with the tested compounds, 20 mL of MTT solution (5 mg/mL) was added to each well for further 4 h, after which 80 mL of the lysis buffer (225 mL DMF, 67.5 g SDS, 275 mL distilled water) were added. Optical density of the culture wells was measured after 24 h with a spectrophotometric microplate reader (Spark 10M, Tecan) at reference wavelengths of 570 nm. Percentages of metabolic active cells were determined according to the following formula: [1-(ODt-ODm/ODc-ODm)]x 100, where ODt is the optical density of formazan formed in treated cells, ODc is the optical density of formazan formed in control (untreated) cells and ODm is the optical density of medium only. The results are mean values ± SD obtained from three independent experiments (3 wells each).

2.2.5.4. Statistical analysis. All data are shown as means with standard deviations (SD). Statistical analyses were performed with STATISTICA 13.3 (StatSoft, Polska) and illustrated by R Foundation for Statistical Computing (R Core Team., 2018). The results were considered significant when $p < 0.05$.

3. Results and discussion

3.1. Chemical composition

Analysis of the volatile profile of the JB samples revealed that they are all significantly diversified, both with respect to composition and share of occurring compounds. All identified constituents of JB samples via HS-SPME are presented in Table 2. Since JB1, JB2 and JB3 were produced by the friars from the same order (O.H.) and even JB1 and JB3 have the same raw materials composition declared on the label (Table 1), this result is truly surprising.

HS-SPME analysis of JB1, JB2 and JB3 showed that main components of these samples are monoterpenes, monoterpenoids and esters of aromatic and aliphatic short chain acids – mainly bornyl acetate, *p*-cymene, menthone, α-thujone and thymol. Those and other identified components match the main raw materials declared on the labels of JB1, JB2 and JB3 samples which are *Artemisia abshintum* (wormwood) – artemisia ketone, β-thujone (Abad et al., 2012); *Matricaria chamomilla* (camomile) – bisabolol oxide B (Shams-Ardakani et al., 2006); *Mentha piperita* (peppermint) – menthone, limonene, eucalyptol (El-Zaeddi et al., 2016; Park et al., 2016); *Thymus vulgaris* (thyme) – *p*-cymene, γ-terpinene, thymol (Borugă et al., 2014; Hosseini Behbahani et al., 2013); and *Valeriana* sp. – camphene, borneole, bornyl acetate, maaliol, valerenal (Huynh et al., 2013; Raal et al., 2007). The recipe for JB2 which includes thirteen raw materials used by O.H. friars is similar to the JB formulas reported by Amar and Lev (2005) or Schnittny (2015),

Table 1
Raw material composition of JBs - declared on labels.

SAMPLE				
JB1	JB2	JB3	JB4	JB5
RAW MATERIAL COMPOSITION				
Amara tinct. Hippocastani intr. Taraxaci intr. Salviae tinct. Farfarae tinct. Thymi fluidum extr. Chamomillae tinct. Valerianea tinct.	Menthae piperitae folium Tiliae inflorescentia Inulae radix Hyssopi herba Salviae folium Verbasci flos Lichen islandicus Pini gemmae Thymi herba Farfarae folium Althaeae radix Chamomillae extr. Thymi extr.	Amara tinct. Hippocastani intr. Taraxaci intr. Salviae tinct. Farfarae tinct. Thymi fluidum extr. Chamomillae tinct. Valerianea tinct.	Myrrh Olibanum Styrax balsam Balsam of Peru	–

Table 2
Volatile profile of JB samples obtained by HS-SPME analysis.

Compound	RI[1]	RI[2]	RI[3]	JB1	JB2	JB3	JB4	JB5
Acetaldehyde, diethyl acetal	724	–	726	+		+		+
Propanoic acid, 2-methyl-	770	–	772					+
Butanoic acid, 2-methyl-, methyl ester	775	–	775	+		+		
Hexanal	799	801	800	+		+	+	
Butanoic acid, ethyl ester	800	802	802					+
Lactic acid, ethyl ester	810	–	815					+
Furfural	830	828	833					+
Isovaleric acid	832	827	–	+		+		
trans-Crotonic acid, ethyl ester	838	–	835	+				
Butanoic acid, 2-methyl-, ethyl ester	843	–	849	+	+	+		+
Isovaleric acid, ethyl ester	847	849	854	+		+		+
Butanoic acid, 3-methyl-, ethyl ester	848	–	854		+			
Isobutyraldehyde, diethyl acetal	855	–	859	+		+		+
1-Hexanol	865	863	868	+		+		+
5-Hexen-2-one, 5-methyl-3-methylene-	883	–	880					+
Butyraldehyde, diethyl acetal	889	–	901	+				
Styrene	889	–	893				+	+
Pentanoic acid, ethyl ester	901	901	900	+		+		
Tricyclene	920	921	925			+		+
2-Butenoic acid, 3-methyl-, ethyl ester	920	921	925			+		
α-Thujene	925	924	929		+	+		
α-Pinene	929	932	937	+	+	+	+	+
Fenchene	929	932	937			+		
Camphene	942	945	950	+	+	+		
Isovaleraldehyde, diethyl acetal	943	946	952	+	+	+		+
Butane, 1,1-diethoxy-2-methyl-	950	–	953		+			
Butyraldehyde, 2-methyl-, diethyl acetal	950	–	955			+		
Benzaldehyde	953	952	962	+			+	+
Verbenene	965	961	967	+	+			
Sabinene	972	969	974			+	+	
β-Pinene	973	974	979	+	+			
1-Octen-3-ol	976	974	980	+	+	+		+
3-Octanone	984	979	986	+	+	+	+	+
2-Octanone	988	988	990	+	+	+	+	+
3-Octanol	988	988	990	+	+	+		
5-Hepten-2-ol, 6-methyl-	991	989	993				+	+
Hexanoic acid, ethyl ester	998	997	1000	+	+	+	+	+
3-Hexenoic acid, ethyl ester	1006	1003	1005	+				
3-δ-carene	1008	1008	1011	+	+	+	+	+
α-Terpinene	1008	1008	1011			+		+
Acetic acid, hexyl ester	1012	1007	1011				+	
p-Cymene	1021	1020	1025	+	+	+	+	+
Limonene	1025	1024	1030	+	+	+	+	+
Eucalyptol	1027	1026	1032	+	+	+	+	+
Benzyl alcohol	1030	1026	1036				+	+
cis-β-Ocimene	1037	1032	1038	+		+	+	+
Benzeneacetaldehyde	1038	1036	1045	+		+	+	
trans-β-Ocimene	1047	1044	1049	+		+	+	+
2-Furancarboxylic acid, ethyl ester	1050	–	1049					+
γ-Terpinene	1054	1054	1060	+	+	+	+	+
Ether, benzyl ethyl	1057	–	1048				+	
Artemisia ketone	1058	1056	1062	+	+	+		
Acetophenone	1061	1059	1065				+	
cis-Sabinene hydrate	1063	1065	1070	+	+	+		+
1-Octanol	1068	1063	1071				+	
Hexanoic acid, 5-methyl-, ethyl ester	1068	–	1072					+
Hexanoic acid, 2-propenyl ester	1071	1079	1076	+				+
1-Nonen-3-ol	1078	–	1080			+	+	+
m-Cymenene	1080	1082	–		+			
Artemisia alcohol	1082	1080	1084	+		+		
Terpinolene	1085	1086	1088			+	+	
p-Cymenene	1088	1089	1090	+	+			
Benzoic acid, methyl ester	1092	1088	1094				+	+
Hexanal, diethyl acetal	1092	–	1092	+	+		+	+
Hexanal, diethyl acetal + Sabinene hydrate, trans	1094/1096	−1098	1092/-			+		
Heptanoic acid, ethyl ester	1099	–	1097					+
Linalool	1100	1095	1099	+	+	+	+	
α-Thujone	1103	1101	1103	+	+	+	+	+
Propene, 1,3,3-triethoxy-	1107	–	1102					+
β-Thujone	1114	1112	1114	+	+	+		+
trans-p-Menth-2,8-dien-1-ol	1117	1119	1124	+		+		+
cis-p-Menth-2-en-1-ol	1119	1118	1122		+			
α-Campholenal + 3-Octanol, acetate	1122	1122/1120	1125/1123	+	+			
unknown	1127				+			

