

Novel comprehensive characterization of PELLAMAR sapropelic extract and implications for therapeutic applications

Matei Tom Iacob

University of Bucharest

Adriana-Elena Bălan

University of Bucharest

Cornelia Nichita

University of Bucharest

Elena Nedelcu

Elena.Nedelcu@ucsf.edu

University of California

Article

Keywords:

Posted Date: July 16th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-9903126/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background

The therapeutic value of natural sapropels from Romanian lakes has been known since Dacian-Roman times, and they have been used to treat a variety of disorders. Although PELLAMAR products are rooted in medical therapeutic tradition and have been used in balneotherapy for decades, the therapeutic mud extract has never been characterized by modern techniques. This novel study aimed to provide a comprehensive characterization of the PELLAMAR therapeutic mud extract.

Methods

Core analytical and physicochemical methods used for characterization of PELLAMAR sapropelic mud extract were performed using Fourier-Transform Infrared Spectroscopy (FT-IR) and Raman Spectroscopy (RS) for molecular composition, and Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) for thermal stability. These methods provide a comprehensive multi-modal assessment of sapropelic muds.

Results

FT-IR analysis demonstrates a mineral-dominated composition characterized by strong Si–O–Si stretching vibrations ($\sim 1090\text{ cm}^{-1}$) indicative of quartz, carbonate bands ($\sim 1317\text{ cm}^{-1}$) consistent with calcite, and sulfate bending modes (~ 636 and 611 cm^{-1}) confirming the presence of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). Broad O–H stretching bands ($3857\text{--}3473\text{ cm}^{-1}$) and H–O–H bending ($\sim 1657\text{ cm}^{-1}$) reflect significant hydration and a colloidal matrix. Minor contributions from organic functional groups suggest the presence of humic substances, supporting a mixed organic–mineral structure typical of sapropelic materials. Raman spectra indicate that the sapropelic mud extract contains a heterogeneous organic–mineral matrix, characterized by strong silicate, sulfate, and carbonate bands, with a smaller but detectable organic contribution reflected in O–H/ H_2O and aliphatic C–H-associated features. Strong bands in the $2900\text{--}3000\text{ cm}^{-1}$ region indicate abundant aliphatic C–H groups consistent with lipid-rich organic matter, whereas the $3634\text{--}3706\text{ cm}^{-1}$ bands are consistent with hydroxylated humic structures. In addition, carotenoids, aromatic carbon structures, kerogen-like material, and alkynes or related unsaturated compounds are found. TGA/DSC analysis is consistent with a rich mineral phase conferring heat retention and thermal stability.

Conclusions

This study, to our knowledge, is the first characterization of the PELLAMAR sapropelic mud extract by modern spectroscopic and thermal analytical techniques. Findings and provide scientific validation of its

quality and demonstrate that the extract is structurally consistent with high-quality peloid content, suitable for therapeutic and cosmeceutical applications.

BACKGROUND

Sapropelic muds (peloids) refer to a type of organic-rich sediment that forms at the bottom of stagnant or low-oxygen water bodies (like lakes, lagoons, or saltwater pools), mainly comprising decomposed plant and animal material that has accumulated over thousands of years in anaerobic (oxygen-poor) conditions. The therapeutic value of natural sapropels from Romanian lakes has been known since Dacian-Roman times [1, 2], and they have been used to treat musculoskeletal, gynecological, endocrine, dermatological, and hepatobiliary diseases [3, 4]. Such lakes, Techirghiol, Amara, Ursu, and Balta Alba, have been harvested for sapropelic mud used in balneotherapy protocols.

Unlike others [5], the sapropelic mud or extract from Balta Alba has not been characterized.

Balta Albă lake is typically described in balneological and hydrochemical classifications used for Romanian therapeutic lakes as an inland brackish-saline Na–Cl lake with sapropelic mud deposits. It is less mineralized than hypersaline lakes such as Lake Techirghiol or Lake Ursu, and its peloid is known to be rich in salts (e.g., calcium, magnesium, sulfur) and amino acids, giving it biotrophic, regenerative, anti-inflammatory, analgesic, sedative, and vasodilatory effects. The mud extract from this lake is a proprietary bioactive complex, patented in the late 1970s by the Romanian physician and professor, Dr. Ștefan Ionescu-Călinești, as a topical therapeutic agent primarily used in balneotherapy and dermatology. The therapeutic mud (paste) is used in spas or at home for joint pain, rheumatic conditions, tendonitis, eczema, psoriasis, varicose veins, and post-traumatic recovery or processed into a soluble bioactive extract, removing insoluble particles and making it suitable for cosmetic formulations. Core ingredients of the PELLAMAR extract are minerals: Calcium (Ca), Magnesium (Mg), Potassium (K), Sodium (Na), sulfates and chlorides; humic and fulvic acids, amino acids, fatty acids, lipids and organic sulfur compounds, and trace elements. Humic and fulvic acids are known for their anti-inflammatory, regenerative, and detoxifying properties.

The use of the peloid extract is also the foundation of all PELLAMAR products [6]. PELLAMAR (also styled Pell Amar) is a Romanian ISO-certified (ISO 9001 & GMP/ISO 22716) brand built around this unique therapeutic ingredient, which under high-quality manufacturing [7] uses the mineral-rich therapeutic mud extract combined with other natural ingredients for products used in skincare (anti-aging creams, acne treatments, moisturizers), haircare (shampoos and scalp treatments), spa treatments (body wraps, face masks) or for clinical use for joint and muscle therapy (for arthritis or muscle pain) or dermatology (psoriasis, seborrheic dermatitis, acne-prone or sensitive skin). Other ingredients (depending on the product), depending on the purpose of the product, include botanicals and natural additives (propolis, honey, aloe vera, calendula, sea buckthorn); active cosmetic agents (hyaluronic acid, collagen, peptides, salicylic acid (in acne products), vitamin E, A, or C), base components and emulsifiers: aqua (purified water), glycerin, plant-derived oils (jojoba, almond, etc.) and

emulsifying waxes and stabilizers (cosmetic-grade); PELLAMAR does not contain parabens or synthetic colorants and is often marketed as hypoallergenic and dermatologically tested; some products may include essential oils or bee products. The exact content can vary by product (e.g., face cream, body lotion, mud mask), but the core ingredient is consistently the PELLAMAR extract, the proprietary bioactive complex.

Although PELLAMAR products are rooted in a rich therapeutic tradition, clinical studies are mostly conducted regionally, and neither the mud nor the extract has been characterized using modern techniques. This novel study aimed at addressing this gap.

MATERIALS AND METHODS

Fourier-Transform Infrared Spectroscopy

Fourier-Transform Infrared (FT-IR) spectroscopy is widely used to identify functional groups in the organic and mineral components of sapropelic muds, enabling qualitative comparison of organic-matter maturity and aromaticity across samples [8]. Infrared radiation interacts with molecular vibrations, and the resulting absorption spectrum provides characteristic peaks corresponding to bonds such as O–H (hydroxyl), C = O (carbonyl), C–H (aliphatic), and Si–O (silicate). Typical spectra of humic-rich sediments exhibit strong absorption near 3400 cm^{-1} associated with O–H stretching, around $1650\text{--}1660\text{ cm}^{-1}$ corresponding to carbonyl or aromatic amide groups, and approximately $1400\text{--}1450\text{ cm}^{-1}$ related to aliphatic C–H deformation, reflecting the presence of humic substances and clay minerals [9].

FT-IR spectra were recorded on a Bruker ALPHA spectrometer in transmission mode. The sample was prepared by depositing it onto a Potassium Bromide (KBr) window without mixing or pressing it into a KBr pellet. Spectra were initially acquired over the range $400\text{--}4000\text{ cm}^{-1}$ at a spectral resolution of 4 cm^{-1} and subsequently trimmed to $500\text{--}4000\text{ cm}^{-1}$ for presentation. Data were collected in absorbance mode and converted to transmittance.

Raman Spectroscopy

Raman spectra were acquired using a JASCO NRS-3100 spectrometer with 785 nm laser excitation. Spectra were collected in backscattering geometry using a 20× objective lens at a spectral resolution of 4 cm^{-1} . Each measurement consisted of 100 accumulations, each with an exposure time of 5 s. To obtain optimal signal quality and resolution in the regions of interest (full spectral-range scans yielded suboptimal results), acquisitions were performed at selected Raman shift centers of 1450, 2250, 3000, and 3300 cm^{-1} , corresponding to characteristic vibrational modes of organic functional groups.

Thermogravimetric Analysis, Derivative Thermogravimetry, and Differential Scanning Calorimetry

Thermogravimetric Analysis (TGA), Derivative Thermogravimetry (DTG), and Differential Scanning Calorimetry (DSC) evaluate thermal stability, water content, and the behavior of organic decomposition. DSC measures the heat flow associated with physical or chemical transitions as the sample is heated, revealing endothermic peaks from moisture loss and exothermic peaks from the oxidation of organic matter. TGA monitors weight changes as temperature increases, typically showing a multi-step loss: initial dehydration (below 150°C), organic degradation (200–400°C), and mineral decomposition (> 500°C). Together, they characterize the energy capacity, thermal resistance, and phase transitions of the sapropel matrix, parameters that correlate with its heat-retention capability in therapy [11].

TGA/DGT/DSC analysis was performed using a TGA/SDTA instrument (Mettler Toledo, model Star, Greifensee, Switzerland) in air at a heating rate of 10°C/min in the temperature range of 25–1000°C. DSC curves were obtained under nitrogen at a flow rate of 80 ml/min and a heating rate of 10°C/min in the temperature range 25–450°C. The 10 mg sample was sealed in an aluminum pan. Indium was used as a calibration reference.

