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Physico-chemical studies of Siberian pine (*Pinus sibirica* Du Tour) derived chewing gum

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ABSTRACT

In this work, 'Siberian chewing gum', a natural product derived from the resin of *Pinus sibirica* Du Tour, to which healing effects on mouth, stomach and duodenum chronic ulcers are attributed, has been characterized by Attenuated Total Reflection Fourier-Transform Infrared spectroscopy (ATR-FTIR) and thermal analysis techniques. With regard to the vibrational spectrum, the band at 1693 cm^{-1}, ascribed to ν(CO) terpenic oxo groups, suggests the presence of diterpenes, while the existence of hydroxystilbenes and their glycosides is consistent with the absorption bands in the 3380-3080 cm^{-1}, 1800-1300 cm^{-1} and 1000-450 cm^{-1} regions. On the other hand, the thermal behavior of the Siberian chewing gum, elucidated by thermogravimetry (TG), derivative thermogravimetry (DTG) and differential thermal analysis (DTA) techniques, resembles that of arabinogalactan, albeit with a more delayed decomposition.

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1. Introduction

Chewing gum, with a 5,000-year history, may be defined as a soft, cohesive substance designed to be chewed without being swallowed. Stone-age gum samples from western Finland suggest that birch bark tar may have been chewed due to its antiseptic properties, derived from its content in phenols (BBC News, 2007). Similar antiseptic properties are also attributed to Siberian pine chewing gum, which is the pure resin from the *Pinus sibirica* Du Tour. The Siberian pine or Siberian stone pine, often erroneously referred to as 'Siberian cedar', provided that its Russian name-Сибирский кедр, tr Sibirsky kedr-is prone to mistranslation in English.

P. sibirica is a member of the white pine group from Pinaceae family, genus *Pinus* subgenus *Strobus* growing both in lowland areas along the great Siberian rivers and in the mountains to an altitude of around 2,400 m, where it forms dense, pure forests. In the lowlands, it occurs with *P. sylvestris*, *Larix gmelinii* or *L. sibirica* and *Betula pendula* on drier sites that regularly burn, and with *Abies sibirica* and *Picea obovata* and *Betula* sp. on more mesic sites in the river basins, in bog margins, and uplands. This species is one of the major forest-forming conifers in the Siberian taiga and is estimated to cover ca. 45 million hectares (Farjon, 2010a; Farjon, 2010b; Farjon, 2013).

In relation to its resin, the inhabitants of Siberia and the Urals long ago discovered both the special properties and the healing power of the chewing gum made by gently filtering and drying *P. sibirica* wild-crafted larch resin. In a similar fashion to birch bark tar, *chicle*, mastic gum, spruce sap, etc. in other indigenous cultures, the chewing gum from *P. sibirica* has been historically used by Siberian people to clean and freshen the mouth, promoting a good dental hygiene. Unlike synthetic rubber-based chewing gums on the market, it survives

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Fig. 1. Siberian pine resin-derived chewing gum sample.

as a natural product made by traditional procedures without added chemicals. The Siberian chewing gum is not sweet and needs to be warmed/softened in the mouth before chewing. However, it retains its pine-like flavor much longer than synthetic chewing gums and has, as noted above, healing power: *P. sibirica* resin and its derivatives have rightfully been called *zhivitsa* which is related to *zhizn*, the Russian word for life for their ability to heal wounds. Scholars have established that, in addition to its balsamic properties, it possesses bactericidal, epithelializing and anti-inflammatory properties, thus being suitable for the treatment of mouth, stomach and duodenum chronic ulcers (Shikov et al., 2008).

