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ADVANCING ASPHALTUM PUNJABIANUM SHILAJIT THERAPEUTICS: A COMPREHENSIVE STRATEGY FOR CHEMO-STANDARDIZATION, SAFETY VALIDATION, AND BIOAVAILABILITY ENHANCEMENT VIA A NOVEL PHOSPHOLIPID COMPLEX

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ABSTRACT

Asphaltum punjabianum Shilajit, a revered substance in Ayurveda, faces significant challenges in its transition to modern therapeutics due to quality variability, safety concerns, and poor bioavailability. This project aimed to address these limitations through a comprehensive strategy involving chemo-standardization, safety validation and bioavailability enhancement. Fifteen Shilajit batches from different geographical regions were analyzed using FTIR, HPTLC and a newly developed and validated HPLC method for quantification of key biomarkers like dibenzo-α-pyrone and fulvic acid. ICP-MS confirmed that purified batches contained heavy metals below WHO/USP safety limits. To overcome bioavailability issues, a novel Shilajit-phospholipid complex was developed, which demonstrated a ~3.5-fold increase in lipophilicity and a remarkable enhancement in *in vitro* dissolution (>85% release in 4 hours vs. 35% for native Shilajit). The complex showed optimal nanometric particle size (215.4 nm), high stability (Zeta potential: -32.6 mV), and remained stable under accelerated storage conditions. This research establishes robust standardization protocols and introduces an advanced delivery system for Shilajit, effectively bridging traditional use with evidence-based modern medicine and paving the way for its reliable therapeutic application.

1. INTRODUCTION

Shilajit (Asphaltum punjabianum), a tar-like exudate obtained from high-altitude rock crevices of mountain ranges such as the Himalayas, Altai, Caucasus and Pamir, is one of the most revered rasayana (rejuvenative) substances in Ayurvedic medicine.[1] It has been traditionally consumed for centuries across India, Nepal, Tibet, Russia and neighbouring regions as a general tonic used for enhancing vitality, longevity and resistance to disease.[2] Modern interest in Shilajit has resurged due to increasing global demand for evidence-based herbal and mineral supplements that can complement conventional therapeutics.[3] Chemically, Shilajit is a highly complex phytomineral matrix formed over long periods through the microbial degradation and humification of specific plant materials under unique geoclimatic conditions.[4] Its organic fraction is dominated by humic substances (fulvic acid, humic acid and humins), which together account for approximately 60–80% of the material and are accompanied by a wide spectrum of non-humic compounds including dibenzo-α-pyrones (DAPs) and their chromoprotein complexes, organic acids, polyphenols, amino acids, lipids, sterols and small peptides.[5] The inorganic fraction comprises more than 80 minerals and trace elements such as selenium, magnesium, iron and zinc, typically present in ionic or chelated forms that may favour biological uptake. Among these constituents, fulvic acid is widely regarded as the principal bioactive carrier molecule due to its low molecular weight, high solubility and strong chelating and antioxidant properties, while DAPs are considered key pharmacological markers associated with cognitive and energy-enhancing effects.[6]

A growing body of experimental and clinical evidence has begun to substantiate several of Shilajit's ethnomedicinal claims. In vitro and in vivo studies report antioxidant, anti-inflammatory, immunomodulatory, antidiabetic, anti-ulcerogenic, antifungal and analgesic activities, as well as potential neuroprotective actions linked to inhibition of tau protein aggregation by fulvic acid.[7] Clinical investigations further suggest that Shilajit

supplementation can improve muscle strength, endurance and post-exercise recovery, reduce fatigue and support bone health in selected populations, alongside reported benefits for male reproductive parameters. Despite these promising findings, significant methodological limitations and heterogeneity across studies have impeded definitive conclusions and delayed broader regulatory acceptance.[8]

One of the major obstacles to the rational therapeutic use of Shilajit is its pronounced variability in composition across different geographical origins, collection periods and processing methods. Differences in altitude, flora, microbial consortia and environmental conditions lead to marked batch-to-batch variation in humic content, fulvic acid levels, DAP concentration and mineral profile, which in turn may influence both efficacy and safety.[9] At the same time, the global market is flooded with products of uncertain provenance, often with inadequate labelling or authentication, making it difficult for clinicians and consumers to rely on consistent quality. This complex and dynamic composition poses a serious challenge for standardization, quality control and regulatory oversight.