(*continued on next page*)

Table 2 (*continued*)

Compound	RI[1]	RI[2]	RI[3]	JB1	JB2	JB3	JB4	JB5
p-Menth-2-en-1-ol	1127	1118	1122			+	+	+
unknown	1130	–	–				+	
Limonene epoxide	1131	1132	1133		+			
3-Thujanol	1132	1134	1136	+		+		+
trans-Pinocarveol	1134	1135	1139	+	+			
Camphor	1137	1141	1145	+	+	+		+
cis-Verbenol	1142	1137	1142	+	+			
Menthone	1148	1148	1158	+	+	+		+
trans-Pinocamphone	1153	1158	1160			+	+	+
trans-3-Pinanone	1154	1158	1160	+	+			
Pinocarvone	1156	1160	1164	+	+	+		
Isomenthone	1160	1158	1164		+			
Borneol	1161	1165	1167	+	+	+		+
Lavandulol	1164	1165	1170			+		
Benzoic acid, ethyl ester	1166	1169	1171				+	+
Benzoic acid, ethyl ester + Isocamphopinone	1166/1168	1169/1172	1171/1173	+	+	+		
Terpinen-4-ol	1171	1174	1177	+	+	+	+	
p-Cymen-8-ol	1178	1179	1183		+			
Verbenyl ethyl ether	1180	–	1186	+	+		+	
Butanedioic acid, diethyl ester	1180	–	1182		+			+
α-Terpineol	1187	1186	1189	+	+	+	+	
Myrtenal	1190	1195	1193	+	+			
Myrtenol	1191	1194	1195	+	+	+		
Estragole	1194	1195	1196	+	+			
Octanoic acid, ethyl ester	1195	1196	1196	+	+	+	+	+
unknown	1198	–	–				+	
Dihydrocarveol	1202	1193	1192				+	
Verbenone	1201	1204	1204	+	+			
Diethyl methylsuccinate	1207	–	1205					+
Acetic acid, octyl ester	1210	1211	1210				+	
trans-Pulegol	1213	1213	1213	+	+			
Carveol	1215	1215	1219	+	+			
Coahuilensol methyl esther	1217	1219	–	+	+			
Fenchyl acetate	1230	1229	1223	+	+			
Borneol formate	1222	–	1226			+		
Isothymol methyl ether	1227	1230	–	+		+		
Thymol methyl ether	1232	1232	1235	+	+	+	+	+
Cumic aldehyde	1235	1238	1235	+	+			
Carvone	1238	1239	1242	+	+			
Carvacrol methyl ether	1239	1241	1244	+	+	+	+	+
Thymoquinone	1245	1248	1250	+	+	+		
Piperitone	1248	1249	1254	+	+			
Carvenone	1251	1255	1255			+		
Benzoic acid, allyl ester	1252	–	1254				+	+
Geraniol	1253	1249	1255	+	+			
trans-Cinnamaldehyde	1263	1267	1270					+
Salicylic acid, ethyl ester	1265	1266	1270	+	+			
p-3-Menthen-7-al	1269	1273	1269	+	+			
p-Ethylguaiacol	1274	–	1282				+	+
Anethole	1280	1282	1286				+	+
Bornyl acetate	1280	1284	1285	+	+	+		
Cumic alcohol	1285	1289	1299			+		
Menthyl acetate	1290	1294	1295	+	+			
2-Undecanone	1291	1293	1294				+	+
Thymol	1294	1289	1291	+		+	+	+
Nonanoic acid, ethyl ester	1296	–	1296		+			
Carvacrol	1300	1298	1299	+	+	+	+	+
unknown - thymol/carvakrol isomer	1305				+			
Myrtenyl acetate	1322	1324	1327	+		+	+	
Silphiperfol-5-ene	1332	1326	1331					+
7-epi-Silphiperfol-5-ene	1343	1345	1348					+
δ-EIemene	1344	1335	1338	+	+	+	+	+
Citronellol acetate	1351	1350	1354				+	
Eugenol	1353	1356	1357	+		+		+
Cyclosativene	1360	1369	1368			+	+	
Nerol acetate	1362	1361	1364				+	
Ylangene	1369	1373	1372				+	+
Longicyclene	1370	1371	1374	+		+	+	+
β-Patchoulene	1376	1379	1381					+
β-Bourbonene	1378	1387	1384			+	+	+
Isocomene	1382	1387	1386	+		+	+	+
β-Elemen	1386	1389	1391	+			+	
Isoitalicene	1398	1401	1395					+
β-Longipinene	1402	1400	1403			+	+	
cis-α-Bergamotene	1412	1411	1415					+

(*continued on next page*)

Table 2 (*continued*)

