wjpmr, 2025, 11(7), 82-89

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH
www.wjpmr.com

SJIF Impact Factor: 6.842

Review Article

e-ISSN: 2455-3301

p-ISSN: 3051-2557

SHILAJIT: A COMPREHENSIVE SCIENTIFIC REVIEW OF ITS NATURE, HEALTH BENEFITS, CHEMISTRY, AND FUTURE PROSPECTS

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Article Received on 25/04/2025 **Article Revised on 15/05/2025** **Article Accepted on 03/06/2025**

ABSTRACT

Shilajit, a complex biogeological substance formed over centuries from plant and microbial matter, has gained significant attention in modern nutraceutical research. This comprehensive review examines its geological origins, identification methods, multifaceted health benefits (particularly for cognitive, immune, and gastrointestinal systems), chemical constituents including bioactive dibenzo-α-pyrones and potentially concerning N-nitrosamines, and future research directions. As global interest in traditional medicine compounds grows, understanding Shilajit's scientific foundations becomes increasingly important for harnessing its therapeutic potential while ensuring safety and standardization.

KEYWORDS: Biogeological exudate, Fulvic acid-dominant resin, Cognitive bio enhancer, Immunomodulatory adaptogen, Structural-activity relationships, Global market CAGR, N- nitrosamines contamination.

1. INTRODUCTION: Nature and Historical Significance

Shilajit (known variously as salajit, shilajatu, mummiyo, or asphaltum) is a sticky, tar-like exudate that emerges from cracks in high-altitude rock formations during warm months, particularly in Himalayan ranges between 1000-5000 meters elevation. This natural phytocomplex forms through the slow decomposition of plant material (notably Euphorbia royleana and Trifolium repens) and microbial metabolites under extreme pressure and temperature conditions over centuries. Its colour ranges from pale brown to blackish-brown, with a characteristic coniferous or camphor-like Odor depending on geographical origin.

In Ayurvedic medicine, shilajit has been revered for millennia as a "Rasayana" (rejuvenator) believed to increase longevity, restore energetic balance, and prevent disease. The Sanskrit term translates to "conqueror of mountains and destroyer of weakness," reflecting its esteemed status in traditional healing systems. Contemporary scientific investigation now seeks to validate these traditional claims through rigorous biochemical and clinical research.

2. Geographical Sources and Identification

2.1 Primary Sources

Shilajit is primarily sourced from:

- **The Himalayas** (Nepal, Northern India, Bhutan)
- **Altai Mountains** (Russia, Mongolia)
- **Caucasus Mountains**
- **Andes Mountains** (notably "Andean Shilajit" in Chile)

The **chemical composition** varies significantly by region due to differences in source vegetation, microbial activity, mineral content, and geological conditions. Himalayan Shilajit typically exhibits higher fulvic acid content and antioxidant capacity (ORAC index: 50-500 Troop units/g), exceeding values found in blueberries and noni fruit.

2.2 Identification and Authentication

Genuine Shilajit can be identified through several characteristic properties:

- **Solubility**: Fully soluble in water at various pH levels (distinguishing it from humins)
- **Physical properties**: Density 1.01-1.28 g/cm³; pH 6.7-7.0 for 1% aqueous solution
- Chemical tests
- Positive reaction with $FeCl_3$ (indicates phenolic content)
- Frothing test with sodium bicarbonate.
- Characteristic UV absorption at 270-280 nm.

Purification is essential because raw Shilajit may contain mycotoxins, heavy metals (lead, mercury, arsenic), free radicals, and polymeric quinones. Proper processing removes these contaminants while preserving bioactive components. Table 1summarizes key identification characteristics.

Table 1: Shilajit Types and Identification Markers.