Safety is an equally critical concern. Raw or improperly processed Shilajit may contain substantial levels of toxic heavy metals (such as lead, mercury, arsenic, cadmium and thallium), mycotoxins, microbial contaminants and extraneous inorganic material derived from the surrounding rock matrix.[10] Recent analyses have reported that some commercial supplements may even show higher levels of certain toxic elements than the corresponding raw material, highlighting deficiencies in purification protocols and manufacturing practices. In the absence of harmonized pharmacopoeial standards, there is an urgent need for robust, validated analytical workflows that can authenticate genuine Shilajit, quantify key biomarkers such as fulvic acid and DAPs, and rigorously assess contamination against international safety limits (WHO/USP).[11]

Beyond issues of quality and safety, Shilajit's therapeutic translation is further constrained by its unfavourable biopharmaceutical properties. The native resin is sticky, poorly water-soluble and chemically heterogeneous, factors that can limit dissolution, membrane permeation and oral bioavailability of its active constituents.[12] Conventional dosage forms based on crude or minimally processed Shilajit may therefore deliver suboptimal or highly variable systemic exposure, undermining its clinical potential. Advanced drug-delivery strategies that enhance solubility, protect labile constituents and improve absorption are therefore essential to fully realize the benefits suggested by preclinical and clinical studies.[3]

Phospholipid-based delivery systems, such as phytosomes and drug–phospholipid complexes, have emerged as a promising approach to improve the biopharmaceutical performance of poorly soluble phytoconstituents and herbal extracts. By forming molecular complexes with phosphatidylcholine, bioactive molecules can acquire amphiphilic character, exhibit increased lipophilicity in biorelevant media, and demonstrate enhanced membrane affinity and stability, without the need for synthetic surfactants. Applying this concept to Shilajit offers a rational strategy to address both its solubility constraints and the need for a more reproducible, scalable formulation platform.[13]

In this context, the present study was designed as an integrated pipeline to advance Asphaltum punjabianum Shilajit from a traditional rasayana to a more standardized and pharmaceutically acceptable therapeutic candidate. Fifteen Shilajit batches from diverse geographical regions were first subjected to a comprehensive chemo-standardization workflow using FTIR, HPTLC and a newly developed and validated HPLC method for the quantification of fulvic acid and DAP-type biomarkers, alongside ICP-MS-based heavy metal profiling to ensure compliance with WHO/USP safety limits. The best-performing batch was then used to develop a novel Shilajit–phospholipid complex via solvent evaporation, which was extensively characterized for physicochemical properties, in vitro dissolution and accelerated stability. By combining rigorous quality and safety assessment with an advanced delivery system in a single study, this work aims to provide a comprehensive framework for the development of safe, effective and bioavailability-enhanced Shilajit-based therapeutics that can bridge traditional use and modern evidence-based medicine.

2. MATERIALS AND METHODS

2.1. Materials

Crude Shilajit (Asphaltum punjabianum) raw material from multiple geographical regions (India, Nepal, Russia, Iran and Kyrgyzstan) was procured through a commercial vendor (Total Trading Solutions, New Delhi, India). Analytical grade methanol, ethanol, chloroform, n-hexane, ethyl acetate, acetic acid, orthophosphoric acid and other solvents were used as received. Reference standards of fulvic acid and representative dibenzo-α-pyrone (DAP) derivatives were employed for chromatographic calibration and method validation. Simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4) were prepared using standard compositions. Soya phosphatidylcholine was used for preparation of the Shilajit–phospholipid complex.