Compound	RI[1]	RI[2]	RI[3]	JB1	JB2	JB3	JB4	JB5
Caryophyllene	1414	1417	1419	+	+	+	+	+
tert-Butylhydroquinone, dimethyl ether[a]	1417	–	1417			+		
γ-Elemene	1431	1434	1433				+	
Thymohydroquinone dimethyl ether	1424	–	1422	+		+	+	
β-Copaene	1427	1430	1432	+		+		
Aromandendrene	1433	1439	1440	+	+			
α-Guaiene	1437	1437	1439	+		+		+
Isogermacrene D	1442	–	1448				+	+
Neoclovene	1444	1454	1454				+	
epi-Cedrane	1447	1447	1440	+				+
Humulene	1452	1452	1454			+	+	
Dihydropseudoionone	1455	1456	–	+				
Alloaromadendrene	1457	1458	1461	+		+	+	+
Ethyl cinnamate, trans	1466	1465	1463				+	+
γ-Muurolene	1476	1478	1477	+	+	+	+	+
Germacrene D	1480	1480	1481	+	+			+
α-Curcumene	1482	1479	1483			+	+	+
β-Selinene	1484	1489	1486	+	+			
Viridiflorene	1485	1496	1493	+	+			
β-Ionone	1486	1487	1491			+	+	
epi-Cubebol	1491	1493	1493	+	+			
Valencene	1491	1496	1492		+	+	+	+
Undecanoic acid, ethyl ester	1495	–	1494	+				
α-Muurolene	1496	1500	1499		+	+	+	+
cis-α-Bisabolene	1503	1505	1504	+	+	+	+	
γ-Cadinene	1509	1513	1513	+	+	+	+	+
β-Cadinene	1520	–	1518	+	+	+	+	+
unknown sesquiterpene	1531			+		+	+	
α-Cadinene	1537	1536	1538	+	+	+	+	
α-Calacorene	1541	1544	1542				+	
Italicene epoxide	1546	1547	1549	+	+	+		
Germacrene B	1555	1559	1557				+	
Myrtenyl 2-methyl butyrate	1558	–	1560			+		
Maaliol	1564	1566	1574	+	+	+	+	
Spathulenol	1574	1577	1576	+	+	+	+	
Caryophylene oxide	1581	1581	1581	+	+	+		
Viridiflorol	1589	1592	1591	+	+	+	+	
Humulene epoxide II	1604	1608	1606	+	+	+		
Isospathulenol	1628	–	1638	+		+		
epi-α-Cadinol	1643	1638	1640		+	+		+
Benzyl ether	1652	–	1653				+	
Bisabolol oxide B	1657	1656	1655	+	+	+	+	
Intermedeol	1675	1665	1667			+	+	
α-Santalol	1683	1674	1681	+	+	+		
Valerenal	1744	–	1736	+				
Benzyl Benzoate	1813	1759	1772	+			+	+
Eudesma-5,11(13)-dien-8,12-olide	1925	–	1916	+				
Cembrene	1946	–	1939				+	
Kaur-16-ene	2046	–	2041				+	

R[1] Relative retention indices calculated against n-alkanes; R[2] Retention indices according to Adams (2012); R[3] Retention indices according to NIST14 database.
[a] Plastic impurities.

that include up to forty ingredients. This is different from the JB4 recipe, which has a composition that is more similar to the recipe found in Saint Savior Monastery in Jerusalem (Amar and Lev, 2005).

HS-SPME analysis showed that in the JB4 and JB5 samples, groups of analytes appear (e.g. benzoic acid derivatives) which were not observed in JB1-3 samples. The main constituents in JB4 sample were acetic acid octyl ester, benzyl benzoate and styrene, while the dominant constituents in JB5 sample were benzoic acid ethyl ester and β-bourbonene presented in Table 2. A more careful look at the volatile constituents identified in the JB4 sample reveals that there are constituents characteristic to the raw materials declared on its label. Using the HS-SPME technique such compounds like styrene, benzaldehyde, benzyl alcohol, benzoic acid methyl ester and vanillin were identified in earlier studies on benozin reported by Castel et al. (2006) and Lizzani-Cuvelier et al. (2005). Further, regarding olibanum, acetic acid octyl ester and 1-octanol confirm its presence (Hamm et al., 2005; Hayashi et al., 1998; Wahab et al., 1987). Also, characteristic constituents for myrrh β-bourbonene, β-elemene, γ-elemene and δ-elemene are present (Morteza-Semnani and Saeedi, 2003). The constituent last pointed out on the label of JB4 was Balsam of Peru which, according to Custódio and Veiga-Junior (2012), is characterized by cinnamic acid, benzoic acid and a number of their derivatives, including benzyl benzoate and ethyl cinnamate and these were also well identified in this study.

Even, if the composition of JB5 sample was not available, by comparison with other JB samples volatiles composition, it is possible to develop a potential raw material composition. First, the presence of styrene, benzaldehyde, benzyl alcohol or vanillin may suggest that benzoin – *Styrax* spp. was used. Then, the presence of benzoic acid and its derivatives (benzyl benzoate) and cinnamic acid and its derivatives (cinnamyl cinnamate or ethyl cinnamate) may be the evidence for Balsam of Peru. Also, olibanum (*Boswellia* spp.) is expected to be a raw material for original JB; however, acetic acid octyl ester and 1-octanol, which are characteristic for olibanum, were not found in JB5 sample. It is worthy of note, however, that diterpene – cembrene was identified in JB5. According to Hamm et al. (2005), diterpene-cembrene may be found in some *Boswellia* spp. without the accompanying presence of

acetic acid octyl ester and 1-octanol; therefore, it is possible that some olibanum was utilized. Moreover, literature (Amar et al., 2005) also suggests that mastic (*Pistacia lenticus)* and myrrh (*Comiphora molmol* Engl.) should be used for original JB production but no clear evidence for mastic utilization was found. However, the presence of β-burbonene may be an indication for myrrh. Finally, as in JB1, JB2 and JB3 samples, the presence of menthone, limonene, eucalyptol and β-thujone may be some indication for the use of peppermint (*Mentha piperita*) or wormwood (*Artemisia abshintum*). Further research would be needed to confirm this, especially in face of lack of artemisia ketone.

As a result of hexane:ethyl acetate extracts GC-MS liquid injection, a group of cinnamic acid derivatives, like cinnamyl cinnamate or ethyl cinnamate, a group of sesquiterpenes, diterpene – cembrene and benzoic acid and its derivatives were identified for both JB4 and JB5 samples. Also, the presence of compounds like benzyl benzoate and vanillin was confirmed. Most of these findings align with the results of Kurkiewicz et al. (2017). In the case of less volatile or non-volatile compounds, fifteen compounds were identified in total (*Non-volatile profile of JBs.pdf* in supplementary materials).

3.2. Cytotoxic activity

The next step in the comparison of the properties of original and contemporary available JBs was the study of their cytotoxic activity. First, the effect of the tested compounds on the metabolic activity of normal cell lines was examined. The results revealed only a slight effect on the metabolic activity of the cells of said cell lines even at concentrations as high as 125 μg/mL. The mentioned concentration inhibited the cellular metabolic activity only in about 20% of all tested compounds (Fig. 1). Cells observation using a light microscope revealed no dead and detached cells, which may indicate that the observed decrease in metabolically active cells was not synonymous with cell death but only with their slowed metabolism and/or proliferation rate (data not shown). Interesting results were provided by research using cancer cell lines. This study showed that one of the samples - JB5 has a strong cytotoxic effect. At a concentration of 125 μg/mL, after 72 h of incubation, the percentage of metabolically active cells was 21.62 ± 2.57 for the CLB70 and 10.20 ± 1.87 for the CLBL-1 cell line. Cells evaluation using light microscope indicated the presence of disintegrated and dead cells (data not shown). This means that the MTT result observed as a decrease in absorbance in JB5-treated cells compared to the control is an effect of cell mortality and not due to an inhibition of cell growth or their metabolic activity. At the same time, the remaining compounds did not show or showed small (about 20%) cytotoxic activity (Fig. 2). The difference in the strength of action of JB5 on normal and cancer cell lines at the concentration of 62.5 μg/mL and 125 μg/mL was statistically significant ($p < 0.005$). Nevertheless, the cytotoxic effect of JB5, although distinct in comparison to other balms, is incomparable to the potency of typical anticancer drugs (IC_{50} for doxorubicin for CLBL-1 and CLB-70 is < 0.05 μg/mL).