Classification Basis	Types	Key Characteristics	Bio active compounds
By Odor	Gomuthira Shilajit	Distinct coniferous smell	Higher fulvic acid content
	Karpura Shilajit	Camphor-like aroma	Different terpenoids profile
By miner source	Lauha Shilajit (iron-rich)	Reddish-brown hue	Iron complexes with Fluvic acid
	Tamra Shilajit (Copper-rich)	Bluish tint	Copper –Fluvic acid complexes
	Rajat Shilajit (Silver-rich)	Whitish appearance	Silver nanoparticles
Authen tication Tests	Water solubility	Dissolves completely	Low molecular weight fractions
	Frothing test	CO_2 release with $NaHCO_3$	Acid functional groups
	UV Spectroscopy	Peak at 270 nm to 280nm	Aromatic / conjugated system

3. Health Contributions: Cognitive, Immune, and Gut Health

3.1 Cognitive Health Mechanisms

Shilajit's most promising application lies in **Neuroprotection and cognitive enhancement**, primarily attributed to its **fulvic acid (FA) content** (60-80% of bioactive components). FA exerts multiple beneficial effects:

- **Tau Protein Modulation**: Inhibits abnormal aggregation of tau proteins into neurofibrillary tangles, a hallmark of Alzheimer's pathology.
- **Mitochondrial Support**: Enhances cellular energy production by protecting mitochondria from oxidative damage and improving electron transport chain efficiency.
- **Metal Chelation**: Binds neurotoxic metals like copper and iron, reducing oxidative stress in neural tissue
- **Neurotransmitter Regulation**: Animal studies suggest acetylcholine enhancement.

Clinical evidence includes a 24-week trial where Alzheimer's patients receiving Shilajit/B- vitamin combinations showed significantly less cognitive decline than placebo groups. In vitro studies demonstrate hippocampal neuron development enhancement, with increased neurite outgrowth and axon-like processes.

3.2 Immune System Modulation

Shilajit functions as a **natural Immunomodulatory** through several mechanisms:

- **Complement System Activation**: Fulvic acid components stimulate the complement system, enhancing opsonization and pathogen clearance.
- **Phagocytosis Enhancement**: Increases macrophage activity and microbial clearance capacity
- **Anti-inflammatory Action**: Suppresses pro-inflammatory cytokines (TNF-α, IL-6) while promoting anti-inflammatory IL-10 production.
- **Antiviral Activity**: Shilajit extracts demonstrate dose-dependent inhibition against HSV-1, HSV-2, human cytomegalovirus, and respiratory syncytial virus, though humic substances from other sources show stronger anti-HIV activity.

Notably, Shilajit enhances immune function without excessive inflammation, characteristic of adaptogenic substances.

3.3 Gut Health and Microbiome Interaction

Emerging research reveals Shilajit's significant **gastro protective and microbiome- modulating effects**:

- **Mucosal Protection**: Enhances mucopolysaccharide biosynthesis, strengthening the gastric mucus barrier against ulcerogens like ethanol, NSAIDs, and stress.
- **Anti-ulcer Activity**: Reduces gastric lesions by 40% in animal models through antioxidant mechanisms and prostaglandin regulation.
- **Microbiome Modulation**: Humic components selectively inhibit pathogenic bacteria while promoting beneficial species
- **Bile Acid Metabolism**: May improve bile acid reabsorption and reduce bile acid- induced diarrhea.

The fulvic acid in Shilajit also supports gut barrier integrity, preventing "leaky gut" syndrome and systemic inflammation.

Health Domain	Study Findings	Mechanism	Clinical evidence level
Cognitive Health	Reduced cognitive decline in Alzheimer's patients	Tau anti-aggregation, mitochondrial protection	Human pilot studies
Male Fertility	60% increase in sperm count af 90 days	Testosterone optimization, antioxidant effects	Randomized human trial
Bone Health	Reduce bone loss in	Collagen synthesis, anti-	Human RCT (n=60)

	postmenopausal women	inflammatory action	
Altitude Sickness	Reduced symptoms (Insomnia, headache, fatigue)	Oxygen utilization improvem mitochondrial support	Animal and observationa studies
Chronic Fatigue	Improved swimming enduranc rats	Mitochondrial ATP enhancem	Preclinical studies
Iron Deficiency	Increased haemoglobin, haematocrit, RBC's	Iron absorption facilitation, mineral delivery	Animal studies

4. Chemical Constituents and Structure-Activity Relationships

4.1 Primary Bioactive Components

Shilajit's complexity arises from its **humic substance framework** containing hundreds of bioactive molecules.