2.2. Procurement and preliminary authentication of Shilajit batches

For this comprehensive study, fifteen batches of Shilajit Raw material were procured from various geographical regions including India, from the Vendor Total Trading Solutions, New Delhi, Nepal, Russia, Iran and Kyrgyzstan. These samples were selected to represent the diversity of Shilajit available in the market and from

traditional sources. Each sample was subjected to rigorous authentication procedures including organoleptic evaluation (color, odor, texture), determination of physicochemical parameters (solubility, pH, ash value, loss on drying), and preliminary screening for contaminants.

The authentication process included traditional validation methods such as solubility testing (genuine Shilajit should be completely soluble in water), specific gravity determination and response to specific chemical reagents. Samples were also screened for heavy metal contamination using X-ray fluorescence (XRF) spectroscopy as an initial rapid screening.

2.3. Extraction and purification of Shilajit

Raw Shilajit resin was first cleaned to remove physically adherent impurities such as dirt, sand and rock fragments. For resin purification, the material was dissolved in purified water, passed through a multistage filtration system and subjected to chromatographic and ultraviolet treatment to eliminate insoluble debris, microbial contaminants and extraneous inorganic matter while preserving humic substances and bioactive constituents.

For preparation of Shilajit extract powder, finely ground Shilajit (40 g) was dispersed in aqueous media containing citric acid and subjected to controlled heating in a water or steam bath for several hours, followed by filtration to obtain supernatant fractions. Sequential extractions were combined, centrifuged to remove residual particulates and concentrated under reduced pressure (Fig. 1). The concentrated extract was then dried using a pilot-scale spray dryer to yield a free-flowing Shilajit extract powder, which was stored in a cool, dry place until further use.[14]

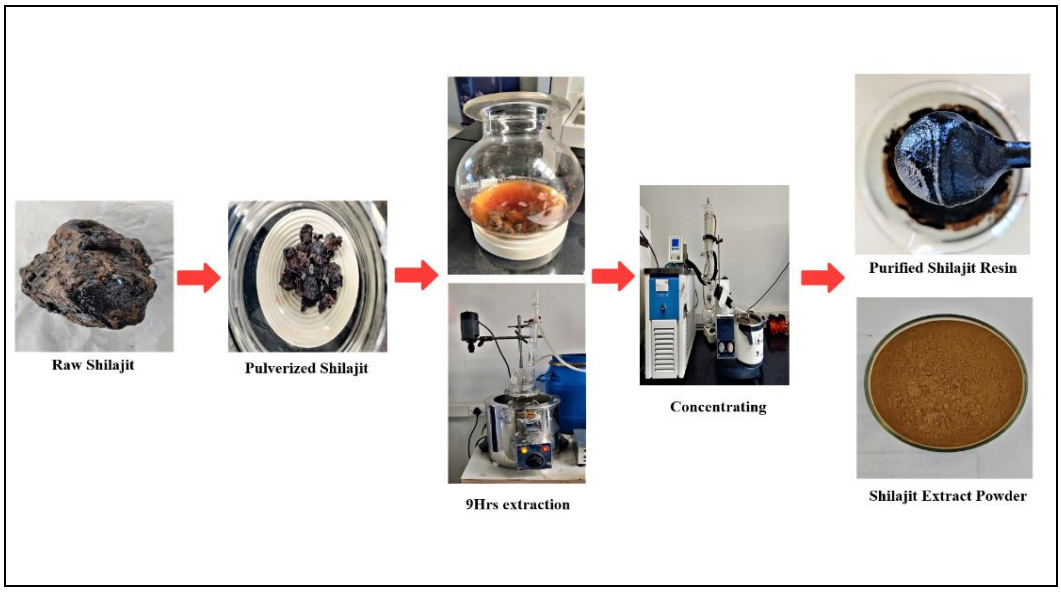

Fig. 1: Preparation of Shilajit extract powder.

