Understanding the Mechanism of *Shilajit* by Using Network Pharmacology Approach

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INTRODUCTION: *Shilajit* is a blackish-brown exudate from high mountain rocks and is produced over a couple of mineral years from organic matters and plants that have been trapped between the layers of rocks in mountains in a couple of regions, including Tibet, China, India and Altay. With time, the pressure from the weight of the mountains causes the materials to be transformed into a rich mineral mass which then oozes out of the rocks [1].

Traditional medicine is an integral part of the health care system in a number of developing countries including India. There are a number of natural remedies, which have been in use for ages in Asian countries but unfortunately lack systematic scientific evaluation and documentation. However, these remedies can only find a place for themselves in the mainstream medicine if their claims are evaluated scientifically and documented systematically. *Shilajit* is one such remedy. It has been ascribed a number of pharmacological activities and has been used for ages as a rejuvenator and for treating a number of disease conditions. Modern scientific research has systematically validated a number of properties of *Shilajit* and has proven that *Shilajit* is truly a panacea in Oriental medicine [2].

Network pharmacology is an emerging discipline useful in formulation discovery, which integrates recent advances in omics technologies and systems biology through computational biology. Many drugs used in specialties like oncology, cardiology and psychiatry, have effects on multiple targets. To better understand the underlying complex biological and pharmacological processes for chronic diseases like asthma, cancer, and neurodegenerative diseases, it is important to know the systems network of different pathways where the drugs are likely to act [3].

CHEMICAL CONSTITUENTS: The major organic mass of *Shilajit* comprised humus (60–80%) along with other components such as benzoic acid, hippuric acid, fatty acid, ichthyol, ellagic acid, resin, triterpenes, sterol, aromatic carboxylic acid, 3,4-benzocoumarins, amino acids and phenolic lipids [4]. The major physiological action of *Shilajit* was found to be due to the presence of the bioactive dibenzoalpha-pyrones along with humic and fulvic acids which acted as carrier molecules for the active ingredients [2]. On performing an HPLC analysis of *Shilajit*, some of the polyphenols determined were tannic acid, gallic acid, ferulic acid [6]. The composition of *Shilajit* is influenced by factors such as the plant-species involved, the geological nature of the rock, local temperature profiles, humidity and altitude, etc.

METHODOLOGY: All the available structures of the bio-actives were taken from PubChem and were queried in Binding database or Binding DB for identifying their targets using special tool 'Find my compounds' targets'. Binding DB is a web based free database that covers protein interactions with small drug-like molecules. It searches for the exact or similar compounds in the database and retrieves the target information of those compounds. The similarity search gives the structurally similar compounds with the degree of similarity to the queried structure as scores, where 1 is the highest possible value. A score of 1 indicates that either the exact queried compound is present or it gives a 100% structural similarity to another compound in the database.

The bio-actives were also queried in the DrugBank database and STITCH database. The DrugBank database is a unique bioinformatics and cheminformatics resource that combines detailed drug data with comprehensive drug target information. STITCH is a resource to explore known and predicted interactions of chemicals and proteins. Chemicals are linked to other chemicals and proteins by evidence derived from experiments, databases and the literature.

The targets of the bioactives were searched in the Therapeutic Targets Database (TTD) for their association with any disease or indication. This database provides information about known and explored therapeutic protein and nucleic acid targets, the targeted disease, pathway information and the corresponding drugs directed at each of these targets [3].

For the target interaction network, the gene names of all the collected targets were input into the interaction prediction database STRING (Search Tool for the Retrieval of Interacting Genes/Proteins). It contains information from numerous sources, including experimental data, computational prediction methods and public text collections. It is freely accessible and it is regularly updated. On obtaining the predicted interaction network, the interactions were downloaded in a tabular form.

A pharmacology network is made up of nodes, the points of communication or redistribution, and edges, the lines of communication joining the nodes. The network is analyzed and visualized using Cytoscape 3.7.1; a java based open source software [7]. The interaction information files were imported into Cytoscape to create the networks.

HYPOTHESIS: Pharmacological network of *Shilajit* will depict the interaction of bio-actives with molecular targets of cancer.

OBJECTIVES:

- To find the bio-actives of *Shilajit*
- To predict the targets of the components of *Shilajit*
- To construct a pharmacology network of the mechanism of the formulation of *Shilajit*
- To validate findings

SCOPE: Network pharmacology aims to understand the network interactions between a living organism and drugs that affect normal or abnormal biochemical function. It tries to exploit the pharmacological mechanism of drug action in the biological network, and helps to find drug targets and enhance the drug's efficacy.

PROPOSED METHODOLOGY:

- Literature Mining
- Using Binding Database and Therapeutic Targets Database
- Using STRING and Cytoscape to construct the pharmacology network
- Using docking studies and cancer cell lines for validation

PLAN OF WORK:

Phase	**Period**	**Work to be executed**
1	Literature Mining	Jan 25th – Feb 10th
2	Finding Bioactives & targets	Feb 11th – Feb 28th
3	Network Construction	Feb 28th – April 1st
4	Validation	April 1st – April 20th

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