- **Fulvic Acid (FA)**: Low molecular weight (≈2 kDa) fraction soluble at all pH conditions. Contains phenolic hydroxyl, quinone, ketone, and carboxylic groups enabling free radical scavenging and metal chelation.
- **Humic Acid (HA)**: Higher molecular weight (5-10 kDa), alkaline-soluble fraction with more aromatic rings
- **Dibenzo-α-Pyrones (DBPs)**: Chromoproteins carriers that facilitate nutrient transport across membranes. Act as molecular "chaperones" for co-delivery of trace minerals.
- **Minor Components**: Triterpenes, sterols, ellagic acid, resins, amino acids, and >40 trace minerals including selenium, iron, and zinc.

Structural analyses reveal FA contains **oxygen-rich aromatic clusters** with alkyl chains and heteroatoms, forming dynamic supramolecular assemblies in solution. Its antioxidant capacity correlates with phenolic content and conjugation systems.

4.2 Key Carrier Molecules: DCPs and DBPs

The **dibenzo-α-pyrone-Chromoproteins** (DBPs) serve critical functions:

- **Structural Features**: Composed of a dibenzo-α-pyrone core with peptide and carbohydrate moieties
- Biological Functions
- Enhance bioavailability of trace minerals
- Facilitate cellular uptake of active components
- Protect antioxidants during gastrointestinal transit
- Regulate enzyme activity through allosteric interactions

Molecular characterization shows DBPs form **stable complexes with metal ions** (Fe^{3+}, Cu^{2+}, Zn^{2+}) through their phenolic and carboxylic groups, functioning as targeted mineral delivery systems.

4.3 Structure-Activity Relationships:

Bioactivity correlates strongly with molecular properties:

- Antioxidant **Capacity**: Proportional to phenolic content and aromaticity ($r=0.87$, $p<0.01$)
- **Anti-HIV Activity**: Humic materials outperform shilajit extracts (EC_{50}: 0.37-1.4 mg/L vs. 14-142 mg/L) and correlates with aromatic carbon content.
- **Neuroprotection**: Dependent on FA's ability to chelate metals and inhibit tau aggregation through specific binding sites
- **Carrier Function**: DBPs with intermediate lipophilicity (logP 2-4) show optimal membrane permeability

The figure below illustrates key structural features and their biological relevanc.

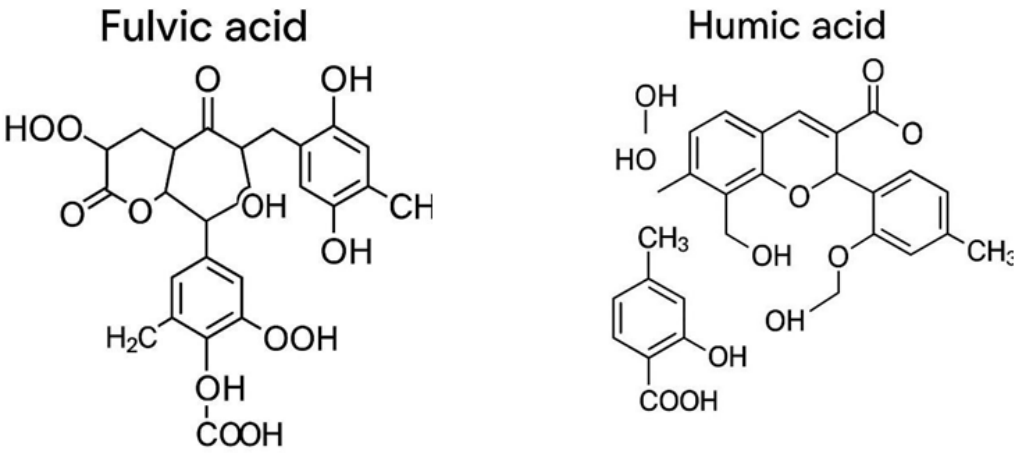

Figure: Key Bioactive Components and Their Structural Features (FA_HA)

