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ORIGINAL ARTICLE

Qualitative Analysis of Baltic Amber Resin by Gas Chromatography Coupled with Mass Spectrometry and the Therapeutic Potential of this Fossil Resin

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ABSTRACT

Objective: To analyze the molecular composition of a sample of Baltic amber taken from an amber bracelet, verify the presence or absence of compounds beneficial to human health, and discuss the therapeutic potential of this fossil resin. **Material and Methods:** For this, a qualitative analysis was performed by gas chromatography coupled with mass spectrometry (GC-MS system), which can perform an automated separation into individual components of amber. **Results:** The samples comprised terpenes and terpenoids: monoterpenoids, sesquiterpenoids, diterpenoids, hydronaphthalene, succinic acid, and isopimaric acid. These have therapeutic potential for several diseases, inhibit several stages of the inflammatory process, and improve the symptoms caused by it. **Conclusion:** The amber of this sample tested is formed by terpenes and terpenoids, substances with anti-inflammatory and analgesic. However, an in-depth study is needed on the release and absorption of substances in human skin or their actual effectiveness.

Keywords: Amber; Chromatography; Mass Spectrometry.

Introduction

The treatment of human diseases is mediated by drugs, mainly produced from natural products and their derivatives, which comprise approximately 40% of all drugs [1]. A natural product is a substance produced by a living organism found in nature and has a pharmacological or biological activity for use in drug discovery. This product can be extracted from plant tissues, marine organisms, or by the fermentation of microorganisms [1].

Natural products correspond to one of the largest groups of secondary metabolites in plants, with thousands of different structures [2]. The beneficial effects of terpenes and terpenoids on inflammatory diseases and other processes in human health have attracted the attention of many researchers, raising the therapeutic possibility of these compounds for various diseases [2].

Amber is a fossil resin [3,4] produced by trees all over the planet, such as Kauri pines (Agathis australis) from New Zealand and some species from East Africa and South and Central America. Its production aims to protect trees from invasion by insects and microorganisms, in addition to assisting in wound healing and preventing the appearance of diseases in the plant [4]. One of the main substances in amber resin is succinic acid, which has anti-inflammatory properties and calming analgesics. It is also responsible for improving the immune system and is a natural therapeutic agent [5].

Therefore, the use of amber necklaces as an alternative to a non-pharmacological treatment has been increasingly popular among those responsible for relieving signs and symptoms of teething. Since non-pharmacological treatments are safer and indicated by pediatricians and pediatric dentists, they do not generate as many side effects compared to pharmacological treatments [6].

Much has been said in the literature about the potential beneficial effects of amber on human health and the relief of signs and symptoms of tooth eruption in babies, and based on this assumption and the need for evidence, the present study aimed to analyze the chemical composition of amber through a commercialized sample; and discuss the therapeutic potential of this fossil resin.

Material and Methods

Sample Preparation

Approximately four resin beads that made up the bracelet (from the Baltic Sea region of Lithuania) were initially washed externally with dichloromethane and then pulverized with a mortar and pestle. The powdered amber sample (sample of a Baltic sea amber bracelet) (ca. 100 mg) was sonicated (3 × 5 min) with dichloromethane: methanol (1:1; v:v). The combined extracts were evaporated under N_2 and transferred before drying to a vial, and the residual solvent was removed under N_2 to obtain a crude extract An aliquot of the total crude extract was converted to trimethylsilyl derivatives by reaction with N-Trimethylsilyl-N-methyl trifluoroacetamide for 30 minutes at 70 ºC, and then a derivatized extract was obtained The derivatization reaction was carried out in a complementary way for better detection of compounds containing acidic groups. The extracts were analyzed by gas chromatography coupled with mass spectrometry (GC-MS).

GC-MS Analyses

The GC-MS system (Agilent Technologies Inc., Santa Clara, CA, USA) used for analysis was a 6890 GC coupled to an HP5973 mass-selective detector. Compound separation was achieved with a DB-5MS fused silica column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The GC oven operating conditions were: 60 to 240 °C at 3 °C min-1 and 240 to 310 °C at 3 °C min-1. Helium was used as a carrier gas. The sample was injected in

split mode (20:1) with the injector temperature at 290 °C. The mass spectrometer was operated in the full scan mode from m/z 40–700 and with electron impact ionization (70 eV). Individual compounds were identified by comparison of mass spectra acquired with literature mass spectra, retention time, elution order, and Nist Mass Spectral Database® (version 2.0, 2005). Comparisons between mass spectra acquired through the Nist MS Database were made considering a minimum similarity of 70% [6]

Results

The total ion chromatogram (TIC) from the GC–MS analyses of the crude amber extract is presented in Figure 1, and the compounds identified in the crude and derivatized extracts are listed in Table 1. The extract contains alkyl hydronaphthalene (6), monoterpenoids (1-4), -sesquiterpenoids (5, 7, 8), and diterpenoids (9-16).