Since only the JB5 sample had shown significant cytotoxic activity it was screened for potentially active chemical constituents. In the first step, volatile profiles of JB1, JB2, JB3 and JB4 were compared, including the concentration of particular compounds, to JB5 HS-SPME profile (Fig. 3). This attempt revealed that contemporary JBs lacked many compounds that were identified in JB5 sample. Nevertheless, the most interesting finding was in the JB4 and JB5 comparison. This is because the JB4 sample had a slightly higher cytotoxic activity than JB1-3 samples. The comparison revealed that a group of overlapping compounds in JB4 and JB5 samples, which were absent in JB1-3 samples. Mainly, these compounds were benzoic acid derivatives (allyl ester; ethyl ester; methyl ester). Furthermore, the concentration distribution of chemical constituents shows that one of them, benzoic acid ethyl ester, is a dominant constituent in JB5 sample and slightly dominating constituent in JB4 sample. These findings may suggest that the constituents responsible for cytotoxic activity of JB5 sample are found among benzoic acid derivatives, although other compounds should not be underestimated.

The distribution of less volatile and non-volatile compounds in JB samples was unequal. Most of them were identified in JB1, JB2 and JB3 samples, and much less in JB4 and JB5 samples (Table 3). Again, two compounds occurred in JB4 and JB5, which were not present in JB1, JB2 and JB3 samples. Those were vanillin and benzoic acid, which confirms GC-MS analysis results.

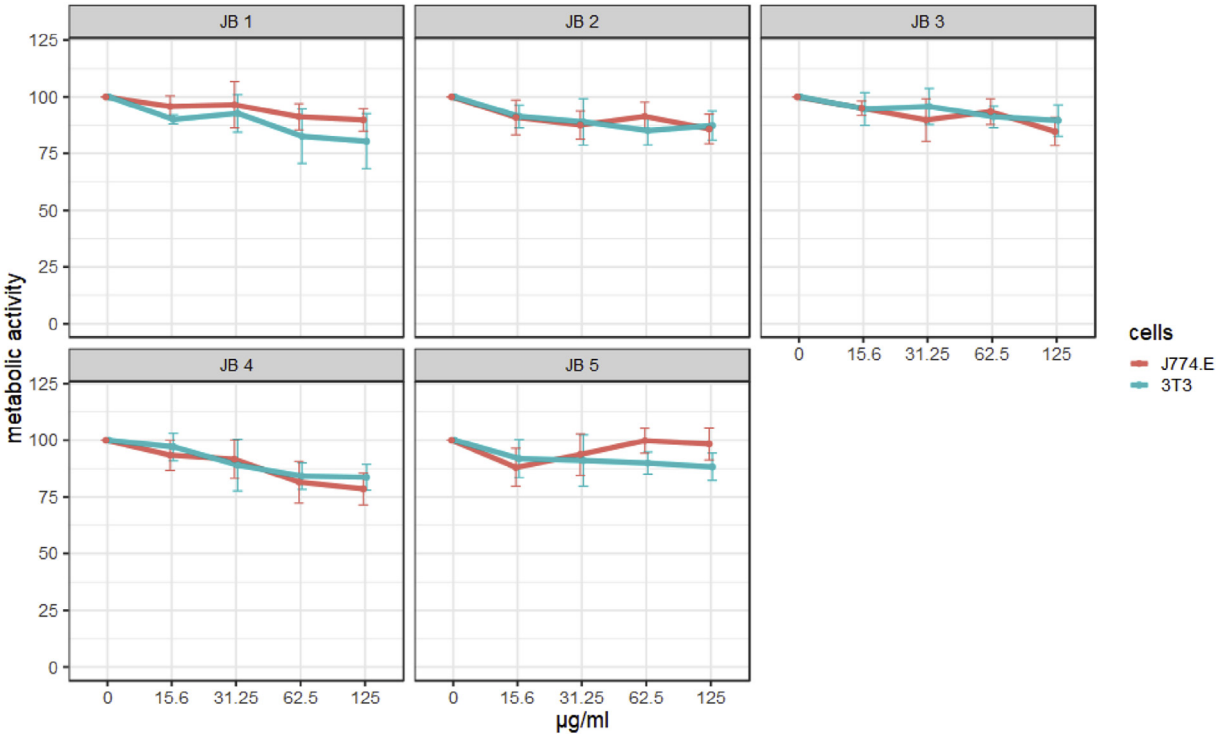

Fig. 1. Impact of JBs on metabolic activity of normal cell lines validated by MTT test.

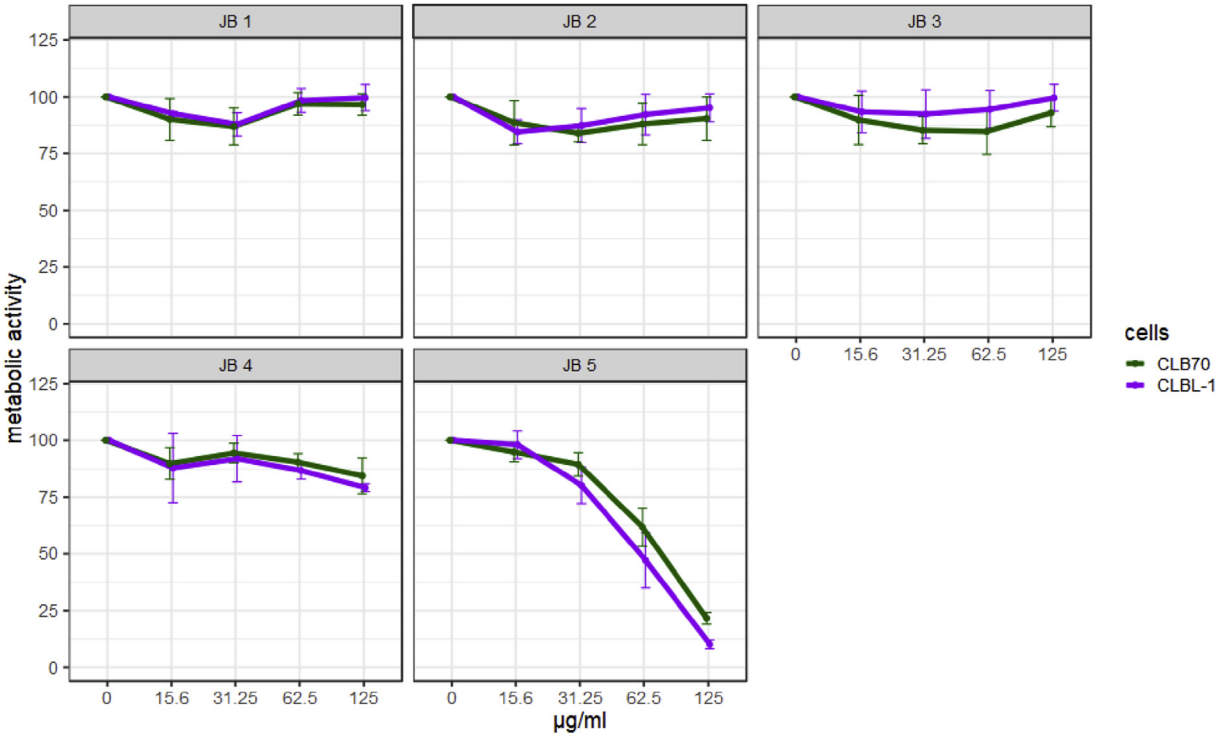

Fig. 2. Impact of JBs on metabolic activity of cancer cell lines validated by MTT test.

