

Spectroscopic and Chemical Insights into Baltic Amber: Structure, Bioactivity, and Clinical Prospects. A Review

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Received 29 June. 2025, Accepted 9 Sep. 2025, published 30 Dec. 2025.

DOI: 10.52113/2/12.02.2025/135-145

Abstract: Baltic amber (succinate) has attracted attention not only as an ornamental material but also for its biological and medical potential. This review summarizes the structural and chemical features of Baltic amber based on spectroscopic analyses (FTIR, Raman, and GC–MS), with emphasis on its bioactive compounds such as succinic acid and diterpenoids. Reported biological activities include anti-inflammatory, antimicrobial, antioxidant, and neuroprotective effects. The objective of this review is to provide an updated overview of spectroscopic and chemical findings, to connect these findings with biological activities, and to highlight possible clinical prospects. The paper also discusses limitations and gaps between laboratory results and clinical applications.

Keywords: Baltic amber spectroscopy FTIR Raman GC-MS Bioactivity clinical prospects.

Introduction

Amber, particularly Baltic succinite, is the fossilized resin of trees that has been used as an ornamental gem throughout our known history and as a scientific material by Homo sapiens for many thousands of years [1]. This object ranges in color from yellow to black with brown and other colors in between. This leads us to ask what kind of entomological or phytological remains are present, or in other words whether it encompasses not only biotic remains but also what we might call a cultural (as opposed to historical) artefact. However, in the last decades,

amber has also been a tool of choice for research in physics, chemistry and biomedical sciences, as well as biological electron microscopy (which will be discussed later). This makes it possible to study in great detail both the structure and biological activity of amber [2]. In addition to its cultural and historical significance, amber has also attracted increasing scientific interest as a complex material with unique structural and chemical properties.

Amber isn't just a pretty rock; technically speaking, it is a cloudy geological macromolecular substance that consists of a polymer network. The matrix consists predominantly of complex terpenoids, succinic acid derivatives and aromatic compounds that have been polymerised to form a structure that has remained stable for millions of years since undergoing pressure relaxation processes after becoming fossil resin [3,4]. Because of its structure, amber possesses properties not found in other synthetic polymers and natural resins. Because of these structural features, various spectroscopic methods have been employed to better characterize amber.

Spectroscopy has also revealed that it contains a large variety of chemicals. Figure 1 does not show the bands that are present in actual Baltic amber, for example, carbonyl bands, hydroxyl bands, and C-O stretching bands. However, FTIR does show these bands in genuine Baltic amber and other kinds of amber that are not genuine [3].

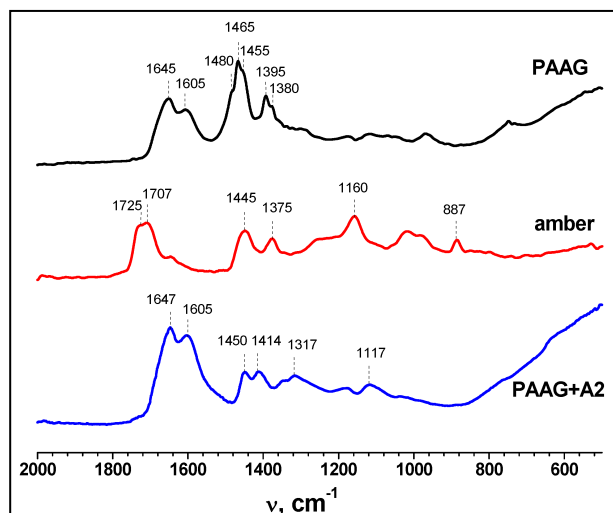


Fig. (1): FTIR spectrum of Baltic amber showing characteristic absorption bands (C=O at $\sim 1725\text{ cm}^{-1}$, C-H

at $1445\text{--}1375\text{ cm}^{-1}$, and C-O stretching at $\sim 1160\text{ cm}^{-1}$), confirming its structural fingerprint [5].