2.4. FTIR spectroscopy for spectral fingerprinting

Fourier transform infrared spectroscopy was employed to obtain characteristic spectral fingerprints of different Shilajit batches. Samples were prepared using the KBr pellet method, and spectra were recorded over the range of 4000–400 cm^{-1}. The obtained spectra were analyzed for characteristic absorption bands corresponding to functional groups including O–H stretching, C–H stretching of aliphatic chains, C=O stretching, C–H bending, C–O stretching and C–O–C stretching. Comparative evaluation of spectral patterns among batches was carried out for preliminary authentication and detection of possible adulteration.[15]

2.5. HPTLC fingerprinting

HPTLC fingerprinting was carried out to establish the organic constituent profile of Shilajit. The sample was prepared by dissolving Shilajit in methanol at a concentration of 10 mg/mL, and 5 µL of the solution was applied as spots on silica gel F254 plates. Chromatographic separation was performed using a mobile phase consisting of n-Hexane: Ethyl Acetate: Acetic Acid (5:4:1, v/v), optimized for effective resolution of compounds. After plate development, visualization was carried out under UV light at 254 nm and 366 nm, followed by derivatization using anisaldehyde-sulphuric acid reagent for enhanced detection of organic constituents.[16]

2.6. HPLC method development and validation

A reversed-phase high-performance liquid chromatography (RP-HPLC) method was employed for the quantitative analysis of fulvic acid and DAP biomarkers in Shilajit samples. Chromatographic separation was carried out using a Phenomenex Luna C18 column (250 mm × 4.6 mm, 5 µm). The mobile phase consisted of solvent A containing 0.1% orthophosphoric acid in water and solvent B consisting

of methanol, delivered under gradient conditions of 40–85% B over 25 min. The flow rate was maintained at 1.0 mL/min with an injection volume of 20 µL and column temperature of 30 °C. Detection was performed using a photodiode array detector at 280 nm for DAP biomarkers and 254 nm for fulvic acid fractions. The developed method was validated according to ICH Q2(R1) guidelines by evaluating parameters such as linearity, precision, accuracy, limit of detection and limit of quantification.[17]

2.7. Heavy metal analysis by ICP-MS

To ensure safety, purified Shilajit batches were subjected to heavy metal analysis using inductively coupled plasma mass spectrometry (ICP-MS). Elements including lead, mercury, arsenic, cadmium, thallium and other relevant toxic metals were quantified, and the results were compared against World Health Organization (WHO) and United States Pharmacopeia (USP) limits for herbal products. Only batches with heavy metal concentrations below these safety thresholds were considered suitable for further development.[16]

2.8. Preparation of Shilajit–phospholipid complex

To enhance bioavailability, a Shilajit–phospholipid complex (phytosome) was prepared using a solvent evaporation technique. Shilajit extract and soya phosphatidylcholine were accurately weighed in a 1:1 mass ratio and dissolved in chloroform. The mixture was refluxed for 2 h under controlled conditions to facilitate complex formation between Shilajit constituents and phospholipids. The solvent was then evaporated under reduced pressure, and the resulting dry complex was scraped, collected and stored in a desiccator until further evaluation.[18]

2.9. Physicochemical characterization of the complex

Complex formation was confirmed by FTIR spectroscopy by comparing the spectra of Shilajit extract, phospholipid, their physical mixture and the final complex. Shifts in characteristic absorption bands, particularly in O–H and P=O regions, were interpreted as evidence of molecular interactions between Shilajit components and phosphatidylcholine.

The solubility of the Shilajit–phospholipid complex was evaluated in water and n-octanol and compared with that of native Shilajit extract to assess enhancement in lipophilicity. Particle size, polydispersity index and zeta potential of the complex were determined using a particle size analyser, and the complex exhibited nanometric size (~215 nm) with a zeta potential around −32.6 mV, indicating colloidal stability.[19]

2.10. In vitro drug release studies

In vitro release of Shilajit biomarkers from the phospholipid complex was studied using the dialysis bag method in simulated gastrointestinal media. Accurately weighed amounts of the complex and an equivalent dose of native Shilajit extract were each placed in dialysis bags and immersed in SGF (pH 1.2) and SIF (pH 7.4) maintained at 37 ± 0.5 °C under constant stirring. At predetermined time intervals, aliquots of the dissolution medium were withdrawn and replaced with fresh medium to maintain sink conditions. Samples were filtered and analysed using the validated HPLC method to quantify the release of key biomarkers (e.g. DAP and fulvic acid equivalents). Cumulative percentage release was plotted against time and compared between native extract and phospholipid complex, and the release data were fitted to kinetic models such as the Higuchi model to elucidate the mechanism of release.[20]