In accordance with GC-MS analysis via HS-SPME and liquid injection techniques, plentiful groups of essential oils components and less volatile compounds were identified in JB5 sample. According to literature, the main identified compounds like benzoic acid and cinnamic acid derivatives, sesquiterpenes or diterpene-cembrene may be linked to the high cytotoxic activity. Anantharaju et al. (2017) had investigated the anticancer activity of various benzoic acid derivatives (mainly with additional hydroxy groups and methoxy groups). Based on the result, they concluded that some of those derivatives are potent agents inducing cancer cell death, what corresponds to the mechanism observed in this study. Then, Balachandran et al. (2011) had found other benzoic acid derivatives, 2-hydroxy-, ethyl ester in group to bind well with breast cancer protein. Also, cinnamic acid derivatives, obtained from natural source (propolis) showed anticancer activity according to the study of Akao et al. (2003). It was investigated on human cancer cells and found that the mechanism is also based on inducing apoptosis. Similar results were later reported by De et al. (2011) in a wide topic review. Also, other major constituents of JB5 like sesquiterpenes or cembrene are reported as compounds with potential anticancer activity. For instance, Silva et al. (2018) found sesquiterpenes and diterpenoids along with diterpene - cembrene as the main constituents of essential oils in *Croton matourensis* leaves, which in turn were shown to have promising cytotoxic activity against HepG2 cells. In an earlier study, Hegazy et al. (2017) examined cembrene-like diterpenoids activity against various type of cell lines, including HepG2, with satisfying results. Vanillin is also considered to be a potential anticancer agent. Bezerra et al. (2016) in a broad overview highlighted the cytotoxic activity of vanillin via different pathways, like apoptosis or reducing the proliferation, although this is linked to high concentrations of the compound. However, Raghavan et al. (2015) obtained more promising results by using the vanillin as a core to obtain potent anticancer agents.

More recently, other herbal formulations and plant derivatives were examined for their pharmacological activity. Gurbuz et al. (2013) and de Oliveira et al. (2018) had investigated the gastroprotective activity of styrax balsams and *Sedum dendroideum* derivatives, respectively. In both studies the plant derivatives had shown activity in the case of ulcer prevention or eradication. In addition, Gurbuz et al. (2013) identified a group of cinnamic acid derivatives in styrax balsam, which were also found in JB5 sample. Regarding the anticancer activity Du et al. (2016) had formulated silver nanoparticles incorporating benzoin extract and proved their activity against HeLa (human cervical cancer), A549 (human lung cancer) cells, while Wang et al. (2015) investigated the effect of herbal formulation Huanglian-Jiedu decoction on the hepatocellular carcinoma and obtained highly satisfying results. Nevertheless, not all studies on plant derivatives show positive biological activity of plant derivatives. de Groot (2019) underlines the high ratio (4–8%) of positive patch test reactions in patients treated with balsam of Peru, although they do not pin it on its main constituents, benzyl cinnamate and benzyl benzoate, which are present in JB5 sample. Also, the report provided by Brito et al. (2010) demonstrates that the, sometimes, beneficial effect is highly dependent on the dosage. In their study they had shown, that a large dose of copaiba balsam (*Copaifera officinalis*) may stimulate cancer growth.

Nevertheless, consumers should be aware that safety and toxicity of essential oils and their constituents should be always considered. Preedy (2016) stresses that even well-known and used widely essential oils like thyme oil (*Thymus vulgaris*), rich in thymol and carvacrol, or basil oil (*Ocimum basilicum),* rich in estragole, in some cases may be toxic or cancerogenic. In addition, JB may be prepared using some raw materials suspected of undesirable activities due to their chemical constituents. Among them are plants rich in thujones (neurotoxic activities) and estragole (cancerogenic) or *Tussilago farfara,* rich in pyrrolizidine alkaloids (PA), which are highly toxic. Fortunately, recent studies have shown that the risk of these threats are low. Radulovic et al. (2017) applied multivariate statistical treatment on data obtained during *in vivo* study using *Salvia officinalis* L., *Artemisia absinthium* L., *Thuja occidentalis* L. and *Tanacetum vulgare* L. rich in thujones essential oils. The results showed that there is no clear correlation between the amount of thujones and toxicity of those essential oils. In the case of estragole originating in *Foeniculum vulgare*, Levorato et al. (2018) suggest that its genotoxicity may be reduced when the plant is used as a

Fig. 3. Hit-map – occurrence of chemical constituents in JB5 and other samples along with their share in volatile profile of the sample.

compound of formulation which includes more raw materials. The biggest issue are pyrrolizidine alkaloids from *Tussilago farfara*. In a study by Said et al. (2019) performed on alcoholic extracts, similar to JBs, PAs were found in product made only with *T. farfara*, but were not found in extract where *T. farfara* was just one constituent among others, what again may be a good sign for formulations. In the case of toxicity, Seremet et al. (2018) proved PA toxicity for aquatic organisms; however, in an earlier study performed *in vivo* for 28 days, Şeremet et al. (2016) showed that *T. farfara* extract administrated orally does not have any toxic effects.

Table 3
Presence of identified non-volatile components in JBs.

Component	RT	JB1	JB2	JB3	JB4	JB5
Caffeoylquinic acid 1	1.15	+	+	+	–	–
Caffeoylquinic acid 2	1.52	+	+	+	–	–
Caffeic acid	2.05	+	+	+	–	–
Ferulic acid derivate	2.74	+	+	+	–	–
Vanillin	3.29	–	–	–	+	+
Dicaffeoylquinic acid 1	3.34	–	+	tr	–	–
Ferulic acid	3.49	+	–	+	+	–
Dicaffeoylquinic acid 2	3.89	+	+	+	–	–
Dicaffeoylquinic acid 3	4.44	+	+	+	–	–
*Caffeic acid dimer	4.74	+	+	+	–	–
Benzoic acid	5.34	–	–	–	+	+
Kaempherol	8.49	+	+	+		–
Cinnamic acid	9.78	–	–	–	+	–
Caffeic acid ethyl ester	9.82	+	+	+	–	–
Apigenin	11.84	+	+	tr	–	–

+ - component present in sample.
tr - component present in trace concentration.
- component absent in sample.

The potential anticancer activity of JB should provide an interesting point of view on this herbal formulation due to the increasing interest in supplements and drugs based on natural products. Complementary and alternative medicine (CAM) is gaining increased followership among customers, especially in the case of prophylactic administration of this kind of products. Study from 2017 carried out in Howard University (Washington, DC, USA) showed that more than 50% of first year pharmacy students (36 out of 49 who filled the questionnaire) had used products qualified as complementary or alternative medicine ones (Hailemeskel et al., 2017). Canizares et al. (2017), Kemppainen et al. (2018) and Peltzer and Pengpid (2015) carried out studies on CAM based on broad research and national surveys in southern Asia, Canada and Europe. Their results show that a significant segment of society is interested in using CAM in addition to conventional treatment. This is a great opportunity for herbal formulations as JB, especially in the face of numerous fake drugs and quackery.