Additional information is obtained from Raman spectroscopy, particularly in the identification of aromatic and conjugated systems as shown in figure 2 [6].

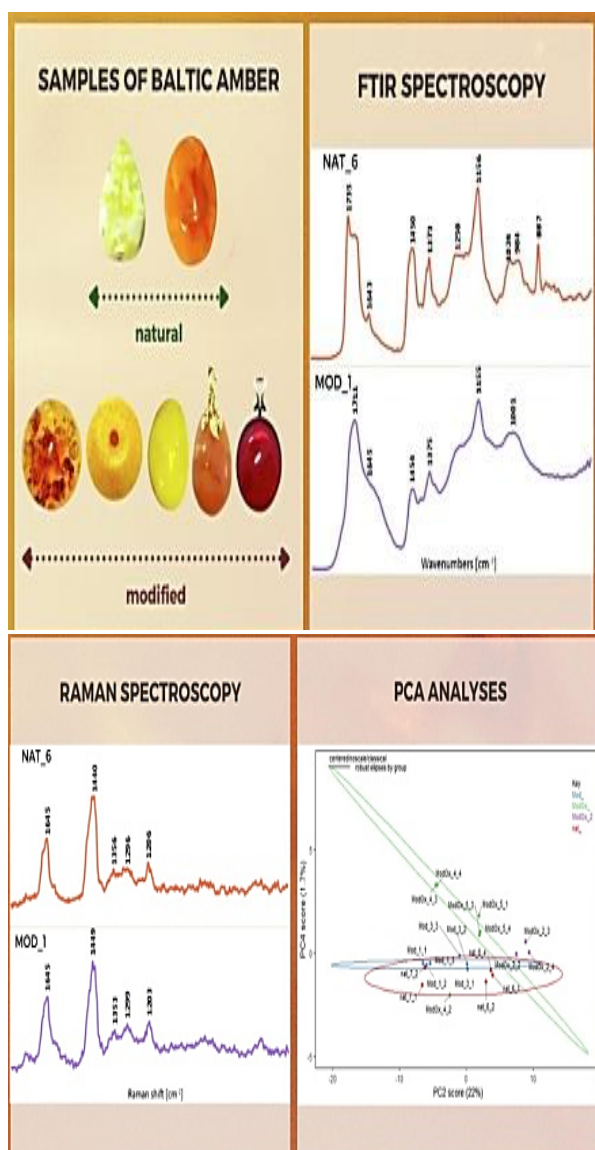


Fig. (2): Natural and heat-treated Baltic amber FTIR and Raman spectroscopy. The variations in the bands and PCA analysis are typical to demonstrate the variations in structural evolution [6].

However, compounds which are semi-volatile or volatile, such as succinic acid and abietane diterpenoids can be analysed by gas chromatography-mass spectrometry (GC-MS). Typical GC-MS profiles of Baltic amber extracts reveal the presence of succinic acid, abietic acid, dehydroabietic acid, and communic acid, which are associated with anti-inflammatory and antimicrobial properties. Besides the interesting application as a means for diagnosing whether an amber belongs to a new species, this kind of research also complements results of structural investigation, according to which there is a correlation between the structure of materials and the functions assigned to them.

Amber extracts have been found to have anti-inflammatory, anti-bacterial, antioxidant and neuroprotective properties [8-10]. Succinic acid is thought to be anti-inflammatory [11] and diterpenoids anti-bacterial and anti-fungal [12]. Prehistoric extract has been proven by animal laboratory studies to have rejuvenation properties. Each amber of the collection is an individual biological and physical sample of nature. On a strictly scientific and technical basis, this amber effect has generated great opportunities for interdisciplinary studies combining materials physics and the life sciences.