P. sibirica chewing gum, in a similar fashion to sandarac and copal, is rich in diterpenoids, in particular in 4-epiisocembrol (Raldugin and Pentegova, 1971). It is also rich in pinostilbene, a stilbenoid found, along with resveratrol, in the bark of *P. sibirica* (Tyukavkina et al., 1972). The resin oil contains α-pinene (39.1%), δ-3-carene (23.0%), and β-pinene (9.1%) with moderate amounts of cembrene (4.9%), β-phellandrene (2.1%), camphene (1.7%) and terpinolene (1.3%) (Grishko et al., 1994; Shatar and Adams, 1996; Rogachev and Salakhutdinov, 2015). These compounds, present also in other essential oils, feature interesting phytochemical and pharmacological properties and medicinal applications (Camilo et al., 2017; Mohammadhosseini, 2017a, b; Mohammadhosseini et al., 2017a, b; Nunes and Miguel, 2017)

Other important components of the Siberian gum are arabinogalactans, which are biopolymers consisting of arabinose and galactose monosaccharides. In fact, arabinogalactan is a major component of many gums, including gum arabic and gum ghatti. They are often found attached to proteins, and the resulting arabinogalactan protein (AGP) functions as both an intercellular signaling molecule and as a glue to seal plant wounds (Zhou et al., 2009).

The main objectives of this work have been to contribute to elucidate the chemical nature of Siberian chewing gum through vibrational and thermal analysis techniques and to valorize this product as an alternative to other chewing gums.

Usual methods for chemical analysis of natural products and their constituents include Nuclear Magnetic Resonance (NMR) spectroscopy, Mass Spectrometry (MS), Infrared (IR) spectroscopy and thermal analysis techniques. IR spectroscopy provides information on chemical functional groups of the sample, which allows the identification of specific compounds and the general characterization of the material. Resins produce vibrational bands which are readily identifiable (Derrick, 1989) with this rapid and accurate method (Huck, 2015). Further, when used in combination with thermal analysis techniques (TG/DTG and DTA) which measure stability and chemical changes (Foreman et al., 1993), valuable information on gums can be retrieved in a facile manner.

2. Experimental

2.1. Samples

Commercial chewing gum samples (Fig. 1) were purchased from живица таёжная (Алтайский край, tr.: Altaiski krai, Russian Federation). According to the manufacturer, they were obtained from pure *P. sibirica* resin, filtered using a low heat method, and no preservatives, artificial ingredients or chemicals had been added.

2.2. Apparatus

The vibrational spectrum in the 400-4000 cm^{-1} spectral range was characterized using a Thermo

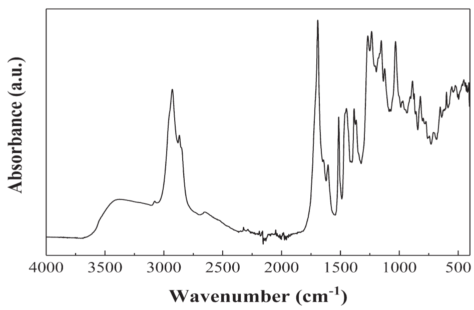

Fig. 2. ATR-FTIR spectrum of *P. sibirica's* resin-based chewing gum.

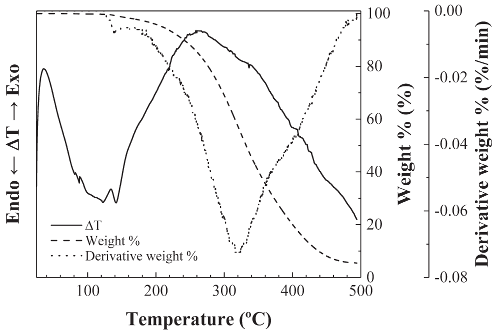

Fig. 3. DTA and TG/DTG curves for the *P. sibirica's* resin-based chewing gum.

Scientific (Waltham, MA, USA) Nicolet iS50 FT-IR spectrometer, equipped with an in-built diamond Attenuated Total Reflection (ATR) system, with a 1 cm^{-1} spectral resolution and 64 scans.

TG/DTG/DTA analyses were conducted with a Perkin-Elmer (Waltham, MA, USA) STA6000 simultaneous thermal analyser by heating the samples in a slow stream of N_2 (20 mL/min) from room temperature up to 500 ºC, with a heating rate of 20 ºC/min. Pyris v.11 software was used for data analysis.

