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# EFFECT OF *BHAVANA* ON QUALITATIVE PHYTOCHEMICAL PROFILE AND ELEMENTAL COMPOSITION OF *SHILAJATHU*: A COMPARATIVE PHARMACEUTICAL STUDY WITH *EKANAYAKA KWATHA BHAVITHA SHILAJATHU*

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## ABSTRACT

*Shilajathu,* a revered Ayurvedic *Rasayana,* is subjected to *Bhavana*, a traditional processing technique. This study investigates the effect of *Bhavana* on *Shilajathu*'s phytochemical profile and elemental composition using *Ekanayak Kwatha Bhavitha Shilajathu*. *Ekanayaka* (*Salacia chinensis*) is a prominently featured drug in various *Kashaya*s recommended for *Prameha Chikitsa* in esteemed Kerala texts, such as *Sahasrayoga* and *Chikitsamanjari. Ekanayaka Kwatha Bhavitha Shilajathu* was prepared through a seven-fold *Bhavana* process. Phytochemical screening revealed a diverse profile of bioactive compounds. HPTLC analysis confirmed the presence of Mangiferin, a standard biomarker for *Ekanayaka* in *Ekanayak Kwatha Bhavitha Shilajathu*, and also revealed enhanced bioactive compounds in *Bhavitha Shilajathu* compared to *Shilajathu* before *Bhavana.* Energy Dispersive X-ray Analysis showed increased weight % of certain elements, C, Na, Mg, Cl, and K in *Bhavitha Shilajathu*. The findings demonstrate enhanced absorption of *Ekanayaka Kwatha*'s active principles into *Shilajathu* during *Bhavana.* The formulation's safety was confirmed by detecting heavy metal levels within permissible limits. The study provides insights into the Ayurvedic concept of *Bhavana* and its effects on *Shilajathu*'s phytochemical and elemental composition.

## INTRODUCTION

In Ayurvedic medicine, *Rasasastra* harnesses the therapeutic potential of mineral-based drugs, including *Shilajathu*, a prominent *Maharasa* group member. Renowned for its anti-diabetic, rejuvenative, and adaptogenic properties, *Shilajathu* is revered in Ayurvedic and Siddha traditions. The reference to *Shilajathu* dates back to ancient times in the *Samhita*s. In *Rasasastra*, the majority of texts have explained *Shilajathu* under *Maharasa*.[1] Combining *Shilajathu* with *Ekanayaka*, a recognized drug in *Prameha chikitsa*, through *Bhavana* in *Ekanayaka Kwatha*, as a form of fortification may enhance its therapeutic potential.

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*Sodhana* of *Shilajathu* removes impurities, enhancing its potency and safety for therapeutic use. *Bhavana* of *Shilajathu,* described in Ayurvedic texts like Ashtanga Sangraha, augments its advantages[2] Susrutha Samhitha mentions using *Shilajathu* with decoctions tailored to individual requirements.[3] Acharya Charaka's verse states *Shilajathu Rasayana* can cure any curable disease when used properly, and can be customized with various *Kashaya*s.[4] The major source of the *Yoga, Sivagudika*'s *Rasayana* properties is the mineral medicine *Shilajathu,* whose advantages are augmented by *Bhavana* in a variety of herbal decoctions.[5]

*Ekanayaka* (*Salacia chinensis*) is traditionally used in Ayurveda and Unani medicine to treat diabetes. Three Salacia species have shown promise in preclinical research and clinical trials for their anti-diabetic properties. The root bark of *S. chinensis* usually exhibits seven circles on cross-section, earning it the name *'Sapta Chakra'*. Bioactive compounds like Salacinol and Mangiferin contribute to S.chinensis's therapeutic effects. [6]

Pharmaceutical modification through *Samskaras*, including *Bhavana*, enhances absorption, assimilation, and therapeutic potency.[7] *Bhavana* involves treating drugs with fluids like *Kashaya* or *Swarasa* to enhance potency and efficacy. The process requires two primary materials: *Bhavya Dravya* (finely powdered drug) and *Bhavana Dravya (Dravadravya)*. Various Ayurvedic texts describe distinct *Bhavana* processes. The objectives of *Bhavana* include maximizing potency, minimizing toxicity, and enhancing bioavailability, and also involve physical, chemical, and pharmacological transformations.[8]

## MATERIALS AND METHODS

### Collection of raw drugs

***Shilajathu:*** 3 samples of *Shilajthu* were purchased, 100gm each from 3 different local markets and their quality assessment was done using the classical methods. Out of these 3 samples, one sample showed almost all the characteristic properties of acceptable *Shilajathu,* mentioned in the classics. 500gm of this sample of *shilajathu* was purchased from the particular local market, reportedly sourced in bulk from Punjab.