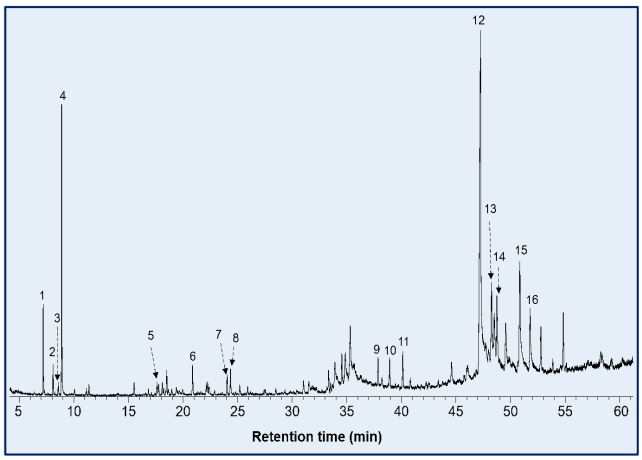
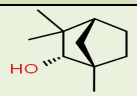
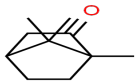
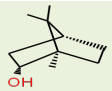
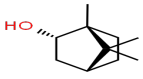
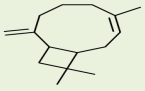
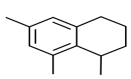
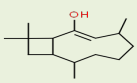
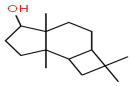
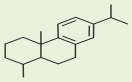
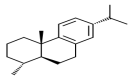
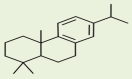
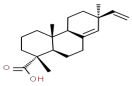
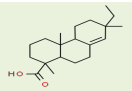
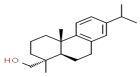
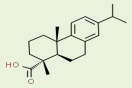
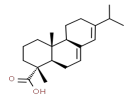
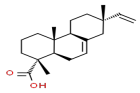


Figure 1. The total ion chromatogram of the amber extract was obtained by GC-MS.

Table 1. Substances tentatively identified in the amber extract. The compounds detected after derivatization were identified as TMS-derivative.

N°	Compound Name	MF[a]	MW[b]	Chemical Structure
1	Fenchol	$C_{10}H_{18}O$	154	
2	Camphor	$C_{10}H_{16}O$	152	
3	Isoborneol	$C_{10}H_{18}O$	154	
4	Borneol	$C_{10}H_{18}O$	154	
5	Humulen-(v1)	$C_{15}H_{24}$	204	
6	Naphthalene, 1,2,3,4-tetrahydro-1,6,8-trimethyl-	$C_{13}H_{18}$	174	
7	Caryophyllenyl alcohol	$C_{15}H_{26}O$	222	

8	2,2,4a,7a-Tetramethyldecahydro-1H-cyclobuta[e]inden-5-ol	$C_{15}H_{26}O$	222	
9	18-norabieta-8,11,13-triene	$C_{19}H_{28}$	256	
10	19-norabieta-8,11,13-triene	$C_{19}H_{28}$	256	
11	Abieta-8,11,13-triene	$C_{20}H_{30}$	270	
12	Pimaric acid	$C_{20}H_{30}O_2$	302	
13	Pimar-8(14)-en-18-oic acid	$C_{20}H_{32}O_2$	304	
14	Abieta-8,11,13-trien-18-ol	$C_{20}H_{30}O$	286	
15	Abietic acid, dehydro-	$C_{20}H_{28}O_2$	300	
16	Abietic acid	$C_{20}H_{30}O_2$	302	
17	Succinic acid (2TMs derivative)	$C_4H_6O_4$	128	
18	Isopimaric acid (TMS derivative)	$C_{20}H_{30}O_2$	302	

[a]Molecular formula; [b]Molecular weight.

Discussion

More than 200 years ago, Friedrich Sertürner chemically isolated from a plant (opium) the first pharmacologically active drug, morphine, derived from the poppy (Papaver somniferum). This shows that while perhaps less than 15% of higher plant species have been screened for bioactivity [7], using plants for medicinal purposes to treat, cure, and prevent disease is one of the oldest medical practices in Humanity [8].

Natural products likely have privileged structures for the discovery of new drugs. However, a barrier to this discovery is the complexity of the natural extract. Therefore, automated separation into individual components coupled with complete spectroscopic identification is the ideal approach to overcome this hurdle before high throughput screening (HTS) [7]. Based on the above, gas chromatography coupled with mass spectrometry, where the amber samples were analyzed. The results revealed that they were composed of monoterpenoids, sesquiterpenoids, diterpenoids, Hydronaphthalene, Succinic Acid (2TMs derivative), and Isopimaric Acid (TMS derivative). Volatile Organic Compounds make up the class of secondary plant metabolites, and most of these organic compounds are Terpenes and Terpenoids. The structure of Terpenes is formed by the binding of Isoprene units (C5H8). It is classified according to the amount of these units [2], which can be hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), sesterterpenes (C25),

triterpenes (C30) and tetraterpenes (C40) [8,9]. Terpenoids are terpenes modified by altering methyl groups or adding functional groups, such as those containing oxygen [10].