4. Conclusions

The potential cytotoxicity in the case of cancer cell lines was strongly dependent on the raw materials composition used for the production of JBs. CLBL-1 and CLB70 cancer cell lines metabolism and viability were affected significantly by the approximately 150-year old JB sample due to its unique chemical composition rich in essential oils constituents. Based on diversified occurrence, concentration of chemical constituents and literature overview obtained results suggest that benzoic acid and its derivatives, cinnamic acid derivatives, vanillin, group of sesquiterpenes and cembrene may be responsible for cytotoxic activity of the original sample. Such evidence-based results, at the time of growing interest in CAM among patients and customers alongside a growing occurrence of quackery, are an important step towards guaranteeing the health benefit of herbal formulations. Identification of possible raw materials used in this formulation may be a great opportunity to improve the beneficial effects of contemporarily produced JBs, which are getting increased interest among consumers.

Author contribution statement

Jacek Łyczko: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Project administration. **Aleksandra Pawlak:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing. **Iwo Augustyński:** Formal analysis, Data Curation, Visualization. **Piotr Okińczyc:** Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Writing - Review & Editing. **Jakub Szperlik:** Methodology, Resources, Writing - Review & Editing. **Anna Kulma:** Methodology, Resources, Writing - Review & Editing. **Henryk Różański:** Resources. **Bożena Obmińska-Mrukowicz:** Resources, Writing - Review & Editing, Supervision. **Antoni Szumny:** Conceptualization, Methodology, Validation, Resources, Writing - Review & Editing, Supervision.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2020.111183.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2020.111183.

References

Abad, M.J., Bedoya, L.M., Apaza, L., Bermejo, P., 2012. The artemisia L. Genus: a review of bioactive essential oils. Molecules 17, 2542–2566. https://doi.org/10.3390/molecules17032542.

Adams, R.P., 2012. Identification of Essential Oils by Ion Trap Mass Spectroscopy. Academic Press, San Diego, CA, USA.

Akao, Y., Maruyama, H., Matsumoto, K., Ohoguchi, K., Nishizawa, K., Sakamoto, T., Araki, Y., Mishima, S., Nozawa, Y., 2003. Cell growth inhibitory effect of cinnamic acid derivatives from propolis on human tumor cell lines. Biol. Pharm. Bull. 26, 1057–1059.

Amar, Z., Lev, E., 2005. An early glimpse at western medicine in Jerusalem 1700-1840. Vesalius 11, 81–87.

Amar, Z., Moussaieff, A., Steinberg, D., Fride, E., Mechoulam, R., Lev, E., Gallily, R., 2005. The Jerusalem balsam: from the Franciscan Monastery in the old city of Jerusalem to Martindale 33. J. Ethnopharmacol. 101, 16–26. https://doi.org/10.1016/j.jep.2005.03.028.

Anantharaju, P.G., Reddy, B.D., Padukudru, M.A., Chitturi, C.M. kumari, Vimalambike, M.G., Madhunapantula, S.V., 2017. Naturally occurring benzoic acid derivatives retard cancer cell growth by inhibiting histone deacetylases (HDAC). Canc. Biol. Ther. 18, 492–502. https://doi.org/10.1080/15384047.2017.1324374.

Balachandran, P., Ajay Kumar, T.V., Partasarathy, V., 2011. Screening of potential anticancer compounds from *Sargassum wightii* to target breast cancer specific HER2 receptor using in-silico analysis. Nat. Prod. J. 21, 1558–1564.

Baran, H., Pietryja, M.J., Kronsteiner, C., Kepplinger, B., 2017. Jerusalem balsam lowers kynurenic acid formation: an in vitro study. J. Tradit. Med. Clin. Naturopathy 6, 224–228.

Bezerra, D.P., Soares, A.K.N., De Sousa, D.P., 2016. Overview of the role of vanillin on redox status and cancer development. Oxid. Med. Cell. Longev. 2016. https://doi.org/10.1155/2016/9734816.

Borugă, O., Jianu, C., Mişcă, C., Goleţ, I., Gruia, A.T., Horhat, F.G., 2014. *Thymus vulgaris* essential oil: chemical composition and antimicrobial activity. J. Med. Life 7, 56–60 Spec No.

Brito, N.M.B., Brito, M.V.H., de Carvalho, R.K.V., de Matos, L.T.M.B., Lobato, R.C., Correa, S.C., Brito, R.B., 2010. The effect of Copaiba balsam on Walker 256 carcinoma inoculated into the vagina and uterine cervix of female rats. Acta Cir. Bras. 25, 176–180. https://doi.org/10.1590/s0102-86502010000200010.

Canizares, M., Hogg-Johnson, S., Gignac, M.A.M., Glazier, R.H., Badley, E.M., 2017. Changes in the use practitioner-based complementary and alternative medicine over