***Ekanayaka:*** 4kg of *Salacia chinensis* was purchased from Karwar, Karnataka, where it was cultivated locally. (Fig 1)

### Quality assessment and authentication of *Shilajathu*

The selected *Shilajathu* sample was authenticated using classical methods, exhibiting characteristic properties as described in the classics. It possessed a distinctive smell of *Gomutra* (cow's urine), was soluble in water, and formed thread-like streaks upon placement on water's surface, followed by dissolution. Additionally, it formed *Lingakara* when subjected to fire and became insoluble in water after heating to *Lingakara.* (Fig 2)

### *Sodhana* of *Shilajathu*

The *Sodhana* of *Shilajathu* was performed using the *Suryatapi* method as explained in Rasatarangini, Taranga 22, verses 69-77. *Triphala kwatha* was prepared according to the '*Prakshepakwathamanam'* specified in Rasatarangini, with a drug-to-water ratio of 1:8, and reduced to ¼th.[9] 500gm of *Triphala* and 4L of water were used to obtain 1L of *Kwatha*. The *Sodhana* process involved combining 500gm of powdered *Shilajathu* with 1L of hot water and 250 ml of *Triphala kwatha,* stirring well, and exposing it to sunlight for 3 hours. The contaminants were removed by filtering, and the mixture was again exposed to sunlight to form a blackish creamy scum layer, which was collected, dried, and stored as *Sodhitha Shilajathu.* This process was repeated until no further scum formation occurred, and the entire process was completed in around 10 days during the summer season.(Fig 3)

### Preparation of *Ekanayaka kwatha*

The *Ekanayaka roots* (4kg) were cleaned, washed, and dried, and their macroscopic characteristics, identity, purity, and strength were evaluated. The roots were then chopped and ground into a coarse powder. *Ekanayaka kwatha*, the *Bhavana dravya,* was prepared by boiling the coarse powder in 8 times water and reducing it to 1/8th.[10,11] For the seven-*Bhavana* process of 180g *Sodhitha Shilajathu*, 1455ml of *Kwatha* was utilized, requiring 1455g of coarsely powdered *Ekanayaka* root.(Fig 4)

### *Bhavana* of *Shilajathu*

*Bhavana* of *Sodhitha Shilajathu* was performed seven times in *Ekanayaka kwatha*. 180gm of *Sodhitha Shilajathu* was powdered and added to an equal quantity of *Ekanayaka kwatha,* and soaked in a wide steel vessel. The vessel was stored indoors at night and exposed to sunlight during the day, covered with a cotton cloth. When the *Kwatha* dried up, subsequent *Bhavanas* were performed by adding filtered and hot *Kwatha* to the *Shilajathu.*[10,11] The seven-stage *Bhavana* process was completed over approximately 2.5 months, with temporal delays due to inclement weather conditions. (Fig 5)

### Preperation of *Ekanayaka Kwatha Bhavitha Shilajathu Gudika*/pills

After the 7th *Bhavana*, the drug had a partially dried, moldable consistency, ideal for pill formation. The mass was manually rolled into 500mg dose pills using a high-precision laboratory weighing balance and clean, dry hands. The pills were arranged in a clean steel tray, dried in the shade, and then stored in a clean, airtight glass container when properly dried. (Fig 6)

### Qualitative Analysis of the Drugs

### Organoleptic Characters

Organoleptic evaluation, based on sensory attributes like colour, consistency, taste, and smell, is a vital step in assessing the quality, authenticity, and purity of a drug. The organoleptic characteristics were observed and recorded for the study drug, *Ekanayaka Kwatha Bhavitha Shilajathu,* as well as the raw *Shilajathu* and *Ekanayaka Kwatha.*

### Macroscopic Evaluation

The raw drug samples were subjected to macroscopic evaluation by observation with the unaided eyes and by tactile and other sensory inspection.

### Powder microscopy

The powdered *Ekanayaka* sample was mounted on microscope slides and treated with Phloroglucinol solution and dilute hydrochloric acid. After gentle stirring, a cover slip was applied, and excess liquid removed. The slides were examined and photographed using a Labomed LX-300 Binocular

microscope, revealing that phloroglucinol staining selectively highlighted lignified tissues, turning them pink, and facilitating clear visualization of the sample's morphological features.[12]

**Phytochemical Screening**

A 10gm powdered sample of raw *Shilajathu* was extracted with distilled water and methanol for 5 hours, filtered, and concentrated to 25ml. Similarly, *Ekanayaka Kwatha Bhavitha Shilajathu* was extracted with distilled water to obtain an aqueous extract, which was then concentrated. The extracts, along with 100ml of concentrated *Ekanayaka Kwatha*, were subjected to phytochemical screening to identify and characterize various phytochemical constituents, including carbohydrates, flavanoids, glycosides, phenols, saponins, terpenoids, tannins, alkaloids, and steroids.