Core Molecular Framework

- **Aromatic-aliphatic backbone**: Fulvic acid (FA) consists of a hybrid structure with benzene rings (aromatic components) connected by aliphatic chains (e.g.methylene groups -CH_2-) and oxygen-containing functional groups.
- **Molecular weight**: Ranges from **1,000–10,000 Da**, significantly smaller than humic acid (HA). This compact size enables deep cellular penetration in plants and animals

Key Functional Groups

FA's bioactivity stems from oxygen-rich functional groups:

- **Carboxyl groups (-COOH)**: Enable metal chelation (e.g. iron, zinc) and nutrient solubilisation.
- **Phenolic hydroxyl groups (-OH)**: Provide antioxidant properties by scavenging free radicals.
- **Carbonyl/quinone groups (C=O)**: Participate in redox reactions and electron transfer.
- **Minor groups**: Methoxy (-OCH_3), amino (-NH_2), and ether bonds

Table: Functional Group Distribution in FA vs. HA

Functional Group	Fulvic Acid (FA)	Humic Acid (HA)
Carboxyl (-COOH)	High (Dominant)	Moderate
Phenolic (-OH)	High	Moderate
Aromatic rings	Low to Moderate	High (Core structure)
Aliphatic chains	Moderate	Low
Amino (-NH2)	Low	High

3. Structural Variability by Source

FA's structure varies significantly based on origin:

- **Herbaceous peat FA**: High carbonyl, amino, methylene, and carboxyl groups.
- **Woody peat FA**: Enriched in aromatic carbons, methoxy groups, and phenols; highest aromaticity.
- **Mossy peat FA**: Dominated by hydroxy, methyl, and phenol groups

4. Supramolecular Assembly

FA molecules self-assemble via non-covalent interactions:

- **Hydrogen bonding**: Between -COOH and -OH groups, creating dynamic aggregates.
- **π-π stacking**: Aromatic rings cluster via hydrophobic interactions.
- **Electrostatic forces**: Carboxyl groups confer negative surface charge (zeta potential: **- 1.7 to +3.3 mV**), influencing solubility and ion binding.

Table: Particle Characteristics of FA in Solution

Property	Range/Description	Functional Impact
Particle size	0.2-69.6 nm	Facilitates root/ leaf absorption
Poldispersity (PDI)	14-183%	Reflects structural heterogeneity
Zeta potential	-1.7 to + 3.3 mV	Governs colloidal stability & aggregation

***5.* Evolutionary Formation Process**

FA evolves from small to large molecules through:

1. **Nucleation**: Small molecules (e.g. quinones, sugars) react via **Maillard pathways**.
2. **Functional group addition**: Carboxyl (-COOH) and hydroxyl (-OH) groups respond first during growth.
3. **Advanced structuring**: Hydrogen bonds and π-π interactions compact FA into dense aggregates, while HA forms looser structures via hydrophobic forces.