time in Canada: cohort and period effects. PloS One 12, 1–17. https://doi.org/10.1371/journal.pone.0177307.
Castel, C., Fernandez, X., Lizzani-Cuvelier, L., Perichet, C., Lavoine, S., 2006. Characterization of the chemical composition of a byproduct from Siam benzoin gum. J. Agric. Food Chem. 54, 8848–8854. https://doi.org/10.1021/jf061193y.
Custódio, D.L., Veiga-Junior, V.F., 2012. True and common balsams. Braz. J. Pharmacogn. 22, 1372–1383. https://doi.org/10.1590/S0102-695X2012005000097.
de Groot, A.C., 2019. *Myroxylon pereirae* resin (balsam of Peru) – a critical review of the literature and assessment of the significance of positive patch test reactions and the usefulness of restrictive diets. Contact Dermatitis 80, 335–353. https://doi.org/10.1111/cod.13263.
de Oliveira, A.F., da Luz, B.B., Werner, M.F. de P., Iacomini, M., Cordeiro, L.M.C., Cipriani, T.R., 2018. Gastroprotective activity of a pectic polysaccharide fraction obtained from infusion of *Sedum dendroideum* leaves. Phytomedicine 41, 7–12. https://doi.org/10.1016/j.phymed.2018.01.015.
De, P., Baltas, P.D.M., Bedos-Belval, F., 2011. Cinnamic acid derivatives as anticancer agents-A review. Curr. Med. Chem. 18, 1672–1703.
Di, L.Q., Xie, T., Lin, L.L., Chen, J. Bin, Shan, J.J., Wang, S.C., Shen, C.S., Xu, J.Y., 2016. Application of traditional Chinese medical herbs in prevention and treatment of respiratory syncytial virus. Evid. base Compl. Alternative Med. 2016, 1–13. https://doi.org/10.1155/2016/6082729.
Du, J., Singh, H., Yi, T.H., 2016. Antibacterial, anti-biofilm and anticancer potentials of green synthesized silver nanoparticles using benzoin gum (*Styrax benzoin*) extract. Bioproc. Biosyst. Eng. 39, 1923–1931. https://doi.org/10.1007/s00449-016-1666-x.
El-Zaeddi, H., Martínez-Tomé, J., Calín-Sánchez, Á., Burló, F., Carbonell-Barrachina, Á., 2016. Volatile composition of essential oils from different aromatic herbs grown in Mediterranean regions of Spain. Foods 5, 41. https://doi.org/10.3390/foods5020041.
Gurbuz, I., Yesilada, E., Demirci, B., Sezik, E., Demirci, F., Baser, K.H.C., 2013. Characterization of volatiles and anti-ulcerogenic effect of Turkish sweetgum balsam (*Styrax liquidus*). J. Ethnopharmacol. 148, 332–336. https://doi.org/10.1016/j.jep.2013.03.071.
Hailemeskel, B., Ziregbe, E., Tran, C., Bwayo Weaver, S., Ahari-Lahagh, A., Kumarra, S., Fullas, F., Habte, A., 2017. Complementary and alternative medicine (CAM) utilization by howard university (HU) first year pharmacy students: survey and review of most commonly used herbs. Curr. Res. Integr. Med. 2, 37–41. https://doi.org/10.4172/2529-797X.1000025.
Hamm, S., Bleton, J., Connan, J., Tchapla, A., 2005. A chemical investigation by headspace SPME and GC-MS of volatile and semi-volatile terpenes in various olibanum samples. Phytochemistry 66, 1499–1514. https://doi.org/10.1016/j.phytochem.2005.04.025.
Hayashi, S., Amemori, H., Kameoka, H., Hanafusa, M., Furukawa, K., 1998. Comparison of volatile compounds from olibanum from various countries. J. Essent. Oil Res. 10, 25–30. https://doi.org/10.1080/10412905.1998.9700833.
Hegazy, M.-E.F., Elshamy, A.I., Mohamed, T.A., Hamed, A.R., Ibrahim, M.A.A., Ohta, S., Pare, P.W., 2017. Cembrene diterpenoids with ether linkages from *Sarcophyton ehrenbergi*: an anti-proliferation and. Mar. Drugs 15, 1–14. https://doi.org/10.3390/md15060192.
Hosseini Behbahani, M., Ghasemi, Y., Khoshnoud, M.J., Faridi, P., Moradli, G., Montazeri Najafabady, N., 2013. Volatile oil composition and antimicrobial activity of two *Thymus* species. Pharmacogn. J. 5, 77–79. https://doi.org/10.1016/j.phcgj.2012.09.002.
Hu, B., An, H.-M., Wang, S.-S., Chen, J.-J., Xu, L., 2016. Preventive and Therapeutic Effects of Chinese Herbal Compounds. Evid. base Compl. Alternative Med. 2, 1–13. https://doi.org/10.4103/0973-8258.42731.
Huynh, L., Pacher, T., Tran, H., Novak, J., 2013. Comparative analysis of the essential oils of *Valeriana hardwickii* Wall. from Vietnam and *Valeriana officinalis* L. from Austria. J. Essent. Oil Bear. Plants 25, 409–414. https://doi.org/10.1080/10412905.2013.828325.
Jiang, Z., Kempinski, C., Chappell, J., 2016. Extraction and analysis of terpenes/terpenoids. Curr. Protoc. Plant Biol. 1, 345–358. https://doi.org/10.1002/cppb.20024.
Kaliora, A.C., Kountouri, A.M., 2012. Chemopreventive Activity of Mediterranean Medicinal Plants. InTech, Rijeka, Croatia.
Kamble, M., Dumbre, R.K., Rangari, V.D., 2009. Hepatoprotective activity studies of herbal formulations. Int. J. Green Pharm. 2, 147. https://doi.org/10.4103/0973-8258.42731.
Kemppainen, L.M., Kemppainen, T.T., Reippainen, J.A., Salmenniemi, S.T., Vuolanto, P.H., 2018. Use of complementary and alternative medicine in Europe: health-related and sociodemographic determinants. Scand. J. Publ. Health 46, 448–455. https://doi.org/10.1177/1403494817733869.
Khazaei, M.R., Makalani, F., Ghanbari, E., Fayzemahdavi, M., 2018. Overview of Effective Herbal and Antioxidant Compounds on Diabetes. 4. pp. 126–133.
Kleiter, M., Christian, M., Hammer, S.E., Willmann, M., Saalmüller, A., de Arespacochaga, A.G., Gerner, W., Schwendenwein, I., Rütgen, B.C., 2010. Establishment and characterization of a novel canine B-cell line derived from a spontaneously occurring diffuse large cell lymphoma. Leuk. Res. 34, 932–938. https://doi.org/10.1016/j.leukres.2010.01.021.
Kurkiewicz, S., Dzierżęga-Lęcznar, A., Pietryja, M.J., Stępień, K., 2017. Badanie składu historycznego Balsamu Jerozolimskiego i współczesnych balsamów klasztornych techniką GC/MS. In: Franciszkańska Konferencja Zielarsko-Farmaceutyczna, pp. 9–19.
Kuropatnicki, A.K., Szliszka, E., Krol, W., 2013. Historical aspects of propolis research in modern times. Evid. base Compl. Alternative Med. 2013, 1–11. https://doi.org/10.1155/2013/964149.
Leong, P.K., Chen, J., Ko, K.M., 2018. Development of Chinese Herbal Health Products for the Prevention of Aging-Associated Diseases, Natural Products and Drug Discovery: an Integrated Approach. Elsevierhttps://doi.org/10.1016/B978-0-08-102081-4.00004-6.
Levorato, S., Dominici, L., Fatigoni, C., Zadra, C., Pagiotti, R., Moretti, M., Villarini, M., 2018. In vitro toxicity evaluation of estragole-containing preparations derived from *Foeniculum vulgare* Mill. (fennel) on HepG2 cells. Food Chem. Toxicol. 111, 616–622. https://doi.org/10.1016/j.fct.2017.12.014.
Lizzani-Cuvelier, L., Loiseau, A.-M., Fernandez, X., Delbecque, C., Jean- Arnaudo, F., Castel, C., Perichet, C., 2005. Volatile constituents of benzoin gums: siam and Sumatra, part 2. Study of headspace sampling methods. Flavour Fragrance J. 21, 59–67. https://doi.org/10.1002/ffj.1502.
Manvi, S., Sharma, R., 2017. Tincture benzoin in dermatology. J. Med. Sci. Clin. Res. 5, 26154–26158.
Milwright, M., 2003. The balsam of Matariyya: an exploration of a medieval panacea. Bull. Sch. Orient Afr. Stud. 66, 193–209. https://doi.org/10.1017/S0041977X03000119.
Milwright, M., 2008. Balsam in the Mediaeval Mediterranean: A Case Study of Information and Commodity Exchange. J. Mediterr. Archaeol. 14, 3–23. https://doi.org/10.1558/jmea.v14i1.3.
Morteza-Semnani, K., Saeedi, M., 2003. Constituents of the essential oil of *Commiphora myrrha* (Nees) Engl. var.molmol. J. Essent. Oil Res. 15, 50–51. https://doi.org/10.1080/10412905.2003.9712264.
Park, Y.J., Baskar, T.B., Yeo, S.K., Arasu, M.V., Al-Dhabi, N.A., Lim, S.S., Park, S.U., 2016. Composition of volatile compounds and in vitro antimicrobial activity of nine *Mentha* spp. SpringerPlus 5. https://doi.org/10.1186/s40064-016-3283-1.
Pawlak, A., Ziolo, E., Kutkowska, J., Blazejczyk, A., Wietrzyk, J., Krupa, A., Hildebrand, W., Dziegiel, P., Dzimira, S., Strzadala, L., Rapak, A., 2016. A novel canine B-cell leukaemia cell line. Establishment, characterisation and sensitivity to chemotherapeutics. Vet. Comp. Oncol. 9, 1–14. https://doi.org/10.1111/vco.12257.
Peltzer, K., Pengpid, S., 2015. Utilization and practice of traditional/complementary/alternative medicine (T/CAM) in southeast asian nations(ASEAN) member states. Stud. Ethno-Med. 9, 209–218. https://doi.org/10.1080/09735070.2015.11905437.
Perry, Y., Lev, E., 2003. The medical activities of the London Jews' society in nineteenth-century Palestine. Med. Hist. 47, 67–88.
Peschel, W., 2016. Quality control of traditional Cannabis tinctures: pattern, markers, and stability. Sci. Pharm. 84, 567–584. https://doi.org/10.3390/scipharm84030567.
Preedy, V.R. (Ed.), 2016. Essential Oils in Food Preservation, Flavor and Safety. Academic Press, London.
Raal, A., Orav, A., Arak, E., Kailas, T., Müürisepp, M., 2007. Variation in the composition of the essential oil of *Valeriana officinalis* L. roots from Estonia. Proc. Est. Acad. Sci. Chem. 52, 67–74.
Radulovic, N.S., Gencic, M.S., Stojanovic, N.M., Randjelovic, P.J., Stojanovic-Radic, Z.Z., Stojiljkovic, N.I., 2017. Toxic essential oils. Part V: behaviour modulating and toxic properties of thujones and thujone-containing essential oils of *Salvia officinalis* L., *Artemisia absinthium* L., *Thuja occidentalis* L. and T*anacetum vulgare* L. Food Chem. Toxicol. J. 105, 355–369. https://doi.org/10.1016/j.fct.2017.04.044.
Raghavan, S., Manogaran, P., Kalpattu Kuppuswami, B., Venkatraman, G., Gadepalli Narasimha, K.K., 2015. Synthesis and anticancer activity of chalcones derived from vanillin and isovanillin. Med. Chem. Res. 24, 4157–4165. https://doi.org/10.1007/s00044-015-1453-2.
Rai, M., Jogee, P.S., Agarkar, G., Dos Santos, C.A., 2016a. Anticancer activities of *Withania somnifera*: current research, formulations, and future perspectives. Pharm. Biol. 54, 189–197. https://doi.org/10.3109/13880209.2015.1027778.
Said, M., Wahrig, B., Beuerle, T., 2019. Fitoterapia Quantitative and qualitative analysis of pyrrolizidine alkaloids in liqueurs, elixirs and herbal juices. Fitoterapia 136, 104172. https://doi.org/10.1016/j.fitote.2019.104172.
Sajkowska-Kozielewicz, J., Wawer, I., Makarova, K., Kozielewicz, P., Paradowska, K., 2016. Analysis of impact of cytotoxic and antioxidant activities of the Jerusalem Balsams using chemometric methods. Planta Med. 81, S1–S381. https://doi.org/10.1055/s-0036-1597059.
Schnittny, H.R., 2015. Balsam Jerozolimski, Polish. In: Herbarium Św. Franciszka, Katowice.
Şeremet, O.C., Bărbuceanu, F., Ionică, F.E., Marginǎ, D.M., Guţu, C.M., Olaru, O.T., Mihaela, I., Veacesla, G., Simona, N., Cornel, C., 2016. Oral toxicity study of certain plant extracts containing pyrrolizidine alkaloids. Rom. J. Morphol. Embryol. 57.
Seremet, O.C., Olaru, O.T., Gutu, C.M., Nitulescu, G.M., Ilie, M., Negres, S., Zbarcea, C.E., Purdel, C.N., Spandidos, D.A., Tsatsakis, A.M., Coleman, M.D., Margina, D.M., 2018. Toxicity of plant extracts containing pyrrolizidine alkaloids using alternative invertebrate models. Mol. Med. Rep. 17, 7757–7763. https://doi.org/10.3892/mmr.2018.8795.
Shams-Ardakani, M., Ghannadi, A., Rahimzadeh, A., 2006. Volatile Constituents of *Matricaria chamomilla* L. from Isfahan, Iran traditionally have been used for the treatment macological effects of chamomile are mainly connected with its essential oil for its antispasmodic, antimicrobial and disinfective and. Iran. J. Pharm. Sci. 2, 57–60.
Silva, V.R., Santos, L.D.S., Soares, M.B.P., Cardozo, N.M.D., Costa, E.V., Silva, F.M.A., Koolen, H.H.F., Bezerra, D.P., 2018. Antitumor effect of the essential oil from the leaves of *Croton matourensis* aubl. (*Euphorbiaceae*). Molecules 23, 1–12. https://doi.org/10.3390/molecules23112974.
Su, G., Hou, Y., Chen, G., Li, W., Tang, Y., Li, N., Chen, R., Zhou, D., 2018. Preventive agents for neurodegenerative diseases from resin of *Dracaena cochinchinensis* attenuate LPS-induced microglia over-activation. J. Nat. Med. 73, 318–330. https://doi.org/10.1007/s11418-018-1266-y.
Teng, L., Zu, Q., Li, G., Yu, T., Job, K.M., Yang, X., Di, L., Sherwin, C.M., Enioutina, E.Y., 2016. Herbal medicines: challenges in the modern world. Part 3. China and Japan. Expet Rev. Clin. Pharmacol. 9, 1225–1233. https://doi.org/10.1080/17512433.2016.1195263.