**HPTLC Fingerprint Profiling Using Mangiferin Biomarker as Standard**

High-performance Thin-Layer Chromatography (HPTLC) fingerprinting was performed on a single plate to analyze the phytochemical profiles of five samples: *Ekanayaka Kwatha Bhavitha Shilajathu* (EKBS), *Sodhitha Shilajathu* (SS), *Ekanayaka Kwatha* (EK), *Ekanayaka Choorna* (EC), and mangiferin. The test solution was prepared by extracting 0.5gm of the sample in 5ml of methanol, and 5µl of the extract was applied onto the HPTLC plate. The plate was developed using the solvent system Ethyl acetate:Methanol:Formic acid (7:2:0.5) which is specifically used for mangiferin, air-dried, and examined under UV light at 254nm and 366nm. After derivatization with Natural Product Reagent (NPR), the plate was scanned at 366nm.

**HPTLC Fingerprint Profiling of EKBS, SS, EK and EC**

HPTLC fingerprinting was repeated for *Ekanayaka Kwatha Bhavitha Shilajathu* (EKBS), *Sodhitha Shilajathu* (SS), *Ekanayaka Kwatha* (EK), and *Ekanayaka Choorna* (EC) using an alternative solvent system. This was necessary because the Mangiferin-specific solvent system, although effective for detecting Mangiferin in EC, EK, and EKBS, failed to produce clear bands for the other phytochemical constituents. The test solution was prepared by extracting 0.5gm of the sample in 5ml of hydroalcohol, and 5µl of the extract was spotted onto the HPTLC plate. The plate was developed using Chloroform: Ethyl Acetate: Formic Acid (5:4:1), air-dried, and examined under UV light at 254nm and 366nm.[13]

**Determination of Levels of heavy metals - Mercury, Cadmium, Arsenic,Lead.**

The heavy metal analysis was carried out according to API (Part 1, Volume 9) using Inductively Coupled Plasma. The process involved preparing a test solution by acid digestion, creating standard solutions, and generating a calibration curve through instrumental analysis. The calibration curve was then used to determine the concentration of heavy metals in the test solution, and the element concentration was calculated in µg/L by multiplying the concentration obtained from the calibration graph by the dilution factor.[14]

**Elemental Composition by EDAX of SS and EKBS**

Energy-Dispersive X-ray Analysis (EDAX) was performed to determine the elemental composition of SS and EKBS samples. Sample preparation involved smearing material onto adhesive carbon tape on specimen stubs. Analysis was conducted using an Oxford XMX N EDS Instrument at 20kV, identifying elemental constituents in specific sample areas, with findings summarized in a table with corresponding peak data.

**OBSERVATIONS AND RESULTS**

***Shilajathu Sodhana***

- Weight of *Sodhitha Shilajathu*- 412gm
- Loss of weight after *Sodhana*- 88 gm

***Bhavana* of *Sodhitha Shilajathu* in *Ekanayaka Kwatha***

The results are depicted in Table 1.

**Table 1: Bhavana-Wise Parameters of *Shilajathu* and *Ekanayaka Kwatha***

| ***Bhavana*** | **Weight of *Shilajathu* taken for *Bhavana* (g)** | **Volume of *Ekanayaka kwatha* utilized (in ml)** | **Resultant Weight of *Bhavitha Shilajathu* (in g)** | **Physical consistency of *Bhavitha Shilajathu*** |
|---|---|---|---|---|
| First | 180 | 180 | 200.5 | Dried, crumbly, yet flexible with a notably wrinkled and unevenly raised surface |
| Second | 200.5 | 201 | 205.5 | |
| Third | 205.5 | 206 | 208 | |
| Fourth | 208 | 208 | 213.5 | |
| Fifth | 213.5 | 214 | 221.6 | |
| Sixth | 221.6 | 222 | 232.1 | |
| Seventh | 232.1 | 233 | 266.7 | Partially dried to a pliable and moldable consistency, ideal for rolling into pills. |

**Organoleptic Parameters**

Organoleptic parameters are summarized in table 2.

**Table 2: Organoleptic Characters of the Samples**

| Organoleptic characters | Raw *Shilajathu* | *Ekanayaka Kwatha* | *Ekanayaka Kwatha Bhavitha Shilajathu* |
|---|---|---|---|
| Colour | Black | Brown | Black |
| Odour | Smell of *Gomutra* | Characteristic | Slight smell of *Gomutra* |
| Touch | Sticky, resinous | liquid | Slightly sticky |
| Taste | Bitter, astringent | Bitter, astringent | Bitter, astringent |

**Macroscopic Evaluation of *Ekanayaka***

The roots are cylindrical, extremely hard, and woody, with a fibrous consistency evident when chopped. The root bark exhibits a bright yellowish outer layer, which is readily detachable by flaking, and the inner surface has a brown colour. On cross-section, the root reveals seven distinct concentric circles accompanied by some indistinct lighter circles in between. (Fig 7)

**Powder microscopy of *Ekanayaka***

Microscopic analysis of EC powder displays calcium oxalate crystals, non-lignified fibres featuring narrow lumens, lignified stone cells, cork cell fragments, brown-colored content, lignified sclereids with wide lumens, bordered-pitted vessels, and starch grains (simple and compound), along with lignified tracheid fragments. (Fig 8)

**Phytochemical screening of Raw *Shilajathu, Ekanayaka Kwatha* and *Ekanayaka Kwatha Bhavitha Shilajathu***

The results are depicted in the Table 3.