Ultraviolet-visible-near-infrared

Ultraviolet-visible-near-infrared (UV-Vis-NIR) is an analytical technique that measures how a material absorbs light across the ultraviolet (UV, ~ 200–400 nm), visible (Vis, ~ 400–700 nm), and near-infrared (NIR, ~ 700–2500 nm) regions of the electromagnetic spectrum. UV-Vis-NIR diffuse reflectance spectra were recorded for the PELLAMAR extract using a JASCO V-570 spectrometer (JASCO Corporation, Tokyo, Japan) in reflectance mode. The PELLAMAR extract powder was pressed directly into the sample holder without further dilution and analyzed using an ISN-470 integrating sphere accessory (JASCO Corporation, Tokyo, Japan) with Spectralon as the 100% reflectance reference standard. Spectra were acquired from 200 to 2000 nm, converted to $\log(1/R)$ (pseudo-absorbance), baseline-corrected, and normalized for comparison.

RESULTS

Fourier-Transform Infrared Spectroscopy

FT-IR analysis of the extract shows the characteristic presence of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), calcite (CaCO_3), and quartz (SiO_2) (Fig. 1). Regions around 3856 and 3473 cm^{-1} correspond to O-H stretching vibrations due to hydration or moisture absorption [11]. Weak disturbances at around 2920 and 2845 cm^{-1} indicate the presence of organic compounds [12], which are more clearly evident in Raman analysis. The 1657 cm^{-1} region is common for H-O-H bending (H_2O) from water crystal formation inside gypsum [11–13]. The strong peak at 1090 cm^{-1} with an additional shoulder at around 1150 cm^{-1} and a sharp peak at 990 cm^{-1} is a signature for SO_4 stretching (ν_3 asymmetric and ν_1 symmetric) in gypsum; this is also confirmed by the 611 cm^{-1} line, which can be assigned to the ν_4 asymmetric SO_4

bend [12–13]. These features are classic for gypsum-dominated peloids. Peaks at 620, 640, and 660 cm^{-1} in Raman analysis can also indicate the presence of gypsum, corresponding to ν_4 SO_4 bending modes [14–16], which may overlap with the TiO_2 anatase mentioned. A broad peak at 1317 cm^{-1} , with shoulders at 1460 and 1400 cm^{-1} , can be attributed to the asymmetric CO_3 stretch of calcite, which extends over a larger spectral range when present in sedimentary form [7, 8]. The ν_3 plain bend at 710 cm^{-1} confirms the calcite [12, 17]. The line at 440 cm^{-1} shows the presence of quartz bending/lattice mode; the presence of Si-O-Si may also broaden the 1090 cm^{-1} peak [17–19].

Raman Spectroscopy

The results of Raman spectroscopy of the PELLAMAR sapropelic mud extract are illustrated in Fig. 2. A to D and explained below.

Region (650–1450 cm^{-1})

Figure 2. A shows a broad, intense band at 651 cm^{-1} (intensity 1.0, FWHM 54 cm^{-1}) corresponding to the E_g mode of anatase (TiO_2 ~640–650 cm^{-1}), a trace detrital or authigenic phase found in bulk sapropels, precipitated under acidic diagenetic microenvironments from organic decay-mediated Ti mobilization. Its presence in this clean extract suggests incomplete separation during processing, reflecting a sedimentary origin in which low-pH diagenesis from organic decay facilitates TiO_2 precipitation [20–24]. Bands at 997 cm^{-1} (intensity 0.17, C– CH_3 rocking), 1152 cm^{-1} (intensity 0.17, C–C stretch), and 1528 cm^{-1} (intensity 0.07, C = C stretch), can be considered carotenoid biomarkers, left by cyanobacterial and algal proliferation in eutrophic, stratified lakes mirroring reported FT-Raman features in Romanian sapropels, where β -carotene or fucoxanthin signals denote preserved phototrophic inputs under anoxia. Weaker aliphatic modes indicated by 1261 cm^{-1} (intensity 0.16, CH_2 twist) and 1314 cm^{-1} (intensity 0.14, CH_2 wag) can suggest polymethylenic chains from lipid-rich kerogen precursors [25–30].

Kerogen immaturity is also evident by the D-band at 1366 cm^{-1} (intensity 0.07, disordered sp^3 carbon) and G-band at 1579 cm^{-1} (intensity 0.07, sp^2 graphitic carbon), yielding a D/G ratio ~ 1, indicative of low thermal alteration. These carbon bands transition seamlessly into mid-region overlaps (e.g., 1585 cm^{-1}), affirming spectral coherence [30–38].

Region (1450–2700 cm^{-1})

The overlap of Fig. 2.A and Fig. 2. B reinforce the carotenoid (1528 cm^{-1}) and kerogen (1579–1585 cm^{-1}) assignments, with positional consistency (± 2 cm^{-1}). A prominent feature at 2115–2119 cm^{-1} (intensities 0.15–1.0, FWHM 13 cm^{-1}) is ascribed to $\text{C} \equiv \text{C}$ stretching in terminal alkynes or polyyne chains ($-\text{C} \equiv \text{C}-$), usually embedded within the sapropelic organic matrix. This band is observed across datasets and suggests an intrinsic sample origin from the diagenetic reconfiguration of aliphatic precursors into sp -

hybridized carbon structures [39–40]. Proximal bands (e.g., 2154 cm^{-1} , intensity 0.11) may represent chain-length variants (longer polyynes shift lower), while alkene modes at 1652 cm^{-1} (intensity 0.27, C = C stretch) and overtone/combination features at 2540 cm^{-1} (intensity 0.37) may indicate transitional unsaturation in sapropel's humic/fulvic fractions [26, 40–42].

Region (2700–3800 cm^{-1})

Figure 2. C and 2.D show robust repeatability, with matched peaks (deviations < 3 cm^{-1}), validating aliphatic dominance. The stretch at 2934–2935 cm^{-1} of asymmetric CH_3/CH_2 is representative for methylated chains from sapropelic lipids (e.g., bacterial hopanoids, algal sterols), correlating with high Hydrogen Index (HI) and Total Organic Carbon (TOC) in Type I kerogen [25]. Peaks at 2880–2892 cm^{-1} (intensities 0.20–0.27, symmetric CH_3) and 3069–3070 cm^{-1} (intensities 0.20–0.26, $=\text{CH}_2$) denote polymethylenic and olefinic structures, with absent aromatic C–H ($\sim 3050 \text{ cm}^{-1}$) aligning with the extract's white hue and limited polyaromatics, favoring aliphatic preservation in oxygen-depleted sapropelic zones. The 3295 cm^{-1} band (intensity 0.94–1.0, FWHM 15–16 cm^{-1}) can be paired with the mid-region $\text{C} \equiv \text{C}$ as $\equiv \text{C}-\text{H}$ stretching in terminal polyynes, reinforcing sp-carbon presence amid diagenetic maturation [43]. Broad O–H modes at 3634 cm^{-1} (intensity 0.97, FWHM 11 cm^{-1}) and 3706 cm^{-1} (intensity 0.73) imply hydroxylated humics or hydrated silicates, repeatable and indicative of sapropel's hygroscopic, clay-associated organics [44].

(A) The low-wavenumber region demonstrates prominent mineral-associated bands, including strong signals compatible with silicate, sulfate, and carbonate structures characteristic of quartz-, gypsum-, and calcite-containing phases. (B) The intermediate spectral region reveals additional inorganic and carbon-associated vibrational modes, suggesting a heterogeneous organic–mineral matrix with structurally complex components. (C) The 2400–3500 cm^{-1} region shows pronounced aliphatic C–H stretching bands near 2900–3000 cm^{-1} , consistent with lipid-rich organic matter and kerogen-like material, together with broad hydroxyl-associated features. (D) The high-wavenumber region demonstrates intense O–H stretching bands between approximately 3300 and 3700 cm^{-1} , reflecting hydrated mineral phases, structurally bound water, and hydroxylated humic substances. Collectively, the Raman spectra support the presence of a complex sapropelic matrix composed of mixed inorganic mineral phases and biologically derived organic constituents relevant to therapeutic pelotherapy applications.

Table 1
Interpretation of Raman Spectroscopy Results of PELLAMAR Sapropelic Extract

Raman Shift (cm ⁻¹)	Assignment	Avg Intensity	FWHM (cm ⁻¹)	Sapropelic Relevance
651	Anatase TiO ₂ – E _g mode	1	54	Trace detrital/authigenic mineral
997	Carotenoid – $\rho(\text{C}-\text{CH}_3)$ rocking	0.17	--	Algal/cyanobacterial pigments
1152	Carotenoid – $\nu(\text{C}-\text{C})$	0.17	--	Polyene chain stretching
1528	Carotenoid – $\nu(\text{C}=\text{C})$	0.09	16.9	Conjugated double bonds
1366	Kerogen D-band	0.07	3.88	Disordered amorphous carbon
1582	Kerogen G-band	0.18	--	Graphitic sp ² carbon
2117	Polyynes / Carbyne – $\nu(\text{C}\equiv\text{C})$	0.58	13	Diagenetic sp-carbon chains
2935	$\nu_{\text{as}}(\text{CH}_3/\text{CH}_2)$	0.93	13	Aliphatic lipids (high HI/TOC)
3295	$\equiv\text{C}-\text{H}$ stretch	0.97	16	Terminal alkyne in polyynes
3634	$\nu(\text{O}-\text{H})$	0.97	11	Hydroxylated humics
3706	$\nu(\text{O}-\text{H})$	0.73	--	Hydrated organics/minerals

Thermogravimetric Analysis, Derivative Thermogravimetry, and Differential Scanning Calorimetry

Results of the thermogravimetric Analysis (TGA), Derivative Thermogravimetry (DTG), and Differential Scanning Calorimetry (DSC) are illustrated in Fig. 3.