Virga, C., Aguzzi, A., Lopez, V., 2018. Propolis: a therapeutic alternative for oral cavity. J. Dent. Sci. Oral Maxillofac. Res. 1, 67–70. https://doi.org/10.30881/jdsomr.00014.

Wahab, S., Aboutabl, E., El-Zalabani, S., Fouad, H., De Pooter, H., El-Fallaha, B., 1987. The essential oil of olibanum. Planta Med. 53, 382–384. https://doi.org/10.1055/s-2006-962745.

Walch, S.G., Lachenmeier, D.W., Kuballa, T., Stühlinger, W., Monakhova, Y.B., 2012. Holistic control of herbal teas and tinctures based on sage (*Salvia officinalis* L.) for compounds with beneficial and adverse effects using NMR spectroscopy. Anal. Chem. Insights 7, 1–12. https://doi.org/10.4137/ACI.S8946.

Wang, N., Feng, Y., Tan, H.Y., Cheung, F., Hong, M., Lao, L., Nagamatsu, T., 2015. Inhibition of eukaryotic elongation factor-2 confers to tumor suppression by a herbal formulation Huanglian-Jiedu decoction in human hepatocellular carcinoma. J. Ethnopharmacol. 164, 309–318. https://doi.org/10.1016/j.jep.2015.02.025.