2.11. Accelerated and refrigerated stability studies

Short-term accelerated stability studies of the optimized Shilajit–phospholipid complex were carried out according to ICH Q1A(R2) guidelines. Samples were stored at accelerated conditions of 40 ± 2 °C/75 ± 5% relative humidity and under refrigerated conditions at 5 ± 3 °C for a period of 3 months. Stability samples were evaluated at predetermined intervals of 0, 1, 2 and 3 months for physical characteristics including colour, odour, texture and ease of redispersion, along with chemical assay by HPLC for DAP content and in vitro dissolution profile at 4 h.[21]

2.12. Chemometric and statistical analysis

All experimental measurements were performed in triplicate, and results are expressed as mean ± standard deviation. Statistical comparisons between groups were carried out using analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons, with $p < 0.05$ considered statistically significant. Chemometric techniques, including principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA), were applied to FTIR and chromatographic datasets to explore multivariate relationships, classify Shilajit samples according to geographical origin and quality and evaluate the discriminatory power of the combined analytical workflow.

3. RESULT AND DISCUSSION

3.1. FTIR analysis

The FTIR analysis revealed characteristic absorption bands that provided a distinctive fingerprint for authentic Shilajit (Fig .2 & Fig .3). All batches showed consistent peaks at ~3400 cm^{-1} (O-H stretching of phenolic and carboxylic groups), ~2920 cm^{-1} & 2850 cm^{-1} (C-H stretching of aliphatic chains), ~1600 cm^{-1} (C=O stretching of quinones and carboxylic acids), and ~1450 cm^{-1} (C-H bending) (Table 1).

The high degree of spectral similarity between batches indicated consistent functional group composition, providing a preliminary method for authentication and detection of adulterants.

Table 1: Characteristic FTIR Absorption Bands of Authentic Shilajit.

Wave Number (cm^{-1})	Assignment	Intensity
~3400	O-H stretching	Strong, broad
~2920 & 2850	C-H stretching	Medium
~1600	C=O stretching	Strong
~1450	C-H bending	Medium
~1230	C-O stretching	Medium
~1050	C-O-C stretching	Weak

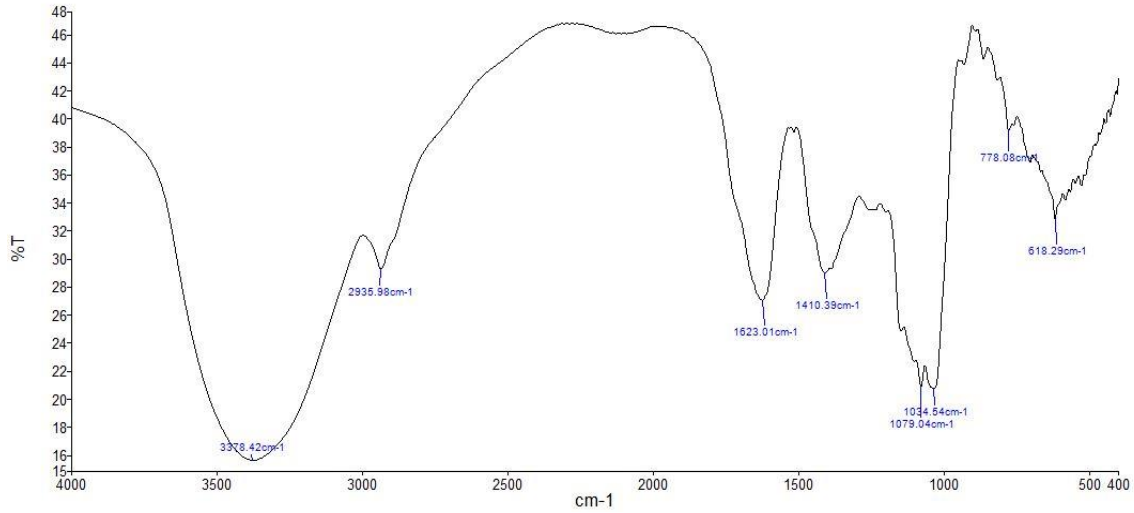

Fig. 2: FT-IR Finger print of Raw Shilajit.