Top Panel: TGA (Weight Loss): The blue curve shows the percentage of mass remaining as the sample is heated from room temperature to 1000°C. Initial weight losses at 25–250°C correspond to the removal of free and interlayer water, while thermal events between 300 and 460°C reflect decomposition of organic matter and dehydroxylation of mineral phases. At 1000°C, approximately 67.3% of the original mass remains, indicating a high mineral content and good thermal stability. The large residual mass at 1000°C (67.3%) confirms the predominance of thermally stable mineral constituents. These results demonstrate the high mineral content, water-retention capacity, and thermal stability of the extract, properties that are important for its therapeutic applications.

Middle Panel: The DTG curve identifies temperatures where weight loss occurs most rapidly.

Three major DTG minima are observed: at 117°C, there is a maximum rate of free-water loss corresponding to dehydration; at 200.6°C, there is a maximum rate of removal of strongly bound water, indicating water associated with clay minerals and organic structures; and at 380.7°C, the maximum rate of dehydroxylation and organic decomposition, reflecting the breakdown of humic substances and structural hydroxyl groups. These peaks confirm that moisture exists in different states within the sapropelic mud matrix.

Bottom Panel: The DSC trace measures heat absorbed or released during heating. Several endothermic transitions are visible: at 116.9°C shows evaporation of free water and corresponds to the first TGA weight-loss region; at 219.3°C shows removal of strongly associated water, indicating possible structural rearrangement of organic-mineral complexes; at 289.7°C shows transformation of organic compounds, likely reflecting decomposition of humic and fulvic fractions; and at 388.5°C demonstrates dehydroxylation of clay minerals and breakdown of hydroxyl groups from silicate structures.

The analyses demonstrate a characteristic multi-stage thermal behavior of an organo-mineral peloid. An initial mass loss of ~ 1.76% below 150°C corresponds to evaporation of free and adsorbed water, followed by a second loss (~ 2.09%) between 150–250°C attributed to removal of bound water and low-molecular-weight organic compounds. A further decrease (~ 1.32%) from 250–400°C reflects decomposition of humic and fulvic substances, supported by DTG peaks at ~ 200.6°C and ~ 380.7°C and an exothermic DSC event near 388°C indicative of organic matter oxidation. The material remains relatively stable up to ~ 800°C, confirming the predominance of a mineral matrix, after which a major mass loss (~ 67.32%) occurs due to carbonate decomposition (e.g., $\text{CaCO}_3 \rightarrow \text{CaO} + \text{CO}_2$). Overall, the profile indicates a predominantly mineral, clay- and carbonate-rich matrix with a modest but thermally active organic fraction, consistent with functional sapropelic muds used in pelotherapy and supporting their thermal stability and heat-retention capacity under therapeutic conditions [45–46].

The detailed stepwise thermal decomposition pattern is shown in Table 2. Between 25 and 150°C, the sample loses 1.76% of its mass, with a DTG maximum at 117.01°C and a DSC peak at 116.88°C. This is assigned to the removal of physisorbed water and bulk interlayer water, meaning loosely held moisture on particle surfaces and water trapped between clay layers. This is the expected first event in hydrated colloidal mud systems. Between 150 and 250°C, a further 2.09% mass loss occurs, with a DTG maximum at 200.61°C and a DSC peak at 219.27°C, corresponding to more strongly bound interlayer water. This indicates that part of the water is more tightly associated with the mineral structure, as is common in layered clay minerals such as smectite or illite. In the 250–300°C interval, there is no clear mass loss reported by TGA, but the DSC shows a peak at 289.70°C, suggesting a structural reorganization rather than major volatilization. This likely reflects the rearrangement of mineral phases and/or the transformation of organic constituents that produces a thermal event without substantial weight change. From 300 to 460°C, the sample shows an additional 1.32% mass loss, with DTG and DSC peaks at 380.68°C and 388.49°C, respectively. This is assigned to dehydroxylation, meaning loss of structurally bound hydroxyl groups from clay minerals and possibly decomposition of part of the organo-mineral fraction. In sapropelic materials, this region may also overlap with the degradation of humic substances.

Between 460 and 1000°C, there is a major mass loss of 28.76%, assigned to carbonate decomposition. This is a strong indication that the material contains a substantial carbonate fraction, most likely calcite or dolomite, which releases CO₂ at elevated temperatures. At 1000°C, the remaining residue is 67.32%, meaning that about two-thirds of the original material persists as a stable inorganic mineral fraction after heating.

Table 2
Thermal analysis of PELLAMAR Sapropelic Mud Extract

Temperature interval (°C)	TGA		DSC	Proposed Thermal Event/Assignment
	Δm_{loss} (%)	$t_{\text{max-rate}}$ ^{**} (°C)	t_{peak} ^{***} (°C)	
25–150	1.76	117.01	116.88	Physiosorbed and bulk interlayer water
150–250	2.09	200.61	219.27	Release of strongly attached interlayer water
250–300	-	-	289.70	Structural transformation of mineral or organic compounds
300–460	1.32	380.68	388.49	Dehydroxylation of mineral-associated hydroxyl groups
460–1000	28.76	-	-	Thermal decomposition of carbonate-rich mineral phases
1000	67.32 [*]	-	-	Residual thermally stable mineral fraction

Legend: ^{*} residue at 1000°C estimated from TGA curve; ^{**} minimum of the 1st derivative of the TGA curve associated with the temperature of maximum decomposition rate; ^{***} the peak temperature on DSC curve.

The UV-Vis-NIR diffuse reflectance spectrum of the sapropelic white extract powder, recorded with an integrating sphere and converted to log(1/R), reveals a characteristic humic-substance profile dominated by both electronic and vibrational transitions. A sharp maximum at 253 nm arises from $\pi \rightarrow \pi^*$ excitations within benzenoid and phenolic aromatic moieties [47–51], while overlapping shoulders at 340 and 380 nm, together with a gently descending tail through the visible region, reflect $n \rightarrow \pi^*$ and charge-transfer contributions, probably, from conjugated quinoid and polyphenolic systems typical of partially humified sapropelic organic matter [47, 49, 52]. Beyond 700 nm, a broad, featureless valley extending to 1400 nm

with only minor second-overtone O–H and C–H features near 1000 and 1200 nm indicates negligible electronic absorption and low scattering [53]. In the near-infrared, a prominent first-overtone O–H stretch at 1470 nm, a clear minimum at 1570 nm separating hydroxyl from amide/aliphatic domains, distinct C–H overtones at 1690 and 1785 nm (aromatic and CH₂/CH₃, respectively), and a deep intervening dip at 1870 nm preceding an intense, split water combination band at 1940–1955 nm together demonstrate the coexistence of phenolic/carboxyl groups, sapropelic lipids, residual protein-like moieties, and strongly hydrogen-bonded adsorbed moisture [53, 54]. The Kubelka–Munk function was also calculated, but provided no significant additional information and is therefore not presented. Data are shown in the 250–2000 nm range. Collectively, the features establish the extract as a humic-rich material that retains the aromatic backbone and oxygenated functionalities of lacustrine sapropel.

DISCUSSION

The present analysis demonstrates that the PELLAMAR sapropelic mud extract retains the essential physicochemical characteristics of Romanian therapeutic sapropels while exhibiting modifications consistent with industrial processing. Spectroscopic and thermal analyses indicate a heterogeneous organo-mineral matrix dominated by mineral constituents, with a biologically relevant organic fraction. FT-IR and Raman spectroscopy identified silicate, carbonate, and sulfate minerals, including quartz, calcite, and gypsum, as well as hydroxylated organic structures. Raman bands at approximately 997, 1152, and 1528 cm⁻¹ indicate the presence of carotenoid-derived compounds, reflecting the preservation of biological input from phototrophic organisms. Additional bands at ~ 1366 and ~ 1582 cm⁻¹ correspond to disordered and aromatic carbon structures characteristic of kerogen-like organic matter, suggesting progressive diagenetic transformation of biogenic material. Strong CH stretching at ~ 2935 cm⁻¹ and O–H bands between 3634 and 3706 cm⁻¹ further support the presence of lipid-associated and humic-type organic components. Collectively, these findings indicate that the extract preserves biologically derived organic signatures despite processing. Diffuse reflectance UV–Vis–NIR spectroscopy revealed a chemically heterogeneous humic-rich matrix characterized by aromatic $\pi \rightarrow \pi^*$ transitions, oxygenated functional groups, aliphatic domains, and structurally bound water. These features are consistent with partially matured organic matter retaining significant biogenic functionality.

Thermogravimetric and calorimetric analyses demonstrated a mineral-dominated composition with characteristic dehydration, dehydroxylation, and carbonate decomposition stages. Limited mass loss below 250°C reflects structured and interlayer water. These findings support favorable heat-retention properties in the extract, a critical characteristic of raw therapeutic peloids. The large residual mass (67.3%) confirms that the extract is predominantly mineral in nature, and

the multiple dehydration steps indicate that water is held at different levels within the extract matrix, supporting prolonged moisture retention. This layered water structure and mineral matrix support gradual heat release of the extract, potentially resulting in tissue warming, vasodilation, increased microcirculation, muscle relaxation, and pain reduction. The presence of these substances in the extract

confirms its sapropelic mud origin and demonstrates retention of the principal organic fractions associated with its bioactive properties.