**Table 3: Phytochemical Screening of Raw *Shilajathu* Sample, *Ekanayaka Kwatha* and *Ekanayaka Kwatha Bhavitha Shilajathu***

| Phytochemicals | Raw *Shilajathu* sample | *Ekanayaka Kwatha* | *Ekanayaka Kwatha Bhavitha Shilajathu* |
|---|---|---|---|
| Carbohydrates | ++ | + | ++ |
| Flavanoids | ++ | + | ++ |
| Glycosides | + | + | **ND** |
| Phenol | ++ | + | ++ |
| Saponins | + | **ND** | + |
| Terpenoids | ND | ND | ND |
| Tannins | ++ | + | ++ |
| Alkaloids | + | **ND** | + |
| Steroids | ND | ND | ND |

**High-Performance Thin Layer Chromatography (HPTLC)**

**Solvent System- Ethylacetate: Methanol: Formic acid (7:2:0.5)**

At 366nm (Derivatized): The major bands of mangiferin standard were found at Rf 0.69(blue), in EKBS, EC, and EK. It was not detected in SS. (Fig 9-11)

**Solvent System - Chloroform: Ethyl acetate: Formic acid (5:4:1).**

EKBS and EK exhibit unique bands that are absent in SS. At 254 nm, EKBS displays two extra black bands (one at Rf~0.69). At 366nm, EKBS shows three additional bands: a yellowish band, a blue-fluorescent band at the starting area, and another blue-fluorescent band at the ending area (Rf~0.85), indicating more bioactive compounds matching *Ekanayaka Kwatha*'s Rf values. (Fig 12-16)

**Elemental Composition by EDAX (Energy Dispersive X-ray Analysis) of SS and EKBS**

The elemental composition of SS and EKBS are depicted in Table 4 and Table 5 respectively (Fig 17)

**Table 4: Elemental Composition by EDAX of SS and Weight of Each Element**

| Element | Line Type | Wt% | Atomic % |
|---|---|---|---|
| C | K series | 54.91 | 62.18 |
| O | K series | 43.8 | 37.23 |
| Na | K series | 0.26 | 0.15 |
| Mg | K series | 0.23 | 0.13 |
| S | K series | 0.24 | 0.1 |
| Cl | K series | 0.26 | 0.1 |
| K | K series | 0.14 | 0.05 |
| Ca | K series | 0.16 | 0.05 |
| Total: | | 100 | 100 |

**Table 5: Elemental Composition by EDAX of EKBS and Weight of Each Element**

| Element | Line Type | Wt% | Atomic % |
|---|---|---|---|
| C | K series | 54.74 | 62.72 |
| O | K series | 41.32 | 35.54 |
| Na | K series | 0.35 | 0.21 |
| Mg | K series | 0.67 | 0.38 |
| Si | K series | 0.4 | 0.19 |
| S | K series | 0.35 | 0.15 |
| Cl | K series | 0.96 | 0.37 |
| K | K series | 1.22 | 0.43 |
| Total: | | 100 | 100 |

**Determination of Levels of heavy metals - Mercury, Cadmium, Arsenic, Lead of *Ekanakaya Kwatha Bhavitha Shilajathu***

The results are depicted in Table 6

**Table 6: Heavy Metal Levels (Mercury, Cadmium, Arsenic, Lead) in *Ekanakaya Kwatha Bhavitha Shilajathu***

| Heavy metal | Unit | Result | Detection Limit | Permissible limits (API) |
|---|---|---|---|---|
| Mercury | ppm | 0.14 | 0.05 | 1 |
| Lead | ppm | 0.67 | 0.05 | 10 |
| Arsenic | ppm | 0.20 | 0.05 | 3 |
| Cadmium | ppm | 0.07 | 0.05 | 0.3 |