3. Results and Discussion

3.1. Vibrational characterization

The ATR-FTIR spectrum of the Siberian pine chewing gum is depicted in Fig. 2. Its main absorption bands are compared with those of other resins in Table 1, and their assignments are presented in Table 2. Upon examination of Table 1, a reasonable doubt could arise about the family of the conifer from which the chewing gum was extracted to check whether it is a *Pinaceae* or a *Cupressaceae*, provided that there is greater overlapping with sandarac, i.e. the resin extracted from *Tetraclinis articulata* cypress (Gerling et al., 2015), than with pine pitch. Nonetheless, this overlap may be due to the content of diterpenoids, which is high in both sandarac and the studied material.

The greatest differences between diverse conifer species products can be observed in the set of terpenic compounds, which are used as chemotaxonomic markers in the literature (Raldugin et al., 1976; Otto and Wilde, 2001). It is known that the band attributable to ν(CO) terpenic oxo groups at around 1700 cm^{-1} appears at different frequencies according to the number of isoprenic units. The absorption for *P. sibirica*-derived chewing gum occurs, as expected for diterpenoids, at 1693 cm^{-1} (as in other resins, such as sandarac, some copals, colophony and Venice turpentine) (Wei et al., 2018).

With regard to the remarkable similarities between the chewing gum and the stilbene (resveratrol) spectra in the 3380-3080 cm^{-1}, 1800-1300 cm^{-1} and 1000-450 cm^{-1} regions, they can be readily explained, for the first two regions, by the fact that they share the O-H stretching absorption bands at 3370-3380 cm^{-1} and the aromatic

Table 1

Comparison of the main absorption bands in the ATR-FTIR spectrum of *P. sibirica's* resin-based chewing gum and those of other resins. Wavenumbers are in cm^{-1}.

Siberian pine chewing gum	Sandarac (rich in diterpenoids)	Black copal	Pine pitch	Amber	Rosin	Mastic	White copal	Hydroxy-stilbenes (resveratrol)	AGP
3380							3477	3380	
3077	3079	3079	3070	3076				3082	
2928	2933	2933	2931	2927	2936	2949	2943		
2868	2873	2873	2873	2867		2874	2868		
2653									
1693	1694	1694	1694	1696	1697	1707	1713	1673	
1644	1643	1643		1643	1650	1650	1641	1634	
1605			1607		1612			1613	
1515	1497		1515		1496			1514	
1449	1449	1449	1453	1454		1459	1453	1444	
1384			1379	1384			1379	1385	
1366					1365			1357	
1267								1265	
1235	1236	1228	1243		1239	1245	1242		
1153	1153	1149		1159		1161		1154	
1124			1125		1130		1137		1134
1078			1114		1107	1115	1095 1067		1094 1075
1032			1033			1046	1036		1045 1018
969	972				980	1008	989	966	
908 888	909	889	909	887	910		879	895	900
858	856							864	
821	823				823	837	819	832	819
797								806	
773								772	
744				744					
709								717	
652								651	
599						580		585	
553				540			547	554	
501							500	499	
451							455	457	
match	****	**	***	***	**	*	****	***	

Note: The number of asterisks reflects the degree of matching of the spectra, i.e., more asterisks indicate that more bands match those of Siberian pine chewing gum.

Table 2
Infrared band assignments. All wavenumber values are in cm^{-1}.

Band	Assignment
3077	ν(O-H) which makes up the gross structure of carbohydrates/ketal forms of saccharides
2928	ν(C-H) from lipid acyl CH_3 and CH_2 groups
2868	ν(C-H) from lipid acyl CH_3 groups
1693	ν(CO) terpenic oxo groups
1644	*ν*(C=C) / exocyclic methylene groups. Typical of resins
1605	*ν*(C=C) aromatic ring / *ν*(C=O) amide
1515	*ν*(C=C). Typical of phenolic resins
1449	*δ*(CH_2), *δ*(CH_3)
1235	*δ*(C-H), ν(C-O-H). Typical of resins
1153	ν(C-O-C), ethers
1124	ν(C-O-C)
1078	ν(C-O-C) of 1→4 glycosidic bonds and *δ*(C-O-H), a characteristic of polysaccharides
1032	ν(C-O)
969	C-O bonds
908	*δ*(C=CH_2)
858	Exocyclic methylene groups/β-anomeric *δ*(C-H) and glycosidic linkages attributable to glucopyranose and xylopyranose units
821	Typical of phenolic resins (after heating)
744	1,2-cis-disubstituted olefin
599	*δ*(C-H) in the furan ring
553	Unsaturated bonds

ring C=C stretching at 1615-1585 and 1510-1514 cm^{-1}.