The 2010 quality report from the National Institute of Rehabilitation, Physical Medicine, and Balneoclimatology further supports the characterization of PELLAMAR extract as a hydrated sulfurous therapeutic mud derived from the Balta Albă sapropelic deposit [10]. The mud exhibited a high moisture content (61.3%), moderate density (1.298 g/cm³), and a specific heat of 0.6903 cal/g/°C, indicating favorable thermal properties for pelotherapy. Chemically, the material contained a predominantly mineral matrix (32.3%) with a smaller but measurable organic fraction (5.5%), including organic carbon, organic nitrogen, humic acids, and lipid-soluble extracts. The aqueous phase was weakly mineralized (2241.6 mg/L) and mildly alkaline (pH 8.5), with chloride, sodium, bicarbonate, sulfate, and carbonate representing the principal dissolved ions. A distinguishing feature was the presence of hydrogen sulfide (110.4 mg%), supporting its classification as a sulfurous mud and providing a potential mechanistic basis for some of its traditionally recognized anti-inflammatory and dermatologic benefits. Importantly, microbiological testing demonstrated the absence of coliform bacteria, *Escherichia coli*, and intestinal enterococci, confirming the material's microbiological safety. Collectively, these findings indicate that the PELLAMAR extract combines a hydrated mineral matrix with sulfur-bearing and humic components, providing physicochemical characteristics consistent with a therapeutically relevant Romanian sapropelic mud (Table 4).

Table 4
Characteristics of the PELLAMAR Sapropelic Mud Extract

Category	Parameter	Value	Significance
Physical Properties	Density	1.298 g/cm ³	Provides appropriate consistency and skin adherence during application
	Moisture content	61.3%	High hydration favors heat retention and gradual thermal transfer
	Specific heat	0.6903 cal/g/°C	Supports sustained heat storage, an important property in pelotherapy
	Sliding resistance	2611 dyne/cm ²	Indicates suitable rheological behavior and plasticity
Chemical Composition	Mineral fraction	32.3%	Forms the structural matrix responsible for thermal and ionic properties
	Organic fraction	5.5%	Provides bioactive organic compounds contributing to therapeutic activity
	Organic carbon	1.54%	Marker of preserved organic matter
	Organic nitrogen	0.71%	Indicates biologically derived organic material
	Humic acids	0.86%	Associated with antioxidant and anti-inflammatory properties
Hydrochemical Characteristics	pH	8.5	Mildly alkaline environment
	Total mineralization of aqueous phase	2241.6 mg/L	Weakly mineralized aqueous solution
	Dominant anion	Chloride (992.8 mg/L)	Principal contributor to ionic strength
	Dominant cation	Sodium (780.4 mg/L)	Major dissolved mineral constituent
	Secondary ions	HCO ₃ ⁻ , SO ₄ ²⁻ , CO ₃ ²⁻ , Mg ²⁺ , Ca ²⁺ , K ⁺	Contribute to buffering and ionic interactions
	Metasilicic acid	41.6 mg/L	May support skin-conditioning effects
Sulfur Components	Hydrogen sulfide (H ₂ S)	110.4 mg%	Basis for classification as a sulfurous therapeutic mud

Category	Parameter	Value	Significance
Microbiological Quality	Coliform bacteria	0 MPN/g	No microbiological contamination detected
	<i>Escherichia coli</i>	0 MPN/g	Meets microbiological safety requirements
	Intestinal enterococci	0 MPN/g	Meets microbiological safety requirements
Official Classification	Therapeutic mud type	Hydrated sulfurous mineral mud with weakly mineralized aqueous phase	Official classification by the Romanian National Institute of Rehabilitation, Physical Medicine and Balneoclimatology

Legend

Data obtained from the 2010 quality report of the National Institute of Rehabilitation, Physical Medicine and Balneoclimatology (Bucharest, Romania) for Balta Albă sapropelic mud. The material is characterized by high moisture content, moderate mineralization, measurable humic and sulfur components, favorable thermal properties, and documented microbiological safety.

An important distinguishing characteristic of the PELLAMAR extract is its sulfurous nature. The Balta Albă quality bulletin identifies the material as a hydrated sulfurous mud extract with alkaline pH (8.5) and measurable hydrogen sulfide content. Sulfur compounds have long been associated with anti-inflammatory, analgesic, keratolytic, and regenerative effects in balneotherapy. Spectroscopic analysis suggests that sulfur is present primarily as sulfate minerals, particularly gypsum, which contributes to the ionic composition of the extract and may influence skin-interactive and osmotic processes during application.

The therapeutically relevant constituents of the PELLAMAR extract can be grouped into several interacting fractions:

- **Hydrated mineral matrix**
 - The extract is dominated by mineral phases, including quartz, calcite, and gypsum, associated with structured and interlayer water. This system confers high heat capacity and gradual heat release, supporting sustained thermal transfer to tissues. The resulting vasodilation, increased microcirculation, and reduction in muscle spindle activity contribute to analgesia, improved joint mobility, and enhanced tissue oxygenation.
- **Interlayer and bound water (colloidal phase)**
 - The presence of O–H functional groups and a two-step dehydration profile (< 150°C and 150–250°C) reflects a hydrated colloidal structure. This phase maintains plasticity and adherence,

ensures uniform heat distribution, and facilitates ion diffusion at the skin interface, thereby improving contact efficiency and the delivery of soluble bioactive components.

- **Carbonate system**

- Carbonate minerals, as evidenced by FT-IR bands, provide local buffering capacity and help stabilize the physicochemical microenvironment. These properties support skin barrier function, reduce irritation, and exert a mild adjunct anti-inflammatory effect.

- **Sulfate minerals (gypsum)**

- Gypsum contributes calcium and sulfate ions, which may exert osmotic and ionic effects at the skin surface, supporting mild keratolytic and skin-conditioning properties.

- **Humic-like organic fraction**

- Although quantitatively limited, this fraction is functionally significant, contributing to modulation of inflammatory pathways (including NF- κ B), antioxidant activity via reactive oxygen species scavenging, and downregulation of pro-inflammatory cytokines (e.g., IL-1 β , TNF- α). These effects support pain reduction and anti-inflammatory responses in conditions such as osteoarthritis and inflammatory dermatoses.

- **Lipid and aliphatic organic components**

- Aliphatic structures contribute to skin barrier interactions and membrane stabilization, and may act as carriers for other bioactive compounds, thereby improving skin hydration and tolerability of topical application.

- **Biogenic organic signatures**

- The presence of carotenoid and kerogen-like components reflects preserved biogenic input, with antioxidant activity and the potential to sustain low-level bioactivity.

Comparison with Romanian sapropels from Techirghiol, Amara, and Ursu lakes, as described by Baricz et al., reveals substantial compositional similarities (Table 5). Like these sapropels, the PELLAMAR extract contains a mixed mineral-organic framework composed of silicate and carbonate minerals associated with biologically derived organic matter. However, the extract displays a relatively simplified and mineral-enriched profile, likely reflecting processing-related concentration and partial removal of less stable sedimentary components. Whereas the raw sapropels contain more complex clay assemblages, higher compositional heterogeneity, and fully characterized thermophysical properties, PELLAMAR preserves the principal bioactive features while exhibiting enhanced mineral predominance and thermal stability.

Table 5
Comparative physicochemical characteristics of PELLAMAR extract versus Romanian sapropels (Baricz et al., 2021)

Parameter	PELLAMAR Extract (Balta Albă)	Techirghiol Sapropel	Amara Sapropel	Ursu Sapropel
Material type	Processed sapropelic extract	Raw sapropel (whole sediment)	Raw sapropel (whole sediment)	Raw sapropel (whole sediment)
Mineral phases	Quartz, calcite, gypsum, minor anatase (spectroscopic)	Quartz, calcite, clay minerals (illite-rich)	Quartz, calcite, aragonite, clay minerals	Quartz, halite-rich, clay minerals
Clay mineralogy (XRD)	Not determined	Illite dominant	Mixed illite-smectite, smectite	Mixed illite-smectite, smectite
Halite content	Not determined	Low-moderate	Moderate	High (defining feature)
Organic matter (spectroscopy)	Carotenoids, aliphatic chains, kerogen-like bands, humic-type signals	Kerogen (notably present), organic matter	Organic matter, moderate carotenoids	Strong carotenoid signal, organic-rich
Structural complexity	Reduced; processed, spectroscopically defined matrix	High; heterogeneous sediment	High; heterogeneous sediment	High; heterogeneous sediment
Thermal behavior (TGA/DSC)	Low-moderate mass loss; high residual mineral fraction	Total loss ~ 22–25%	Total loss ~ 22–25% (higher 200–700°C loss)	Total loss ~ 22–25% (higher 700–1000°C loss)
Thermal stability	High (mineral-dominated residue)	Moderate	Moderate	Moderate
Cation exchange capacity (CEC)	Not measured	Measured (clay-dependent)	Measured	Measured
Specific surface area (SSA)	Not measured	Measured	Measured	Measured
Granulometry	Not measured	Fine-grained sediment	Fine-grained sediment	Fine-grained sediment
Major ions / salinity	Not determined	Saline	Saline	Highly saline
Trace elements / heavy metals	Not measured	Quantified; within therapeutic ranges	Quantified	Quantified

Parameter	PELLAMAR Extract (Balta Albă)	Techirghiol Sapropel	Amara Sapropel	Ursu Sapropel
Thermophysical properties (conductivity, retentivity, diffusivity, specific heat)	Not measured	Fully characterized	Fully characterized	Fully characterized
Colloidal properties (ζ-potential)	Not measured	Not reported	Not reported	Not reported
Overall profile	Mineral-enriched, processed extract with preserved bioactive signatures	Clay-carbonate sapropel with kerogen	Carbonate-rich sapropel with mixed clays	Halite-rich sapropel with strong organic signature

Integrated FT-IR, Raman, UV–Vis–NIR, and thermal analyses demonstrate that the PELLAMAR sapropelic mud extract is a complex hydrated organic–mineral matrix enriched with silicates, carbonates, sulfates, humic substances, hydroxyl-containing compounds, carotenoid biomarkers, lipid-rich organic matter, and kerogen-like carbon structures. The UV–Vis–NIR spectrum further supports the presence of humic and fulvic substances, abundant organic constituents, hydrated clay minerals, and extensive organic–mineral interactions, indicating a highly structured and water-retentive material. The thermal analyses reveal multiple dehydration stages, confirming strong water-binding capacity and prolonged moisture retention within the mud matrix. The gradual thermal transitions and the lack of major decomposition below approximately 300–350°C indicate excellent thermal stability and heat-retention properties, characteristics important for therapeutic mud applications. Moreover, the high residual mineral fraction (67.3%) confirms that the extract is predominantly mineral in composition, consistent with its elevated content of silicates, clays, and carbonates. The presence of humic substances and other bioactive organic fractions further suggests potential biological activity. Collectively, these findings confirm the sapropelic origin of PELLAMAR and support its classification as a hydrated, bioactive, organic–mineral matrix with strong water retention, thermal buffering, and potential therapeutic properties.