Amber is also modified in a way that is closely related both to thermal treatment and to hydrothermal treatment. Its chemical structure, colour and shape are all different [15,16]. And at this stage in time, they most likely want to know how they can stop the erosion of fossil amber. In addition, in order to satisfy this need,

the rest of the paper will be dedicated to the description of the evolution of the physical parameters (temperature, pressure, humidity) and internal condition and structure.

It is proposed that the review is aimed at three objectives: (1) to give an overview on the studies on the spectroscopy and analysis of Baltic amber; (2) to correlate the structures identified with the proposed biological application; (3) to clarify the issues for the application of amber as a biofunctional material and its potential exploitation in medicine. In this work, we want to draw attention to the importance of amber as a natural history object and as a modern scientific and technological object, and to present the results of characterization of new materials, biomedical studies and their synthesis.

Chemical Composition of Amber

Amber is a very complex organic chemical compound that started to form as fossilized vegetable resin that polymerized and diagenetically changed over millions of years, Baltic amber (Baltic amber, succinite) is a Class la fossil resin that is most commonly composed of succinic acid (38 wt%) as the dominant constituent or character-forming material. This active component is in the active state in the amber framework of the macromolecular system and is chemically bonded to the amber framework of the resin, this active component is not in the free state [18]. In addition to succinic acid, the terpenoids found in amber include labdane-related diterpenoids, abietane derivatives, communic acids and volatile sesquiterpenes [19,20]. These molecules are the result of more or less geologically intact pre-

existing plant material. Some of these abietane diterpenoids in particular have been linked with anti-inflammatory and antimicrobial properties [21]. Another class of chemicals of interest in amber were also characterized using GC-MS and Raman spectroscopy aromatic hydrocarbons, such as anthracene derivatives and phenanthrene derivatives [22]. The latter are chemicals which are not only present in the spectral fingerprint of amber but which can also interfere with the function of the amber in biology

In general, the amber chemical composition can be divided into three categories:

1. Compounds chemical organisation and action Succinic acid and compounds.
2. Terpenoids (mono-, sesqui- and diterpenoids) - the major biologically active resins
3. Aromatics: small molecules of spectroscopic interest

The action of amber speaks for itself, not only in its permanence, and its color, but also provides the principle of the biological action of amber as we understand it. A survey of the main chemical constituents of Baltic amber may be given in table 1 [1722].

Table (1): main chemical constituents of Baltic amber.

Compound/Group	Example molecules	Molecular formula	Reported role/properties	References
Succinic acid & derivatives	Succinic acid	$C_4H_6O_4$	Structural role in polymeric network; anti-inflammatory effects	[17,18]
Diterpenoids (abietane type)	Abietic acid, Dehydroabietic acid	$C_{20}H_{30}O_2$ (abietic acid)	Antimicrobial, antioxidant, anti-inflammatory	[19–21]
Sesquiterpenes	Communic acid, Cadinene	$C_{20}H_{30}O_2$ (communic acid)	Resin constituents; antifungal & antibacterial activity	[19,20]
Aromatic hydrocarbons	Anthracene, Phenanthrene	$C_{14}H_{10}$ (anthracene)	Spectroscopic markers; possible antioxidant properties	[22]
Esters & Alcohols	Succinic esters, Terpenoid alcohols	Variable (e.g., $C_8H_{14}O_4$)	Influence volatility and fragrance; contribute to FTIR signals	[17,18]
Other minor compounds	Rosin acids, Fatty acids	Variable	Contribute to spectral complexity; possible biological activity	[19,22]

Spectroscopically Amber.

Baltic amber structural description and chemical description: spectroscopic procedure to obtain

required information The most common of these are Fourier Transform Infrared (FTIR) spectroscopy, Raman spectroscopy, and Gas Chromatography-Mass Spectrometry (GC-MS) The techniques each have different data, which

can be used for authentication and provenance studies as well as amber biology.