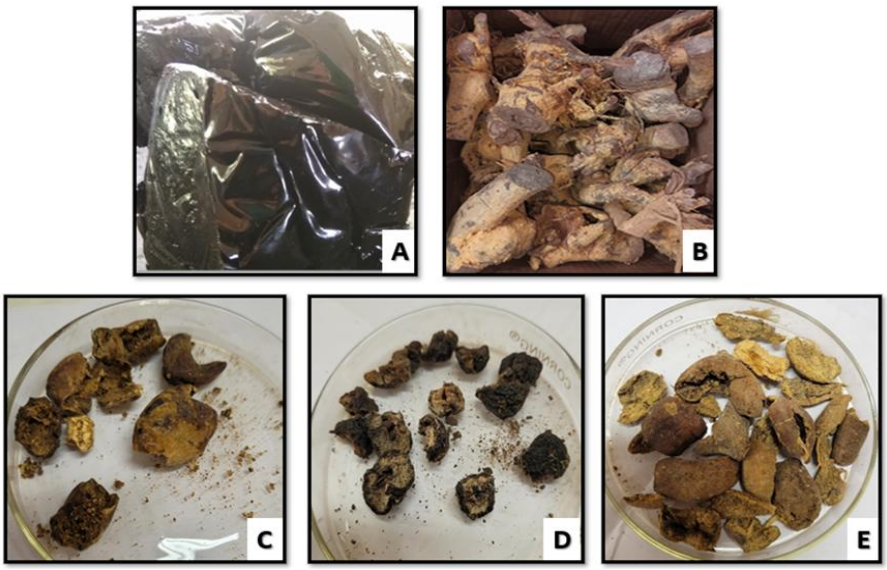



**Fig 1. Raw Drugs**

*(a) Shilajathu, (b) Ekanayaka, (c) Hareetaki, (d) Amalaki, (e) Vibheethaki*

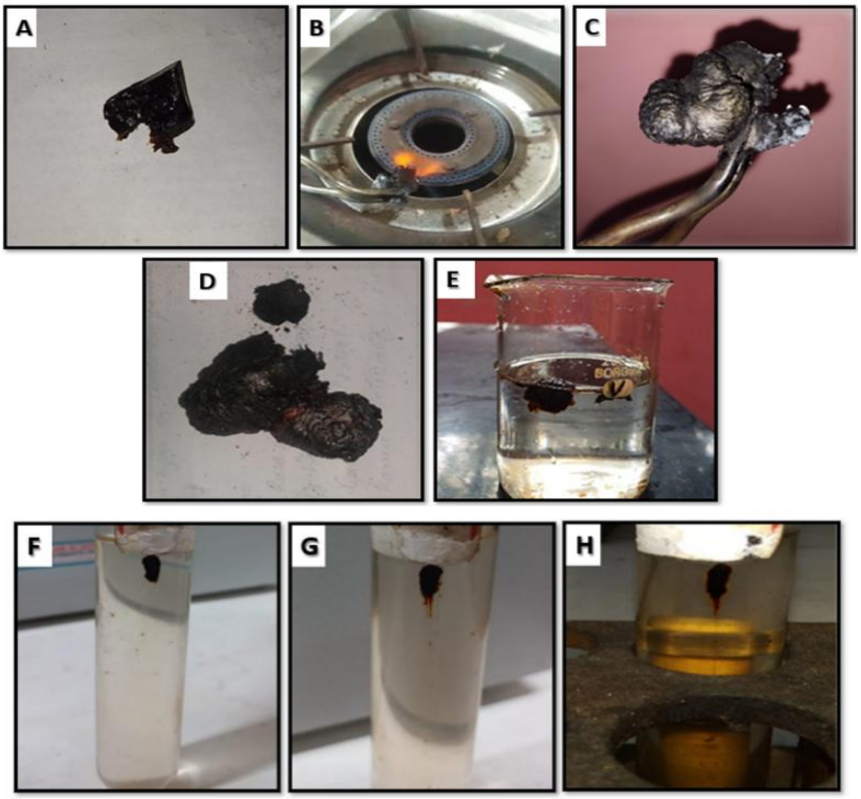



**Fig 2: Quality assessment and authentication of *Shilajathu***

A. A sample piece of the raw *Shilajathu*
B. The raw *Shilajathu* sample being heated in a flame
C. Observation of *Lingakara* formation upon exposure to flame
D. *Lingakara* of *Shilajathu*
E. Residue of burnt raw *Shilajathu* sample exhibiting insolubility in water.
F. A small piece of the raw *Shilajathu* sample placed on the surface of water in a test tube.
G. Thread-like rays emanating from the *Shilajathu* sample in water
H. Thread-like rays from the *Shilajathu* sample reaching the bottom of the test tube and gradually getting dissolved in water.