The intense bands at ca. 1075 and 1045 cm^{-1} can be ascribed to key monosaccharides from arabinogalactan involving galactose and arabinose, respectively, and those at about 1018 cm^{-1} and 1600 cm^{-1} to uronic acids, which usually impart an anionic character to the macromolecule (Wang et al., 2002).

Bands at 1124, 1032 and 908-821 cm^{-1} were probably due to compositional and structural differences between AGPs (Zhou et al., 2009).

3.2. Thermal characterization

The simultaneous TG/DTG and DTA curves for the chewing-gum under study are displayed in Fig. 3. These curves showed that the sample was stable up to 140 °C, when melting (or a glass transition) took place, sensitized by an endothermic peak in the DTA curve and an effect in the DTG. Above this temperature, thermal decomposition occurred up to 470 °C. In a first decomposition step, up to 250 °C, the rate was low, but above this temperature the weight loss rate clearly increased. The first step can be attributed to the loss of water of constitution, while the second step -observed between 250-450 °C and with a maximum at 323 °C in the DTG- would correspond to organic matter oxidation, contributing to the formation of char (Groenewoud, 2001; Bothara and Singh, 2012; Singh and Bothara, 2014; Daoub et al., 2016). The thermal behavior of the chewing-gum was similar to that of arabinogalactan, which exhibited an exothermic peak at 257 °C caused by oxidation and whose decomposition begins at about 260 °C, reaching a maximum at 300 °C (Teratani and Miyazaki, 1968).

It was worth noting that, in a similar fashion to the arabinogalactan polymer, the crystallinity of Siberian pine chewing gum was very low and most domains within it would be amorphous (as confirmed by X-ray powder diffraction, not shown). In a tentative way, the endothermic effect at 140 °C in the DTA curve may be ascribed to melting (T_m at 140 °C) and the previous effects at 43 °C and 55 °C would be associated with glass transitions (T_g at 75 °C).

3.3. Comparison with other chewing gums

The 100% natural, biodegradable composition of the Siberian chewing gum is far from that exhibited by conventional gum brands which contain polyisobutylene, polyvinyl acetate, styrene-butadiene rubber, butyl rubber, paraffin, microcrystalline wax, hydrogenated vegetable oils, lecithin, aspartame, acesulfame K, sucralose, glycerin, sorbitol, maltitol, xylitol, high fructose corn syrup, among others. As a good food product, the main alternative to Siberiam gum would be the Mexican natural chewing gum from Chicozapote trees (*Manilkara zapota* (L.) P.Royen) named *Chicza*, launched in Mexico and UK in 2009. The main difference between both products is that, whereas the Mexican natural gum is rich in a linear polyprenoid (polyisoprene), the Siberiam gum contains more cyclic terpenoids. Further research on the differential behavior of both products is in course.

4. Concluding remarks

The *P. sibirica*-derived products are characterized not only by the presence of hydroxystilbenes (and their glycosides) but mainly by the content in diterpenes. A distinctive feature in the ATR-FTIR spectrum of the natural resin chewing gum under study readily allows to identify *P. sibirica's* diterpenoids *versus* other terpenoids with different number of isoprenic units: the frequency of the band attributable to ν(CO) terpenic oxo groups appears, shifted, at 1693 cm^{-1}. On the other hand, the shape of TG/DTG and DTA thermograms of the chewing gum are similar to those of arabinogalactan, but evince a higher thermal stability. The results of this exploratory work should be followed up by additional studies aimed at the identification of the individual compounds and the determination of their quantitative contributions to the chewing gum composition.

Conflict of interest

The authors declare that there is no conflict of interest.

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