Key Takeaways

- FA's core is a **flexible aromatic-aliphatic network** rich in oxygen-based functional groups.
- Its small size and solubility stem from high -COOH/-OH density and low molecular weight.
- Structural diversity across sources (e.g. woody vs. herbaceous peat) affects functionality.
- Self-assembly into aggregates enables environmental roles (e.g. nutrient delivery, metal chelation)

5. N-Nitrosamines in Shilajit: Analysis and Risk Mitigation

5.1 Origins and Concerns

N-Nitrosamines are potent carcinogens formed when nitrites react with secondary amines. Recent concerns emerged after findings in pharmaceuticals (e.g. 2018 valsartan recall), raising questions about natural products. In Shilajit, potential sources include:

- Natural Formation: Reaction of soil nitrates with amine-containing humic precursors
- Processing Artifacts: Use of nitrogen fertilizers in source regions or nitrite-preserved raw materials
- Contamination: During storage or transportation Regulatory agencies (EMA, FDA) now require risk assessment for nitrosamines in all medicinal products, creating implications for Shilajit-based supplements.

5.2 Detection and Quantification

Analytical approaches include:

- **HPLC-MS/MS: Simultaneous detection of 7 nitrosamine impurities (LOD: 0.03-0.15 ppm)**
- **WHO Nitrosation Assay Procedure (NAP): Evaluates nitrosability potential of amine precursors**
- **Confirmatory Testing: Mandatory for products with identified risk factors.**

Current data suggests purified Shilajit contains nitrosamines below thresholds of toxicological concern (≤0.03 ppm), but crude materials may exceed safety limits.

5.3 Mitigation Strategies

To minimize risks:

- **Source Control**: Avoid Shilajit from agricultural areas with high nitrogen fertilizer use
- **Process Optimization**: Implement low-temperature extraction without nitric acid or sodium nitrite
- **Purification Techniques**: Activated carbon filtration, distillation, or chromatographic separation
- **Quality Monitoring**: Regular testing of raw materials for nitrites, nitrates, and amines Regulatory guidelines now require manufacturers to conduct thorough risk assessments and implement control strategies by October 2023 for EU markets.

5 Future Prospects and Research Directions

5.1 Current Market Trends

Global Shilajit demand is surging (≥18% CAGR) due to:

- **Natural Wellness Movement**: Consumer shift toward Ayurvedic and herbal supplements
- **Social Media Influence**: Promotion as "Himalayan super food" for energy and vitality
- **Aging Population**: Interest in cognitive health products
- **Formulation Diversity**: Availability as resins, capsules, powders, and functional food additives.

Quality concerns persist, with ≈23% of commercial products showing adulteration or heavy metal contamination. Standardization remains challenging due to natural variability.

5.2 Research Imperatives

Critical research needs include:

- **Clinical Translation**: Larger randomized controlled trials (RCTs) for cognitive decline, osteoporosis, and male infertility
- **Mechanistic Studies**: Molecular pathways of DBPs and fulvic acid metabolites
- **Standardization Protocols**: Biomarker-based quality control (e.g. dibenzo-α-pyrones as reference compounds)
- **Delivery Optimization**: Nano-formulations to enhance bioavailability
- **Safety Pharmacology**: Chronic toxicity studies and interaction profiles with common medications.

5.3 Regulatory and Sustainability Challenges

Future development must address:

- **Geographical Protection**: Preventing overharvesting through sustainable collection practices
- **Adulteration Control**: Advanced authentication methods (DNA barcoding, NMR fingerprinting)
- **Regulatory Harmonization**: Establishing global standards for identity, purity, and potency
- **N-Nitrosamine Monitoring**: Implementing ICH M7(R2) guidelines for mutagenic impurities.

6 CONCLUSION

Shilajit represents a fascinating intersection of traditional medicine and modern science. Its complex chemical architecture-centered on fulvic acid and dibenzo-α-pyrones-confirms several traditional applications while revealing novel therapeutic mechanisms. Significant evidence supports roles in cognitive protection, immune modulation, and gut health, though larger clinical trials are needed. The recent focus on N-nitrosamine contamination highlights the importance of quality control in natural products. Future research should prioritize standardization, sustainability, and rigorous clinical validation to fully realize Shilajit's potential as a multifaceted health supplement. As scientific understanding advances, this ancient "destroyer of weakness" may find renewed relevance in addressing modern health challenges, from neurodegenerative diseases to healthy aging.

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