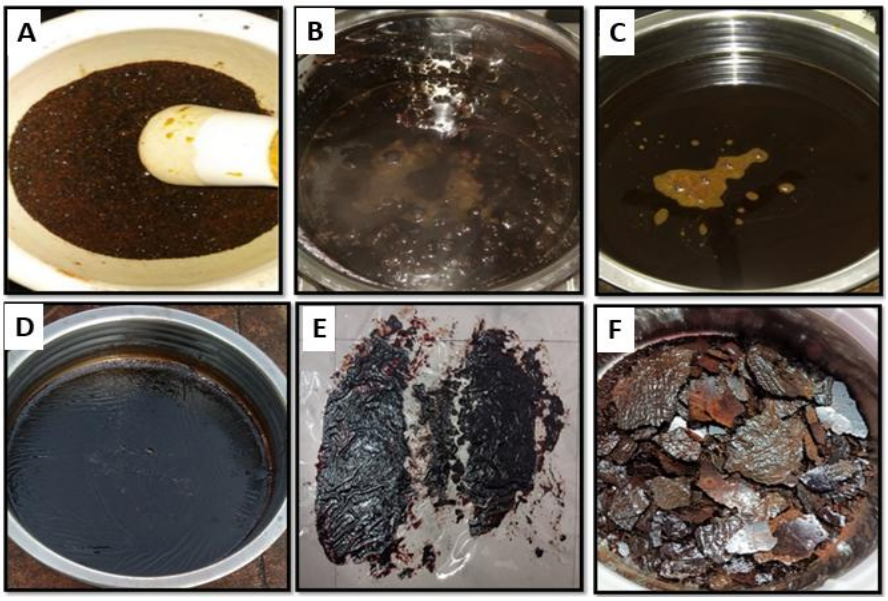



**Fig 3: *Sodhana* of *Shilajathu***

A. Powdered Raw *Shilajathu*
B. Preparation of *Triphala kwatha*
C. Resultant filtrate of *Shilajathu, Triphala kwatha* and hot water mixture, obtained after 3 hrs of exposure to sunlight
D. A layer of blackish creamy scum being formed on the surface of the filtrate.

E. The layers of scum, being collected and spread on a transparent plastic sheet for drying under sunlight.
F. *Sodhitha Shilajathu* (after drying)

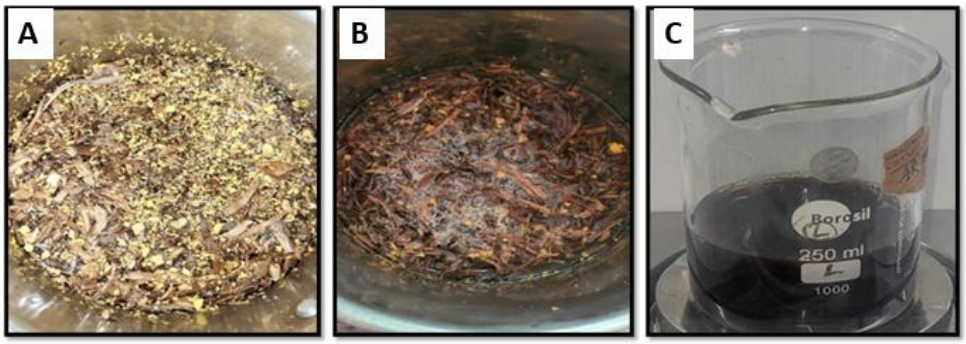



**Fig 4: Preparation of *Ekanayaka kwatha***

A. Chopped and coarsely powdered *Ekanayaka* roots
B. Boiling the coarse powder of *Ekanayaka* root in eightfold water.
C. Filtered *Ekanayaka kwatha*