1. Fourier Transform Infrared Spectroscopy.

FTIR spectra contain a number of diagnostic absorption bands that are characteristic of amber. The most defining feature of the succinits and other fossil resin is the 1 (Baltic shoulder) (1150-1260 cm^{-1}). Other bands of interest are 1735 cm^{-1} (C=O bending), 3400 cm^{-1} (OH bending) and 2940-2850 cm^{-1} (CH vibrations), [24]

2. Raman Spectroscopy

Raman spectroscopy is complementary, and only works for aromatic and conjugated systems. Generally the Baltic amber is relatively strong in Raman 1640 cm^{-1} (C-C stretching) and 1000-1600 cm^{-1} (aromatic vibrations) (25). Amber has also been monitored by Raman after heat or hydrothermal treatment and band changes with an increase or decrease in intensity have been noticed [26]

Table (2): Characteristic FTIR and Raman bands of Baltic amber with corresponding functional groups and their assignments.

Spectroscopic method	Characteristic bands (cm^{-1})	Assignment / Functional group	References
FTIR	1735	C=O stretching (esters, acids)	[23,24]
FTIR	1150–1260 (Baltic shoulder)	C–O stretching (esters/succinate)	[23]
FTIR	2940–2850	C–H stretching (methyl/methylene)	[24]
Raman	~1640	C=C stretching (conjugated systems)	[25]
Raman	1000–1600	Aromatic ring vibrations	[25,26]

3. Gas chromatography mass spectrometry (GC-MS)

Volatile and semi-volatile constituents can be detected by GC-MS analysis of amber extracts.

It has been reported that succinic acid, abietic acid, dehydroabietic acid and communis acid as well as anthracene derivatives are present [27-30]. These compounds not only account for the chemical signature of amber, but also explain their potential antimicrobial and anti-inflammatory activity.

Table (3): Typical GC-MS chromatogram of Baltic amber extract [27].

Compound	Class	Molecular formula	Reported activity	References
Succinic acid	Dicarboxylic acid	C ₄ H ₆ O ₄	Structural role; anti-inflammatory	[27]
Abietic acid	Diterpenoid	C ₂₀ H ₃₀ O ₂	Antimicrobial, anti-inflammatory	[28]
Dehydroabietic acid	Diterpenoid	C ₂₀ H ₂₈ O ₂	Antioxidant, antibacterial	[28]
Communic acid	Diterpenoid	C ₂₀ H ₃₀ O ₂	Antifungal, antimicrobial	[29]
Anthracene derivatives	Aromatic hydrocarbon	C ₁₄ H ₁₀	Biomarkers; spectroscopic markers	[30]

Amber Pro, Biological and Medical.

Indeed, amber and amber extracts have been receiving more and more attention in the biomedical literature for their anti-inflammatory, anti-microbial, antioxidative and neuroprotective properties. The latter bioactivities can be largely explained by succinic acid, diterpenoids and aromatic compounds found in Baltic amber [31]

1. Anti inflammatory activity

Several in vitro studies have shown inhibition of inflammatory mediators in response to the amber extracts. In one published study, amber extract treatment of LPS-stimulated macrophages reduced nitric oxide (NO), reactive oxygen species (ROS), and pro-inflammatory cytokines TNF- α , IL-1 β and IL-6, in significant

amounts [32]. These effects were associated with an inhibition of the NF-KB signalling pathway and may suggest a target for the pathway in the regulation of chronic inflammatory diseases.

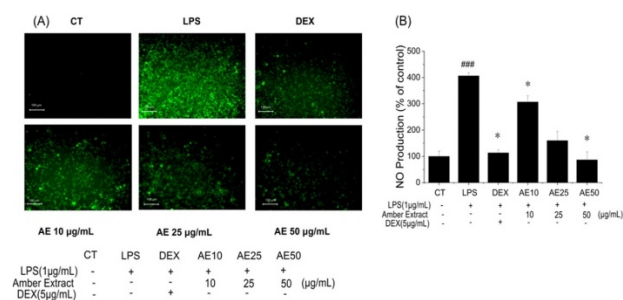


Fig. (3): Effect of amber extract on inflammatory mediators in macrophages, showing suppression of NO and ROS in LPS-induced RAW 264.7 cells [32].