Based on the data above, it is likely that the PELLAMAR extract preserves the sapropel-mediated effect through synergistic thermal, mineral, and organic mechanisms. The hydrated mineral matrix provides efficient heat storage and transfer, promoting vascular and neuromuscular responses. Carbonate minerals contribute buffering capacity and physicochemical stabilization, whereas sulfate minerals provide additional ionic effects. Although quantitatively modest, the organic fraction may play a disproportionately important biological role. Humic-like substances, carotenoid-derived compounds, and kerogen-associated structures possess antioxidant and anti-inflammatory properties that may modulate oxidative stress and inflammatory signaling pathways, including NF- κ B and cytokine-mediated

responses. Together, these mechanisms support the analgesic, anti-inflammatory, regenerative, and skin-conditioning effects traditionally attributed to therapeutic mud applications.

The real potential of the extract lies in transforming a traditional spa material into a standardized, scalable bioactive ingredient for modern medicine, dermatology, cosmetics, and regenerative health products. The extract transforms a traditional therapeutic mud into a scalable, scientifically characterized bioactive platform for modern healthcare applications.

Study Limitations

The comparison is exploratory because the Baricz samples represent whole sediments, whereas PELLAMAR is a processed extract; thus, direct comparison remains limited. Parameters such as clay mineralogy by XRD, cation-exchange capacity, specific surface area, granulometry, thermal conductivity, thermal diffusivity, and trace-element composition were not determined for the extract (Table 5). Consequently, quantitative comparison of functional performance with raw sapropels is not possible. Nevertheless, the available evidence demonstrates that PELLAMAR extract preserves the fundamental organo-mineral framework of Romanian sapropelic muds while exhibiting a processed, mineral-enriched profile characterized by sulfurous properties, alkaline pH, measurable humic content, favorable thermal behavior, and documented microbiological safety. A summary of these characteristics is provided in Table 6. These features support its classification as a therapeutically relevant sapropelic mud derivative suitable for peliotherapeutic and cosmeceutical applications.

Top of Form

Table 6

Physicochemical properties of sapropelic muds/extracts and their associated therapeutic mechanisms

Property / Component	Physicochemical Basis	Primary Biological Targets / Pathways	Mechanistic Effect	Clinical Relevance
Thermal capacity & heat retention	High water content, mineral matrix (clays, carbonates) with high specific heat	Cutaneous thermoreceptors, vascular smooth muscle	Vasodilation, increased microcirculation, reduced muscle spindle activity	Analgesia, muscle relaxation, improved joint mobility
Fine granulometry & plasticity	Clay fraction (illite, smectite), colloidal structure	Skin mechanoreceptors, lymphatic microcirculation	Improved adherence, uniform heat transfer, mild mechanical stimulation	Enhanced therapeutic contact, edema reduction
Clay minerals (illite, smectite)	Layered silicates with high surface area and ion exchange	Cytokine signaling pathways, local inflammatory mediators	Adsorption of pro-inflammatory mediators; modulation of ionic microenvironment	Anti-inflammatory effects in osteoarthritis, soft tissue disorders
Cation exchange capacity (CEC)	Exchangeable ions (Ca^{2+} , Mg^{2+} , Na^{+} , K^{+}) on clay surfaces	Ion channels, keratinocytes, immune cells	Ionic exchange and local mineral delivery; modulation of cellular signaling	Tissue repair support, neuromuscular relaxation
Humic and fulvic substances	Polyphenolic, carboxyl-rich organic macromolecules	NF- κ B pathway, oxidative stress pathways (ROS), cytokines (IL-1 β , TNF- α)	Antioxidant activity; inhibition of NF- κ B activation; cytokine downregulation	Reduction of inflammation, pain, and oxidative stress
Kerogen-like organic fraction	Partially diagenetically transformed organic matter	Redox-sensitive signaling pathways	Sustained release of bioactive organic compounds; redox buffering	Prolonged anti-inflammatory and regenerative effects
Carotenoids and biogenic lipids	Residual algal/bacterial biomolecules	ROS scavenging systems, mitochondrial pathways	Antioxidant activity; membrane stabilization	Protection against oxidative tissue damage
Mineral phases (calcite, quartz)	Structural mineral matrix; carbonate buffering	Local pH regulation, enzyme activity	Buffering of microenvironment; stabilization of organic compounds	Skin barrier support, improved tolerability
Salinity / ionic strength (NaCl, etc.)	Dissolved salts in pore water	Osmotic gradients, ion channels, nociceptors	Osmotic effects, mild stimulation of peripheral nerves	Pain modulation, improved circulation

Property / Component	Physicochemical Basis	Primary Biological Targets / Pathways	Mechanistic Effect	Clinical Relevance
Trace elements (Fe, Zn, Cu, etc.)	Bioavailable microelements in mineral matrix	Enzymatic cofactors, antioxidant enzymes (SOD, catalase)	Enzyme activation, support of tissue repair pathways	Wound healing, anti-inflammatory support
Colloidal stability (ζ -potential)	Negative surface charge (~ - 30 to - 40 mV in reported peloids)	Particle–cell interactions, dispersion stability	Stable suspension, improved spreadability and contact with skin	Uniform application, enhanced bioavailability
Thermal + chemical synergy	Combined heat + mineral + organic components	Neuroendocrine pathways (β -endorphins), vascular and immune systems	Integrated response: vasodilation, cytokine modulation, neuroendocrine activation	Global pain relief, improved function, systemic relaxation

Conclusions

This study, to our knowledge, is the first characterization of the PELLAMAR sapropelic mud extract used in balneotherapy and cosmeceutical production. structurally consistent with high-quality peloid suitable for therapeutic and cosmeceutical applications.

Declarations

Declarations

of interest: None

Declaration

of funding: No funding was used to produce this manuscript.

Declaration

of generative AI and AI-assisted technologies in the manuscript preparation process: During the preparation of this work, the author) used ChatGPT to create figures, review, and edit the content as needed. The author takes full responsibility for the content of the published article.

Declaration

of submission: This manuscript is not under consideration for publication elsewhere, is approved by the author, and, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically, without the written consent of the copyright-holder.

Declaration

of data availability: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contribution

T.M.I., A.E.B., and E.N. wrote the main manuscript text; C.N. prepared the figures; E.N. prepared the tables. All authors reviewed the manuscript.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Bulgăreanu, V. A. C. Protection and management of anthroposaline lakes in Romania. *Lakes Reserv. Res. Manag.* **2**, 211–229. <https://doi.org/10.1111/j.1440-1770.1996.tb00066.x> (1996).
2. Alexe, M. et al. Limnology and plankton diversity of salt lakes from the Transylvanian Basin (Romania): A review. *J. Limnol.* **77**, 17–34. <https://doi.org/10.4081/jlimnol.2017.1657> (2017).
3. Ionescu, E. V. et al. Clinical and biological effects induced by sapropelic mud from Lake Techirghiol in patients with osteoarthritis. *J. Environ. Prot. Ecol.* **15**, 332 (2014).
4. Munteanu, C. *The Therapeutic Mud* (Spa, 2012).
5. Baricz, A. I. et al. Comprehensive mineralogical and physicochemical characterization of recent sapropels from Romanian saline lakes for potential use in pelotherapy. *Sci. Rep.* **11**, 18633. <https://doi.org/10.1038/s41598-021-97904-1> (2021).
6. PELLAMAR. PELLAMAR official website. Available at: <https://www.pellamar.com> (Accessed 2 June 2026).
7. Artri. Artri official website. Available at: <https://www.artri.net> (Accessed 2 June 2026).
8. Socrates, G. *Infrared and Raman Characteristic Group Frequencies: Tables and Charts* 3rd edn (Wiley, 2001).
9. Tkachenko, Y. & Niedzielski, P. FTIR as a method for qualitative assessment of solid samples in geochemical research: A review. *Molecules* **27**, 8846. <https://doi.org/10.3390/molecules27248846> (2022).
10. Institutul Național de Recuperare, Medicină Fizică și Balneoclimatologie (INRMFB). Physical, Chemical, Microbiological, and Therapeutic Characterization of Balta Albă Sapropelic Mud (PELLAMAR) (Technical & Report INRMFB, Bucharest, Romania, (2010).
11. Lei, Y. et al. High-efficiency purification and morphology regulation of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ crystals from phosphogypsum. *Molecules* **29**, 3910. <https://doi.org/10.3390/molecules29163910> (2024).