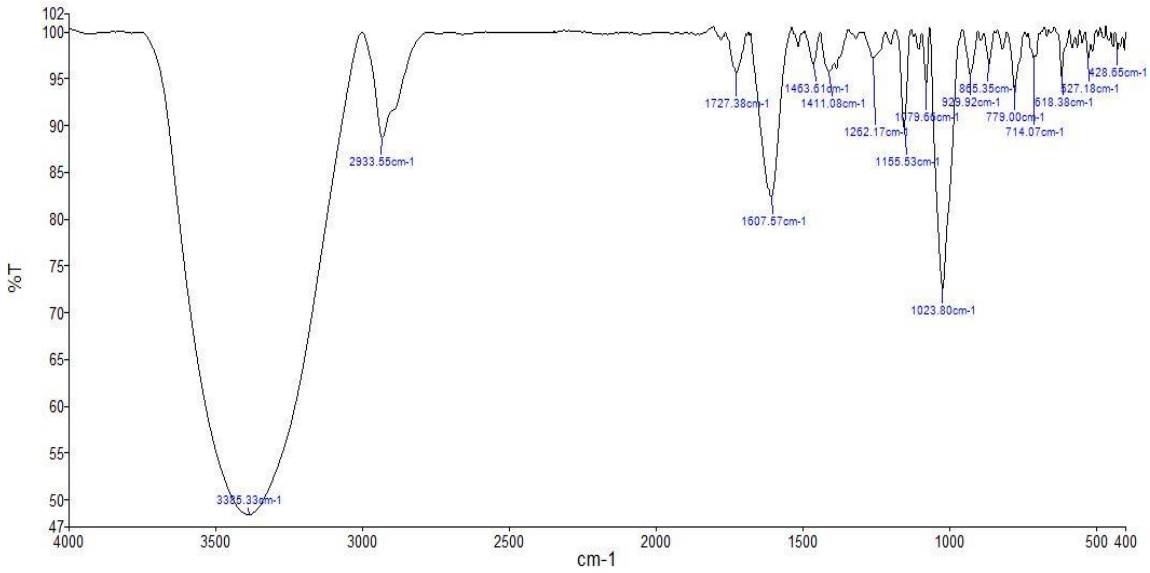

Fig. 3: FT-IR Finger print of Purified Shilajit.

3.2. HPTLC fingerprinting

HPTLC chromatograms provided distinctive profiles of organic constituents, with multiple bands corresponding to humic substances and non-humic components. After derivatization and UV visualization, a prominent band at Rf ≈ 0.45 was consistently observed in all genuine samples and was attributed to dibenzo-α-pyrone (DAP) type compounds based on comparison with standards and literature. The reproducible presence of this band, along with the overall pattern of quenching and coloured zones at 254 and 366 nm, offered a rapid qualitative method for batch-to-batch comparison and screening of suspect material.

3.3. HPLC quantification of DAPs and fulvic acid

The developed RP-HPLC method on a C18 column with a methanol/0.1% orthophosphoric acid gradient successfully resolved fulvic acid and key DAP biomarkers, including 3,8-dihydroxydibenzo-α-pyrone with a retention time of 12.5 ± 0.2 min. Validation according to ICH Q2(R1) demonstrated excellent linearity (R^2 ≈ 0.999), precision (RSD < 2%) and

accuracy (recovery 98–102%), confirming suitability for quantitative standardization. Application to representative batches showed DAP contents ranging from 3.2 ± 0.15 mg/g (Russia, S-3) to 4.5 ± 0.09 mg/g (Nepal, S-2), with Indian and Kyrgyz samples at 3.8 ± 0.12 and 4.2 ± 0.13 mg/g respectively.