2. Antimicrobial activity

As reported by GC-MS studies, abietane diterpenoids (eg, abietic acid, dehydroabietic acid) have a broad spectrum of antimicrobial activity [27]. Laboratory experiments showed that extracts from amber had an inhibitory effect against *S. aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* [33]. Inhibition zones were generally reported in the range of 10–15 mm for common bacterial strains such as *S. aureus* and *E. coli*, indicating a moderate but reproducible antibacterial effect when compared with standard antibiotics. This antimicrobial activity assumes that substances from amber may be exploited as new antibacterial agents in this era of increasing antibiotic resistance. Figure 5 schematic of agar-disc diffusion assay with zones of inhibition, not amber research, just to help illustrate the principle



Fig. (4): Representative agar disc diffusion assay showing inhibition zones around antimicrobial disks (illustrative example).

3. Antioxidant properties

The amber extracts have shown a substantial antioxidant activity as free radical scavengers and oxidative stress mediators in cell models [34]. These results are consistent with the

existence of polyphenolic and aromatic compounds that act as radical scavengers.

4. Neuroprotective effects

Recent studies have shown that amber extracts protect the neuronal cells. Oxidative stress (6-hydroxydopamine) treatment of dopaminergic cells (SH-SY5Y) decreased the intracellular ROS levels and increased the cell survival in vitro in the presence of the amber extracts [35]. Besides that, researchers also reported that amber would induce autophagy and BACE1 expression regulation, which is another possible treatment method for Alzheimer disease [36].

5. Dermatological applications. Cosmetic applications.

Amber extracts have been studied for use in dermatology as skin lightening and anti-aging compounds. Kuji amber extract was shown to have anti-melanin producing effects on B16 cells and pro-collagen producing effects on human dermal fibroblasts [37]. Because of these two effects, these findings may be useful for the treatment of hyperpigmentation and skin aging in cosmetic products.

Table 4: Biological activities of Baltic amber extracts and their reported applications.

Biological activity	Experimental model	Main outcome	References
Anti-inflammatory	LPS-induced RAW 264.7 macrophages	↓ NO, ROS, TNF- α , IL-1 β , IL-6 via NF- κ B inhibition	[32]
Antimicrobial	<i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i>	Inhibition zones in agar diffusion assay	[33]
Antioxidant	In vitro radical scavenging assays	Significant free radical scavenging activity	[34]
Neuroprotective	SH-SY5Y neuronal cells	Reduced ROS, ↑ cell survival, autophagy activation	[35,36]
Dermatological / Cosmetic	B16 cells, fibroblasts	↓ melanin synthesis, ↑ collagen production	[37]

Industrial and Cosmetic Applications of Amber

Amber was once used as an ornamental/decoration material for jewelry and art objects. Baltic amber has always been a part of the jewelry industry,



Fig. (5): Traditional decorative/industrial use of Baltic amber in jewelry (licensed image, CC BY-SA credit: Brocken Inaglory) [38].

In which its unusual optical phenomenon, such as warm colour and fluorescence in the ultraviolet, is highly appreciated [38]. In addition, various heat treatment and pressing techniques have been used to alter the

appearance of amber, make it more transparent or even produce commercial pressed amber [39].

Apart from the traditional applications, there are new applications of amber in modern industry. Extracts and amber powder have been used as ingredients in cosmetic preparations.