Although terpenes and terpenoids have therapeutic potential for several diseases, little is known about their benefits for human health. Most of the anti-inflammatory functions of terpenes and terpenoids are mediated by reducing levels of pro-inflammatory mediators. Furthermore, recent studies have shown that terpenes and terpenoids inhibit several stages of the inflammatory process and improve several symptoms caused by it [2].

Inflammation significantly contributes to pain and malaise [11], a physiological response [10] to infections and tissue damage characterized by vasodilation reactions and recruitment of tissue cells. Defense and proteins to the site of tissue injury or infection [12]. Thus, some inflammatory diseases need the action of steroidal or non-steroidal anti-inflammatory drugs, which can cause unwanted side effects [10].

Pain, in turn, is a problem that requires the action of analgesic drugs to be treated. When we relate to discomforts in the tooth eruption phase, for example, which can generate local pain in the tooth birth region, excessive analgesics can cause side effects and toxicity, even more so when talking about babies whose immune systems are still immature [6]; therefore, other therapeutic and less harmful alternatives can be treatment alternatives, and terpenes are a great option in these cases [9]. Furthermore, the present study found Monoterpenes and sesquiterpenes in smaller amounts in amber. They are very volatile and are lost to the atmosphere when the resin is exuded [10]. In amber samples, they possibly remained in the non-volatile resin matrix [3]. They are cyclized and oxidized in a variety of ways. Due to their low molecular weight, many of them exist in the form of essential oils. They have pharmacological properties such as antimicrobial, anti-inflammatory, antioxidant, antipruritic, hypotensive, and analgesic activities [8].

One of the monoterpenes found in the present study was Borneol, which showed healing and anti-inflammatory activity effects, reducing the migration of leukocytes and antifibrosis activity [13]. Furthermore, this compound did not show cytotoxicity and could suppress mRNA expression of pro-inflammatory cytokines (IL-1β and IL-6) and have inhibitory effects on pathogenic microorganisms [8]. These results may be beneficial to show that in situations with inflammatory processes and pain, such as during tooth eruption or mild fevers, using amber resin is a potential non-pharmacological treatment for this phase.

Another substance found in the chromatographic analysis was camphor, a non-toxic aromatic compound widely known to have anti-inflammatory and analgesic properties [14]. This substance is a monoterpene commonly used in the pharmaceutical and food industry, and studies have indicated autophagy and apoptosis activities depending on the dose of this monoterpene [2].

Like monoterpenes, sesquiterpenes are volatile compounds [15]. For example, Humulen-(v1) is believed to have antimicrobial activity [16-18]. Furthermore, monoterpenes and sesquiterpenes constitute the most innovative and promising analgesic compounds.

The pain relief provided by terpenes is due to their activity in inhibiting the inflammatory cascade, reducing inflammatory cytokines (IL, TNF-a) and PGE-2 levels, and decreasing the expression of COX-2, iNOS and 5- LOX. In addition, these compounds can also act in the nociceptive stimulus, blocking Na+ channels, NMDA [15], and the family of TRP channels, which are involved in several disorders, such as inflammatory and neuropathic pain [9].

Abietic acid has anti-inflammatory, antiallergic, and anticonvulsant activities, stimulates angiogenic properties *in vitro*, and improves skin wound healing in mice [19,20]. A study produced by Ito et al. [21] demonstrated that abietic acid has bacteriostatic potential on the bacterium *Streptococcus mutans* and can act to control oral infections, inhibiting bacterial growth and biofilm formation [20].

In addition, amber has in its composition Succinic Acid [5], which conjugates through ester bonds to the hydroxyl group of other compounds, playing a structural role in amber due to the cross-linking of these compounds, being known for its anti-aging effects. Inflammatory [11]. Thus, amber necklaces have been increasingly marketed as another natural option for therapeutic use [11]. However, there is no evidence of how succinic acid can be released from amber resin to accomplish its effect.

Conclusion

The accomplishment of this study, that Baltic Amber is composed of terpenes and terpenoids, thus presenting anti-inflammatory and analgesic potential; however, in-depth studies on the release and absorption of amber components in contact with human skin and its actual effectiveness, as well as well-designed clinical studies must demonstrate the therapeutic potential of amber.

Authors' Contributions

MSR	https://orcid.org/0000-0002-2711-5620	Conceptualization, Methodology, Data Curation, Writing - Original Draft and Writing - Review and Editing.
FMTC	https://orcid.org/0000-0002-7058-5724	Conceptualization, Methodology, Formal Analysis, Data Curation, Writing - Original Draft, Writing - Review and Editing and Visualization.
RVSS	https://orcid.org/0000-0001-8124-0046	Methodology, Writing - Original Draft and Writing - Review and Editing.
DAA	https://orcid.org/0000-0003-4924-6741	Methodology, Writing - Original Draft and Writing - Review and Editing.
IPS	https://orcid.org/0000-0003-0449-6624	Writing - Original Draft and Writing - Review and Editing.
MMP	https://orcid.org/0000-0002-8418-4139	Conceptualization, Methodology, Validation, Formal Analysis, Writing - Original Draft, Writing - Review and Editing, Visualization, Supervision and Project Administration.

All authors declare that they contributed to a critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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