12. Lupu, R. et al. Physico-chemical characterization of therapeutic muds from Braşov County. *J. Sci. Arts.* **4** (45), 1033–1044 (2018).
13. Bishop, J. L. et al. Spectral properties of Ca-sulfates: Gypsum, bassanite, and anhydrite. *Am. Mineral.* **99**, 2105–2115. <https://doi.org/10.2138/am-2014-4756> (2014).
14. Schmid, T., Jungnickel, R. & Dariz, P. Insights into the CaSO₄-H₂O system: A Raman-spectroscopic study. *Minerals* **10**, 115. <https://doi.org/10.3390/min10020115> (2020).
15. Fines, S. *An Infrared and Raman Spectroscopic Study of Yellow Gypsum Synthesized from LD Slag Fines* (University of Windsor, 2018).
16. Prieto-Taboada, N., Gómez-Laserna, O., Martínez-Arkarazo, I., Olazabal, M. A. & Madariaga, J. M. Raman spectra of the different phases in the CaSO₄-H₂O system. *Anal. Chem.* **86**, 10131–10137. <https://doi.org/10.1021/ac501932f> (2014).
17. Toffolo, M. B. *Infrared Spectroscopy of Archaeological Sediments* (Cambridge University Press, 2025).
18. Andersson, M. P. et al. Infrared spectroscopy and density functional theory investigation of calcite, chalk, and coccoliths - Do we observe the mineral surface? *J. Phys. Chem. A.* **118**, 10720–10729. <https://doi.org/10.1021/jp5053858> (2014).
19. Tyagi, B., Chudasama, C. D. & Jasra, R. V. Determination of structural modification in acid-activated montmorillonite clay by FT-IR spectroscopy. *Spectrochim Acta Mol. Biomol. Spectrosc.* **64**, 273–278. <https://doi.org/10.1016/j.saa.2005.07.018> (2006).
20. Challagulla, S., Tarafder, K., Ganesan, R. & Roy, S. Structure-sensitive photocatalytic reduction of nitroarenes over TiO₂. *Sci. Rep.* **7**, 8783. <https://doi.org/10.1038/s41598-017-08599-2> (2017).
21. Taudul, B., Tielens, F. & Calatayud, M. On the origin of Raman activity in anatase TiO₂ (nano)materials: An ab initio investigation of surface and size effects. *Nanomaterials (Basel)*. **13**, 1856. <https://doi.org/10.3390/nano13121856> (2023).
22. Sacco, A. et al. Quantification of titanium dioxide (TiO₂) anatase and rutile polymorphs in binary mixtures by Raman spectroscopy: An interlaboratory comparison. *Metrologia* **60**, 055011. <https://doi.org/10.1088/1681-7575/ace2df> (2023).
23. Bernardini, S. et al. Morpho-mineralogical and bio-geochemical description of cave manganese stromatolite-like patinas (Grotta del Cervo, Central Italy) and hints on their paleohydrological-driven genesis. *Front. Earth Sci.* **9**, 642667. <https://doi.org/10.3389/feart.2021.642667> (2021).
24. Schulz, H. M., Wirth, R. & Schreiber, A. Nano-crystal formation of TiO₂ polymorphs brookite and anatase due to organic-inorganic rock-fluid interactions. *J. Sediment. Res.* **86**, 59–72. <https://doi.org/10.2110/jsr.2016.1> (2016).
25. Adar, F. Carotenoids - Their resonance Raman spectra and how they can be helpful in characterizing a number of biological systems. *Spectroscopy* **32**, 12–20 (2017).
26. Jehlička, J. et al. Potential and limits of Raman spectroscopy for carotenoid detection in microorganisms: Implications for astrobiology. *Philos. Trans. Math. Phys. Eng. Sci.* **372**, 20140199. <https://doi.org/10.1098/rsta.2014.0199> (2014).

27. Brown, S. D. et al. Black Sea sapropels: Relationship to kerogens and fossil fuel precursors. *Fuel* **79**, 1725–1742. [https://doi.org/10.1016/S0016-2361\(00\)00031-4](https://doi.org/10.1016/S0016-2361(00)00031-4) (2000).
28. Baqué, M. et al. Carotenoid Raman signatures are better preserved in dried cells of the desert cyanobacterium *Chroococcidiopsis* than in hydrated counterparts after high-dose gamma irradiation. *Life* **10**, 83. <https://doi.org/10.3390/life10060083> (2020).
29. Udensi, J., Loughman, J., Loskutova, E. & Byrne, H. J. Raman spectroscopy of carotenoid compounds for clinical applications - A review. *Molecules* **27**, 9017. <https://doi.org/10.3390/molecules27249017> (2022).
30. Schito, A., Muirhead, D. K. & Parnell, J. Towards a kerogen-to-graphite kinetic model by means of Raman spectroscopy. *Earth-Sci. Rev.* **237**, 104292. <https://doi.org/10.1016/j.earscirev.2022.104292> (2023).
31. Wu, D., Wang, C., Liang, W. & Gao, X. Combining Fourier transform infrared and Raman spectroscopy to characterize kerogen molecular structures: Insights into their thermal maturity. *Int. J. Mol. Sci.* **26**, 2696. <https://doi.org/10.3390/ijms26062696> (2025).
32. Khatibi, S., Ostadhassan, M., Tuschel, D., Gentzis, T. & Carvajal-Ortiz, H. Evaluating molecular evolution of kerogen by Raman spectroscopy: Correlation with optical microscopy and Rock-Eval pyrolysis. *Energies* **11**, 1406. <https://doi.org/10.3390/en11061406> (2018).
33. Spötl, C., Houseknecht, D. W. & Jaques, R. C. Kerogen maturation and incipient graphitization of hydrocarbon source rocks in the Arkoma Basin, Oklahoma and Arkansas: A combined petrographic and Raman spectrometric study. *Org. Geochem.* **28**, 535–542. [https://doi.org/10.1016/S0146-6380\(98\)00021-7](https://doi.org/10.1016/S0146-6380(98)00021-7) (1998).
34. Rossi, V., Unitt, R. & McNamara, M. A new non-destructive method to decipher the origin of organic matter in fossils using Raman spectroscopy. *RSC Adv.* **14**, 26747–26759. <https://doi.org/10.1039/d4ra04364b> (2024).
35. Schito, A., Romano, C., Corrado, S., Grigo, D. & Poe, B. Diagenetic thermal evolution of organic matter by Raman spectroscopy. *Org. Geochem.* **106**, 57–67. <https://doi.org/10.1016/j.orggeochem.2016.12.006> (2017).
36. Chabalala, V., Wagner, N. & Malumbazo, N. Application of organic petrology and Raman spectroscopy in thermal maturity determination of the Karoo Basin (RSA) shale samples. *Minerals* **13**, 1199. <https://doi.org/10.3390/min13091199> (2023).
37. Aramendia, J. et al. A new methodology for kerogen maturity estimation based on Raman spectroscopy and chemometric analysis. *Sci. Total Environ.* **887**, 164056. <https://doi.org/10.1016/j.scitotenv.2023.164056> (2023).
38. Tsuji, M., Kuboyama, S., Matsuzaki, T. & Tsuji, T. Formation of hydrogen-capped polyynes by laser ablation of C60 particles suspended in solution. *Carbon* **41**, 2141–2148. [https://doi.org/10.1016/S0008-6223\(03\)00241-0](https://doi.org/10.1016/S0008-6223(03)00241-0) (2003).
39. Romei, M. G. et al. Frequency changes in terminal alkynes provide strong, sensitive, and solvatochromic Raman probes of biochemical environments. *J. Phys. Chem. B.* **127**, 85–94.

- <https://doi.org/10.1021/acs.jpcb.2c06176> (2023).
40. Agarwal, N. R. et al. Structure and chain polarization of long polyynes investigated with infrared and Raman spectroscopy. *J. Raman Spectrosc.* **44**, 1398–1410. <https://doi.org/10.1002/jrs.4300> (2013).
 41. Nyquist, R. A. Alkenes and other compounds containing C = C double bonds. In *Interpreting Infrared, Raman, and Nuclear Magnetic Resonance Spectra* 55–91 Academic Press, (2001).
<https://doi.org/10.1016/B978-012523475-7/50168-6>
 42. Gieleciak, R. et al. Exploring the potential of Raman spectroscopy for characterizing olefins in olefin-containing streams. *Energy Fuels.* **37**, 13698–13709.
<https://doi.org/10.1021/acs.energyfuels.3c02014> (2023).
 43. Volkov, D. S., Rogova, O. B. & Proskurnin, M. A. Temperature dependences of IR spectra of humic substances of brown coal. *Agronomy* **11**, 1822. <https://doi.org/10.3390/agronomy11091822> (2021).
 44. Kök, M. V., Varfolomeev, M. A. & Nurgaliev, D. K. TGA and DSC investigation of different clay mineral effects on the combustion behavior and kinetics of crude oil from Kazan region, Russia. *J. Pet. Sci. Eng.* **200**, 108364. <https://doi.org/10.1016/j.petrol.2021.108364> (2021).
 45. Derkowski, A. & Kuligiewicz, A. Thermal analysis and thermal reactions of smectites: A review of methodology, mechanisms, and kinetics. *Clays Clay Min.* **70**, 946–972.
<https://doi.org/10.1007/s42860-023-00222-y> (2022).
 46. Michalska, J., Turek-Szytow, J., Dudło, A., Kowalska, K. & Surmacz-Górska, J. Evaluation of the applicability of selected analytical techniques for determining the characteristics of humic substances sourced from by-products of the wastewater treatment process. *Sci. Total Environ.* **888**, 164237. <https://doi.org/10.1016/j.scitotenv.2023.164237> (2023).
 47. Albrecht, R., Le Petit, J., Terrom, G. & Périsol, C. Comparison between UV spectroscopy and NIRS for assessing humification during co-composting of sewage sludge and green waste. *Bioresour Technol.* **102**, 4495–4500. <https://doi.org/10.1016/j.biortech.2010.12.053> (2011).
 48. Li, L., Ma, L., Lu, Y., Wang, Y. & Sun, S. Spectroscopic analysis of the effects of alkaline extractants on humic acids isolated from herbaceous peat. *Spectroscopy* **39**, 20–25.
<https://doi.org/10.56530/spectroscopy.wk3774u5> (2024).
 49. Ahmed, Z., Yusoff, M. S., Mokhtar Kamal, N. H., Aziz, A., Roulia, M. & H. & Spectroscopic and microscopic analysis of humic acid isolated from stabilized leachate HSs fractionation. *Agronomy* **13**, 1160. <https://doi.org/10.3390/agronomy13041160> (2023).
 50. Sirotiak, M., Pastierová, A. & Blinová, L. Study into the changes in soil organic matter, caused by laboratory simulated fires in four Slovak soils: 1. UV-VIS spectrum analysis. *Res. Pap Fac. Mater. Sci. Technol. Slovak Univ. Technol.* **29**, 131–142. <https://doi.org/10.2478/rput-2021-0014> (2021).
 51. Hur, J., Jung, K. Y. & Jung, Y. M. Characterization of spectral responses of humic substances upon UV irradiation using two-dimensional correlation spectroscopy. *Water Res.* **45**, 2965–2974.
<https://doi.org/10.1016/j.watres.2011.03.013> (2011).
 52. Zornoza, R. et al. Near infrared spectroscopy for determination of various physical, chemical and biochemical properties in Mediterranean soils. *Soil. Biol. Biochem.* **40**, 1923–1930.