Because of the antioxidant, anti-inflammatory and collagen stimulating properties reported [40]. One case in point is the extracts of amber which are employed in anti aging, skin lightening and skin whitening serums and creams, since the research has already shown a reduction of melanin synthesis and an upsurge of collagen synthesis in the in vitro setting [41]

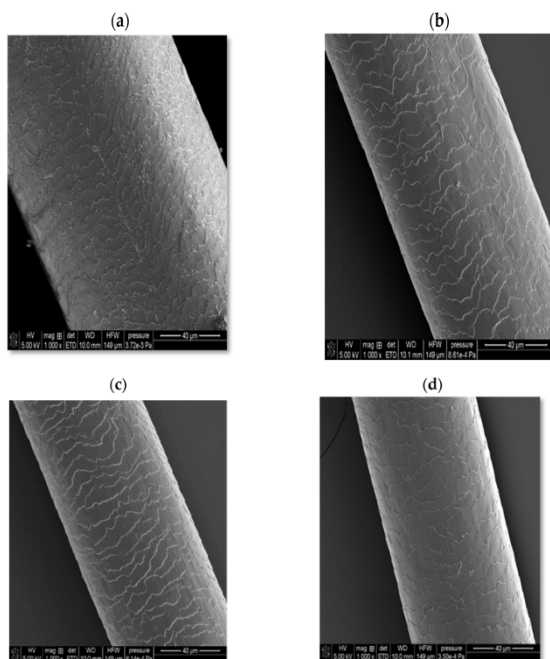


Fig. (6): Cosmetic use-case: (A) SEM images showing hair cuticle smoothing after using a shampoo containing an amber-based composite [41].

Another use of amber in perfumery is as a source of the resinous constituents that are employed as fixatives and fragrance enhancers [42]. Moreover, amber derivatives were also explored in terms of their potential application to the field of polymer science, specifically as natural stabilizers to materials and bio-based fillers to composites [43].

Overall, industrial and cosmetic uses of amber demonstrate the versatility of the material, it is a combination of the ancient usage of the material as a gemstone and its usage in relatively modern times in health, beauty, and material sciences.

Prospects and conclusions

Amber is a fossil resin (and, less often, Baltic succinite) that is extremely rare in the fossil record, and has a very distinct combination of structure, chemical complexity and biological potential. The diagnostic fingerprints and

bioactivity of terpenoid and aromatics, the chemical structure of this compound and the androgenesis of succinic acid has been elucidated using the FTIR and Raman spectroscopy and the GC-MS spectroscopy. Conclusions: Scientific concepts in the literature that describe anti-inflammatory, antimicrobial, antioxidant, neuroprotective, dermatological effects of amber are explained.

Amber is also commonly used as a commercial and cosmetic additive, a jewelry and an ornamental material, an incense and potions spread on the skin in addition to its use in medicine. As researchers could map the impact of physical alteration on bioactivity and stability, it could be introduced in more processes by new hydrothermal or thermal processing techniques. All these do not exclude certain problems. The bulk of what is known in biomedicine has been in vitro or in initial stages of in vivo research and there are no large clinical trials underway at the present. Moreover, the mechanism of action of the bioactive amber active components are unknown. However, it should be noted that most of the reported bioactivities of amber extracts are limited to in vitro cell models or small-scale in vivo studies. These findings, while promising, may not fully represent the complex interactions that occur in living organisms. Differences in extraction methods, sample origin, and compound variability also make direct comparison between studies difficult. Therefore, claims regarding clinical potential must be interpreted with caution until supported by standardized protocols and large-scale clinical trials. The following wave of studies should thus be aimed at:

1. Complete clinical trials to determine the safety and efficacy of amber based preparations.

2. Advance a broad spectrum of recent spectroscopy and nanomaterial technology to enhance insights into the dynamics of the product-biological system formed by amber.
3. General means of extraction calculation with a view of deriving parallels and generalizing research fundings across studies.

Lastly, amber is a culturally and traditionally highly important raw material, and at the same time it could find use in modern medicine, as well as in cosmetics and high technology material sciences. It is also scientifically established that amber can be employed in a bio functional multidisciplinary multipurpose application in addition to its application as an ancient gemstone.

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