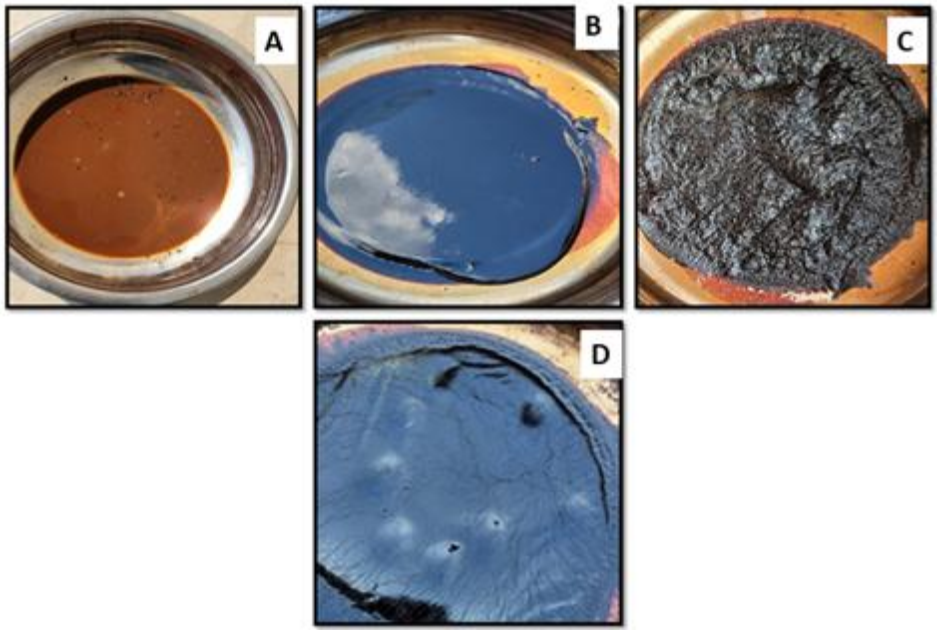



**Fig 5: *Bhavana* of *Shilajathu***

*A.* First *Bhavana: Sodhitha Shilajathu* combined with 180ml *Ekanayaka kwatha*
B. First *Bhavana:* The compound of *Sodhitha Shilajathu* and *Ekanayaka kwatha* getting sun-dried
C. First *Bhavana*: Total drying of the mixture.
D. Seventh *Bhavana:* Partial drying of the compound

**Fig 6. Pills of *Ekanayaka Kwatha Bhavitha Shilajathu***


**Fig 7: Macroscopic Evaluation of *Ekanayaka***

A) *Ekanayka* root bark exhibiting a bright yellowish outer layer

B) Concentric circles revealed on cross-section of the root

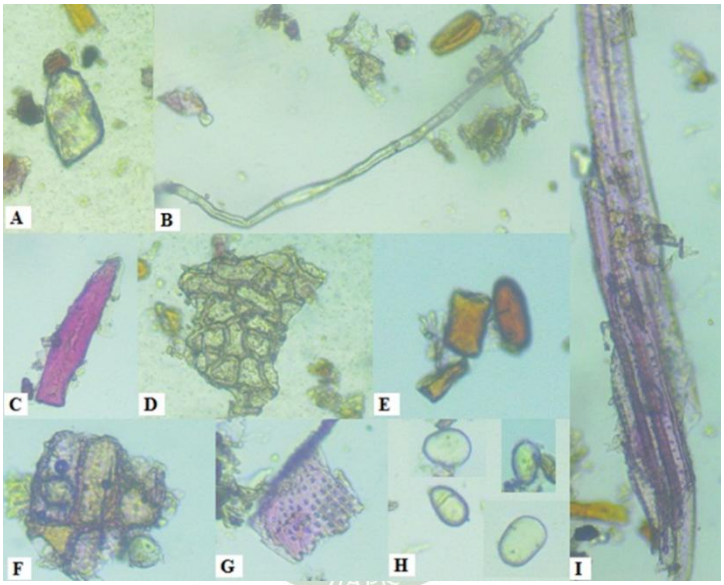



**Fig 8: Powder microscopy of EC**

A. Calcium oxalate crystal
B. Non-lignified fibre
C. Stone cell
D. Fragment of cork cells
E. Coloured content
F. Sclereid
G. Bordered pitted vessel
H. Starch grains
I. Tracheid.

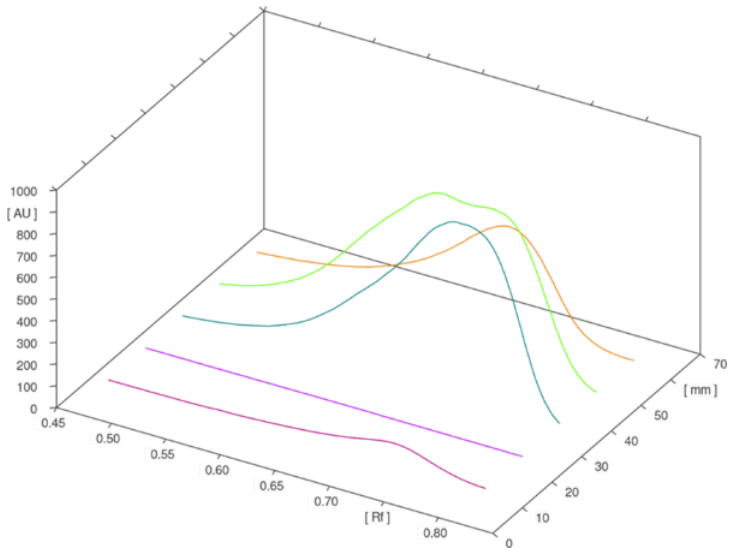
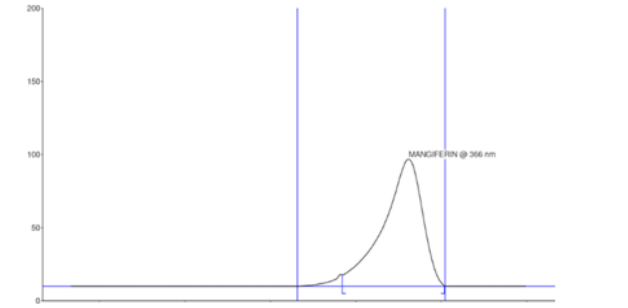
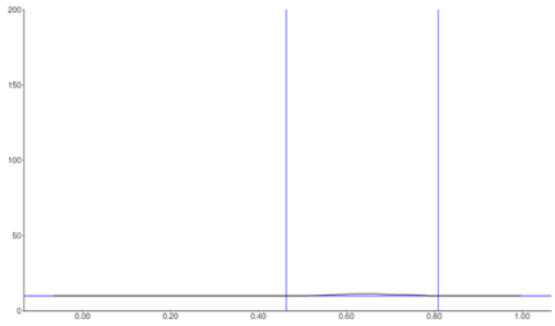