<https://doi.org/10.1016/j.soilbio.2008.04.003> (2008).

53. Caredda, M., Dedola, A. S., Pes, M. & Addis, M. The use of NIR spectroscopy and chemometrics to identify the thermal treatment of milk in Fiore Sardo PDO cheese to detect fraud. *Foods* **14**, 2288. <https://doi.org/10.3390/foods14132288> (2025).

Figures

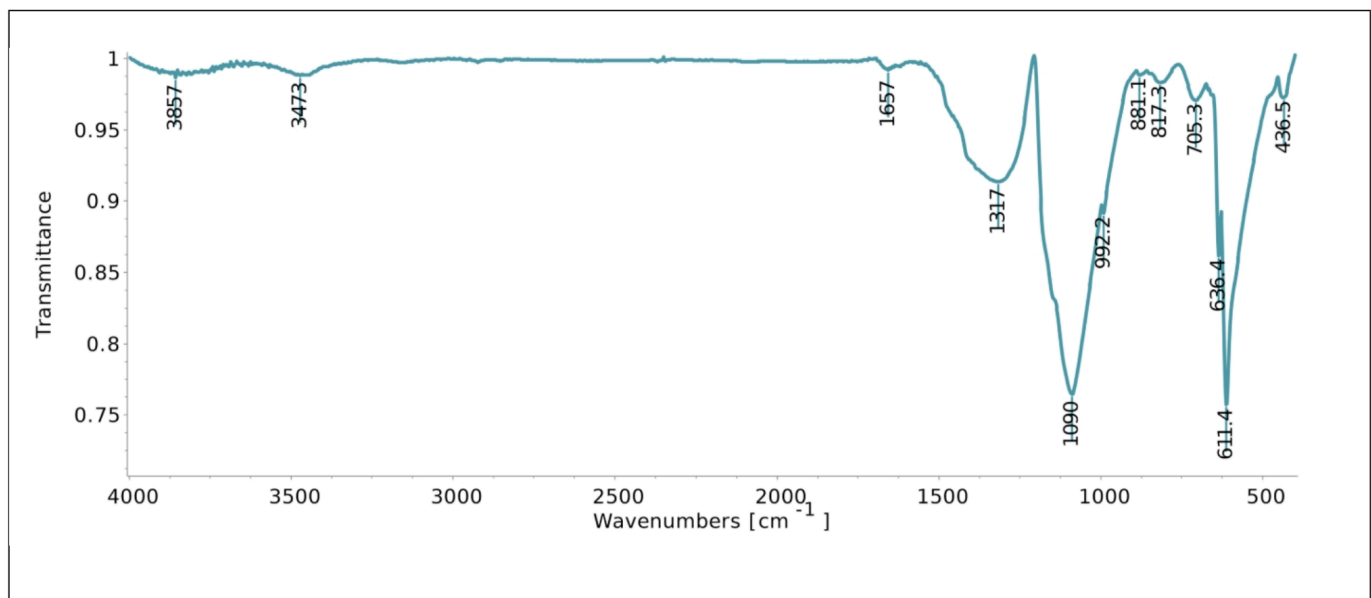


Figure 1

FT-IR analysis of the PELLAMAR extract. The analysis demonstrates a mineral-dominated composition characterized by strong Si–O–Si stretching vibrations ($\sim 1090\text{ cm}^{-1}$) indicative of quartz, carbonate bands ($\sim 1317\text{ cm}^{-1}$) consistent with calcite, and sulfate bending modes (~ 636 and 611 cm^{-1}) confirming the presence of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). Broad O–H stretching bands ($3857\text{--}3473\text{ cm}^{-1}$) and H–O–H bending ($\sim 1657\text{ cm}^{-1}$) reflect significant hydration and a colloidal matrix. Minor contributions from organic functional groups suggest the presence of humic substances, supporting a mixed organic-mineral structure typical of sapropelic materials.

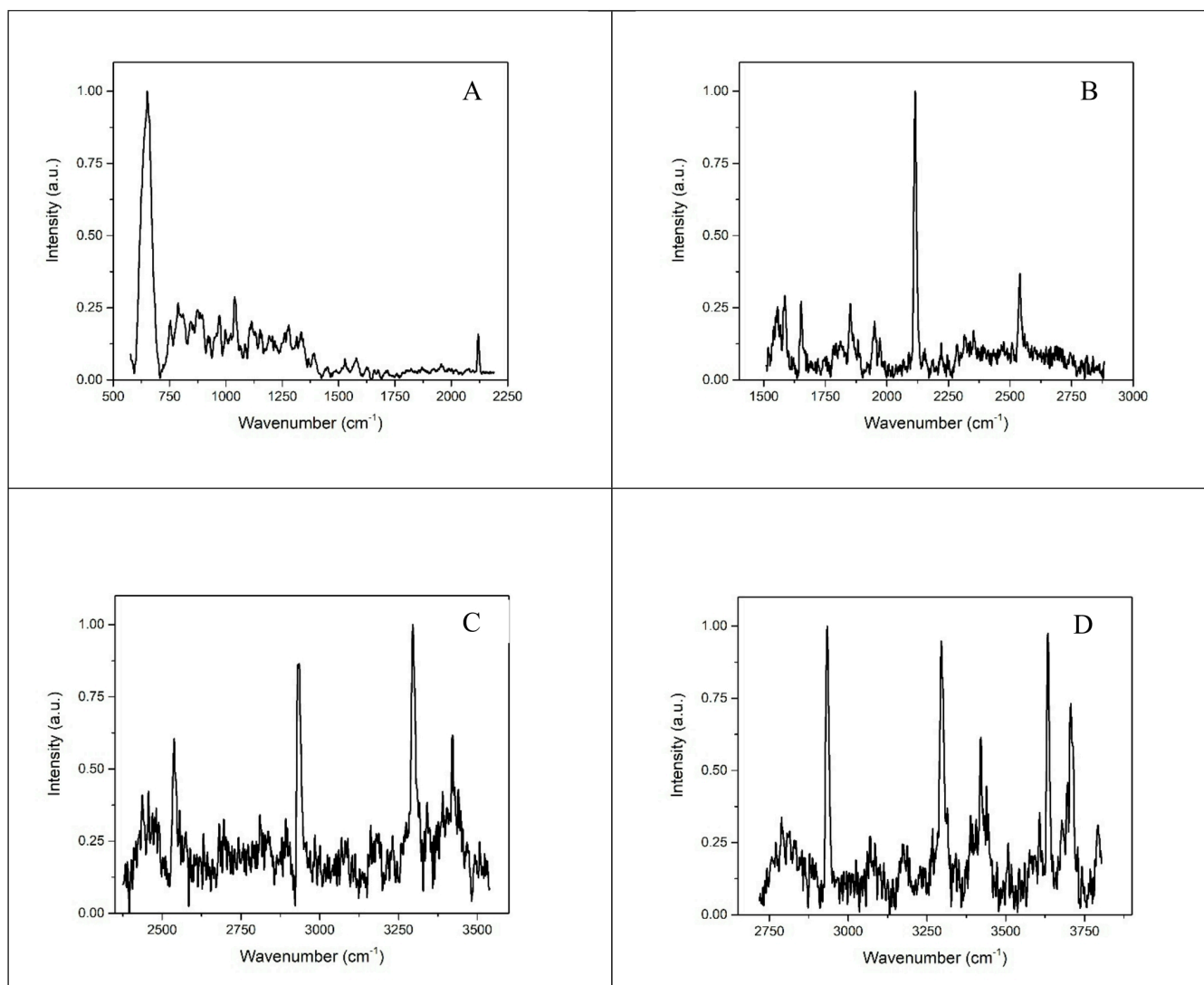


Figure 2

Raman spectroscopic analysis of the PELLAMAR sapropelic mud extract at selected Raman shift regions. 1450 (A); 2250 (B); 3000 (C); 3300 (D).