3.4. Fulvic acid content and batch selection

Total fulvic acid content, quantified using the same HPLC method, varied between 62.5 ± 2.5% and 75.8 ± 2.1% w/w across batches. Batch S-2 (Nepal) exhibited the highest levels of both DAP (4.5 mg/g) and fulvic acid (75.8%), and a moderate positive correlation (R ≈ 0.72) was observed between these markers, indicating that fulvic-rich batches tended to have higher DAP content. On this basis, S-2 was selected as the primary raw material for subsequent phospholipid complex formulation.

Table 2: Content of Key Biomarkers in Different Shilajit Batches.

Batch Code	Geographical Origin	Dibenzo-α-pyrone Content (mg/g) ± SD	Total Fulvic Acid Content (% w/w) ± SD
S-1	India	3.8 ± 0.12	65.2 ± 1.8
S-2	Nepal	4.5 ± 0.09	75.8 ± 2.1
S-3	Russia	3.2 ± 0.15	62.5 ± 2.5
S-4	Iran	3.5 ± 0.11	68.4 ± 1.9
S-5	Kyrgyzstan	4.2 ± 0.13	71.3 ± 2.0

3.5. Heavy metal analysis and safety profiling

ICP-MS analysis confirmed that purified Shilajit batches S-1 (India), S-2 (Nepal) and S-5 (Kyrgyzstan) contained toxic metals well below WHO/USP limits for herbal products. For example, lead, arsenic, cadmium, mercury and thallium were all detected at sub-ppm levels (e.g. Pb ≈ 0.85–1.12 ppm vs 10 ppm limit; Cd ≈ 0.08–0.11 ppm vs 0.5 ppm limit) (Table 3). These results indicate that the purification process effectively removed rock-derived contaminants and that safety concerns surrounding Shilajit are largely attributable to inadequate processing rather than intrinsic toxicity of the material.

Table 3: Heavy Metal Content in Shilajit Batches (ICP-MS Analysis).

Heavy Metal	Permissible Limit (WHO/USP) (ppm)	Batch S-1 (India) (ppm)	Batch S-2 (Nepal) (ppm)	Batch S-5 (Kyrgyzstan) (ppm)
Lead (Pb)	10.0	0.85 ± 0.12	1.12 ± 0.09	0.92 ± 0.11
Arsenic (As)	5.0	1.38 ± 0.15	0.95 ± 0.08	2.10 ± 0.20
Cadmium (Cd)	0.5	0.08 ± 0.01	0.11 ± 0.02	0.09 ± 0.01
Mercury (Hg)	1.0	0.05 ± 0.01	0.03 ± 0.01	0.07 ± 0.01
Thallium (Tl)	1.0	0.22 ± 0.03	0.15 ± 0.02	0.18 ± 0.03

3.6. FTIR evidence of complexation

The Shilajit–phospholipid complex prepared by solvent evaporation appeared as a brown, free-flowing powder distinct from the sticky native resin. FTIR spectra of the complex exhibited broadening and shifting of the O–H stretching band of Shilajit (~3400 cm^{-1}) and a shift of the phospholipid P=O stretching band from 1236 to 1252 cm^{-1}, indicating formation of hydrogen bonds and polar interactions between Shilajit constituents and phosphatidylcholine. These spectral changes confirmed formation of a true molecular complex rather than a simple physical mixture.

3.7. Particle size and zeta potential

Dynamic light scattering showed that the complex formed nanometric particles with a mean hydrodynamic diameter (Z-average) of 215.4 ± 5.8 nm and a polydispersity index of 0.241 ± 0.02, reflecting a relatively narrow size distribution. The zeta potential of −32.6 ± 1.5 mV indicated strong electrostatic repulsion between particles, suggesting good colloidal stability and low risk of aggregation during storage and dispersion (Table. 4).

Table 4: Particle Characterization of the Shilajit-Phospholipid.

Parameter	Result	Interpretation
Z-Average (d.nm)	215.4 ± 5.8 nm	Particle size in the nano metric range.
Polydispersity Index (PDI)	0.241 ± 0.02	Narrow and relatively monodisperse, size distribution.
Zeta Potential (mV)	-32.6 ± 1.5 mV	High electrostatic stability, low aggregation potential.