(A) The low-wavenumber region demonstrates prominent mineral-associated bands, including strong signals compatible with silicate, sulfate, and carbonate structures characteristic of quartz-, gypsum-, and calcite-containing phases. (B) The intermediate spectral region reveals additional inorganic and carbon-associated vibrational modes, suggesting a heterogeneous organic–mineral matrix with structurally complex components. (C) The 2400–3500 cm^{-1} region shows pronounced aliphatic C–H stretching bands near 2900–3000 cm^{-1} , consistent with lipid-rich organic matter and kerogen-like material, together with broad hydroxyl-associated features. (D) The high-wavenumber region demonstrates intense O–H stretching bands between approximately 3300 and 3700 cm^{-1} , reflecting hydrated mineral phases, structurally bound water, and hydroxylated humic substances. Collectively, the Raman spectra support

the presence of a complex sapropelic matrix composed of mixed inorganic mineral phases and biologically derived organic constituents relevant to therapeutic pelotherapy applications.

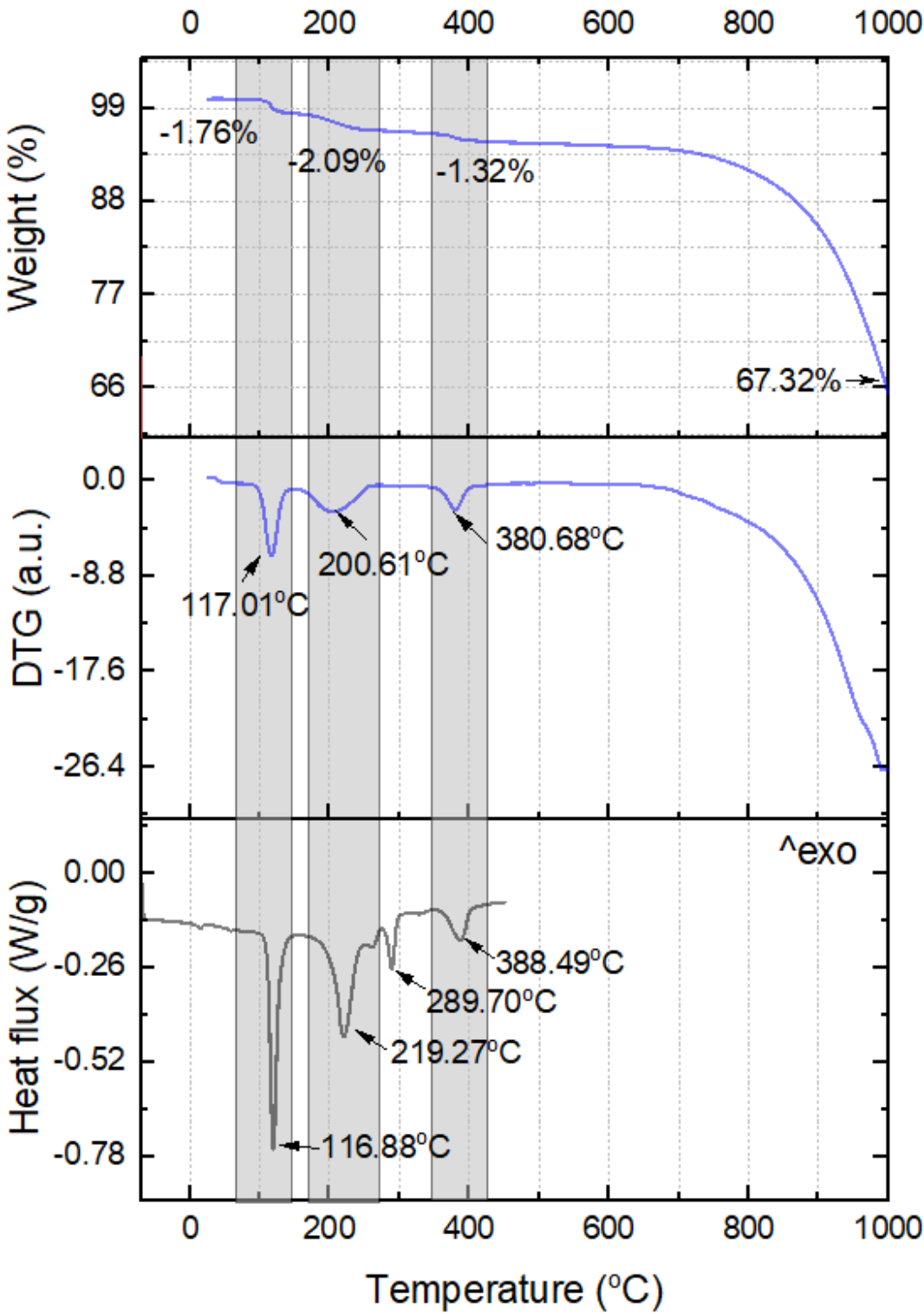


Figure 3

Thermogravimetric Analysis (TGA), Derivative Thermogravimetry (DTG), and Differential Scanning Calorimetry (DSC) of the PELLAMAR sapropelic mud extract. These techniques characterize moisture

content, thermal stability, mineral transformations, and decomposition behavior.

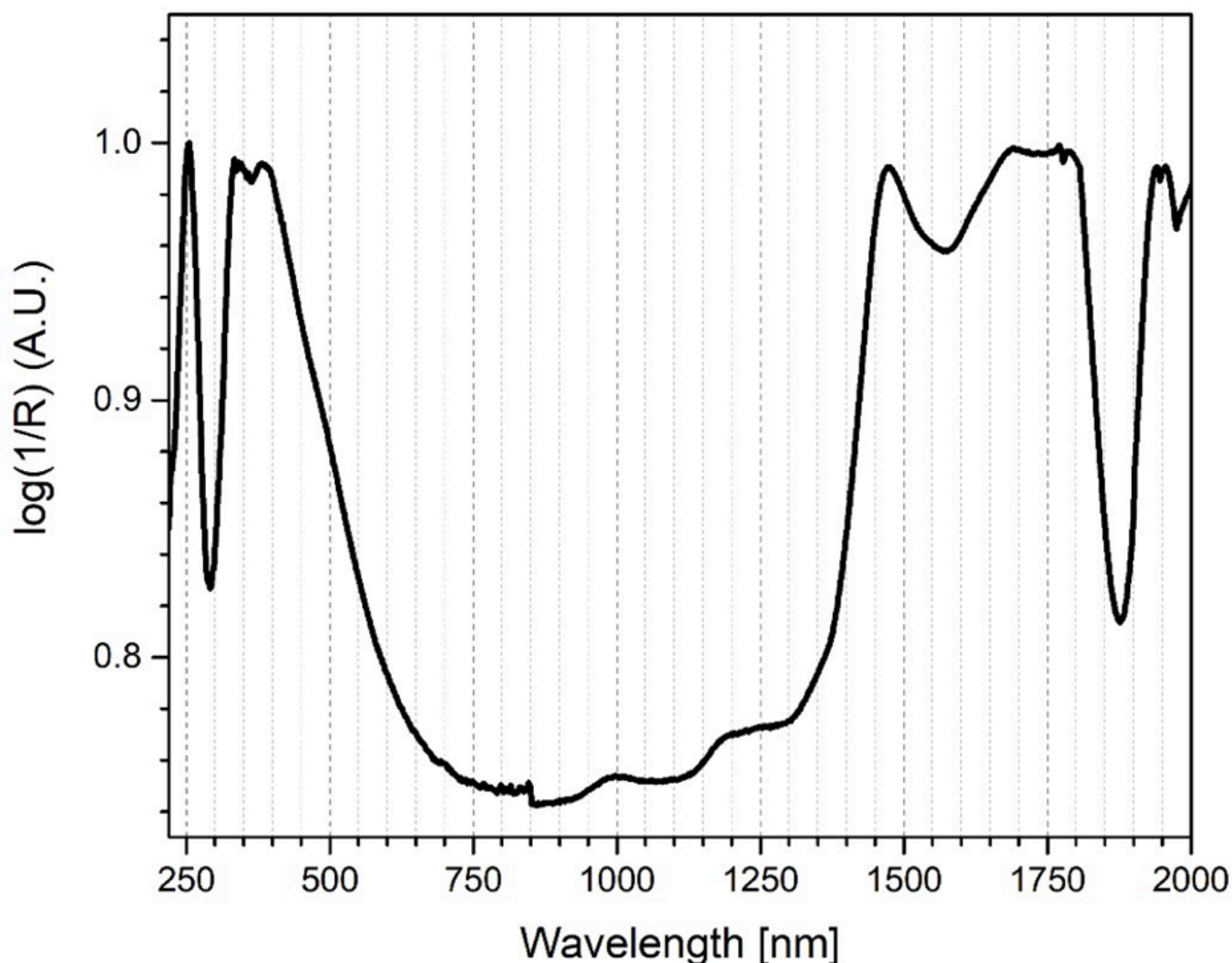


Figure 4

Diffuse reflectance UV-Vis-NIR spectrum of the PELLAMAR sapropelic extract powder. The diffuse reflectance UV-Vis-NIR spectrum, presented as normalized $\log(1/R)$ pseudo-absorbance after baseline correction, demonstrates the complex optical behavior of the sapropelic extract across the 250–2000 nm range. Strong ultraviolet absorption features are consistent with aromatic organic compounds, conjugated carbon structures, and humic-like substances. The gradual spectral profile throughout the visible region reflects the heterogeneous organic–mineral composition of the extract. In contrast, broad absorption bands in the near-infrared region are compatible with hydroxyl groups, structurally bound water, and mineral-associated hydration. Additional NIR features may reflect overtones and combination bands associated with O–H, C–H, and carbonate-containing structures. Collectively, the spectrum supports the presence of a hydrated, structurally complex sapropelic matrix composed of both mineral and bioorganic constituents relevant to peliotherapeutic applications.