3.8. Solubility and lipophilicity enhancement

Equilibrium solubility studies demonstrated an approximately 3.5-fold increase in apparent solubility/lipophilicity of the complex in n-octanol compared with native Shilajit extract. This enhancement is consistent with the amphiphilic nature of the complex, in which the phospholipid envelope facilitates partitioning into lipid phases while maintaining adequate

dispersibility in aqueous media, and is expected to favour intestinal membrane permeation.

3.9. In vitro drug release behaviour

In simulated intestinal fluid (pH 7.4), the Shilajit–phospholipid complex achieved over 85% cumulative release of DAP within 4 h, whereas native Shilajit extract released only about 35% in the same period. This substantial improvement directly addresses the poor dissolution behaviour of native Shilajit and suggests potential for enhanced oral bioavailability of its active constituents. Release profiles for both formulations were best described by the Higuchi model, indicating diffusion-controlled release; however, the complex exhibited a significantly higher release rate constant, confirming the effectiveness of phospholipid complexation in accelerating release while retaining a diffusion-driven mechanism.

3.10. Stability studies of the complex

Accelerated stability studies at 40 °C/75% RH for 3 months showed that the Shilajit–phospholipid complex maintained its physical integrity and chemical assay within acceptable limits. DAP content remained between 95.8 and 100% of the initial value, and 4-h dissolution decreased only slightly from 85.2 ± 2.1% to 82.0 ± 2.5%, with only minor darkening of colour and no caking. Under refrigerated storage at 5 ± 3 °C, assay values (99.5–101.2% of initial) and dissolution profiles remained essentially unchanged, indicating excellent stability under recommended conditions. These findings suggest that the complex is suitable for development into solid oral dosage forms with a practical shelf life, pending longer term ICH-compliant studies.

3.11. Multivariate chemometric analysis

PCA and PLS-DA applied to FTIR and HPLC datasets successfully discriminated Shilajit samples according to geographical origin and quality grade. Data-fusion strategies that combined spectral and chromatographic features yielded improved classification performance, with PLS-DA achieving a test-set accuracy of approximately 0.94 when fused data were used. This demonstrates that multivariate modelling can add a powerful layer of quality assurance on top of conventional univariate specifications for complex natural materials like Shilajit.

4. CONCLUSION

The present work establishes an integrated bridge for advancing Asphaltum punjabianum Shilajit from a traditional rasayana to a pharmaceutically acceptable therapeutic candidate by systematically addressing its three major translational barriers: compositional variability, safety concerns and poor biopharmaceutical properties. A combined FTIR–HPTLC–HPLC workflow enabled robust chemo-standardization of multi-origin Shilajit samples, quantifying key biomarkers such as fulvic acid and dibenzo-α-pyrones and revealing marked inter-batch variability that necessitates stringent batch selection and specification setting. Parallel ICP-MS profiling demonstrated that, when properly purified, Shilajit can comply with WHO/USP limits for heavy metals, indicating that safety risks are largely controllable with appropriate processing and analytical oversight. Building on this standardized and safety-validated starting material, a novel Shilajit–phospholipid complex was successfully developed and characterized, exhibiting nanometric particle size (~215 nm), favourable zeta potential (~ −32.6 mV), a ~3.5-fold increase in lipophilicity and markedly enhanced in vitro dissolution (≈85% vs 35% release at 4 h for native extract), while maintaining good stability under accelerated and refrigerated conditions. Collectively, these findings show that phospholipid complexation is a promising strategy to overcome Shilajit's biopharmaceutical limitations and, when coupled with rigorous analytical standardization and safety validation, provides a comprehensive framework for the development of safe, effective and reproducible Shilajit-based formulations. Future work should focus on in vivo pharmacokinetic and pharmacodynamic evaluation of the complex, extended ICH-compliant stability studies and eventual clinical investigations to confirm the translational benefits suggested by the present in vitro data.

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