**Fig 9: Densitogram- All tracks (EKBS, SS, EK, EC and Mangiferin) at 366 nm**

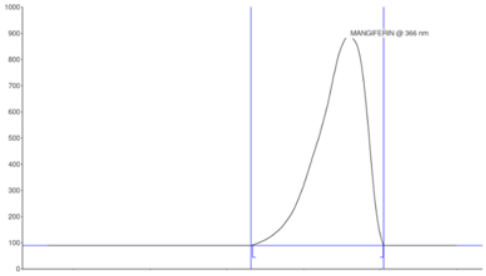



| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.57 | 7.8 | 0.73 | 86.9 | 100.00 | 0.81 | 0.3 | 6905.9 | 100.00 | MANGIFERIN |

A

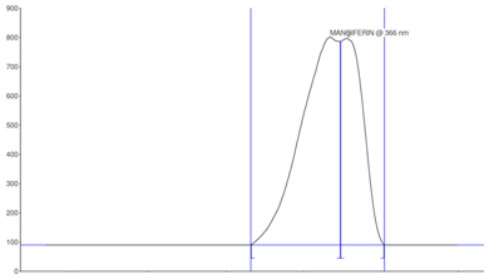


B



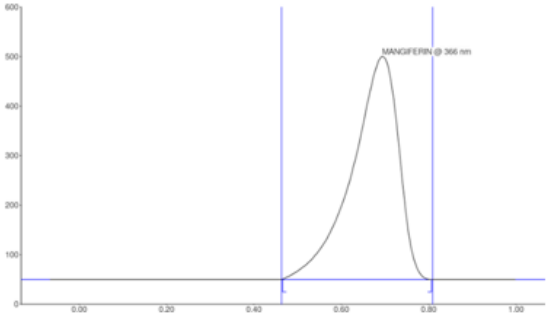

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.47 | 1.8 | 0.72 | 794.5 | 100.00 | 0.81 | 4.8 | 86341.4 | 100.00 | MANGIFERIN |

C




| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.47 | 1.2 | 0.67 | 711.2 | 50.16 | 0.70 | 696.1 | 61239.0 | 63.25 | MANGIFERIN |
| 2 | 0.70 | 696.6 | 0.71 | 706.7 | 49.84 | 0.81 | 1.6 | 35576.0 | 36.75 | unknown * |

D




| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.47 | 1.0 | 0.69 | 450.4 | 100.00 | 0.81 | 0.0 | 41717.0 | 100.00 | MANGIFERIN |

E

**Fig 10: HPTLC peaks at 366 nm: A) Track 1, ID: EKBS, B) Track 2, ID: SS C) Track 3, ID: EK, D) Track 4, ID: EC, E) Track 5, ID: Mangiferin**

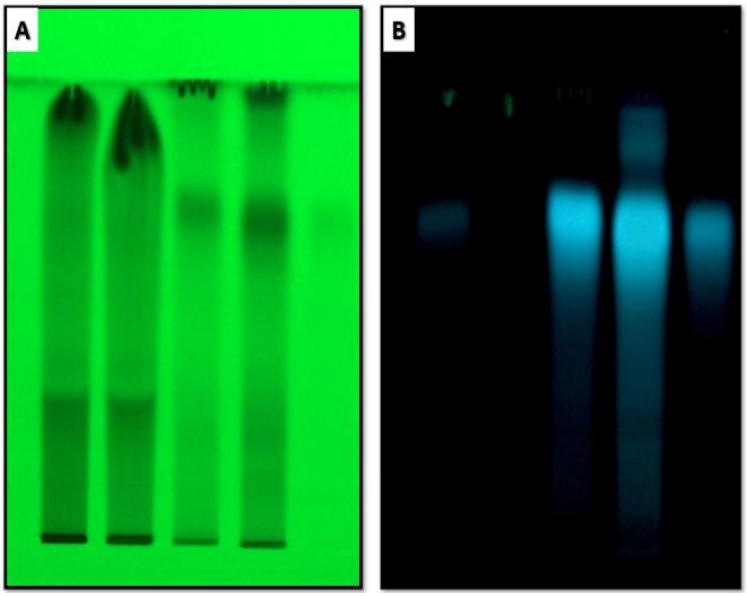



**Fig 11: HPTLC Profile of EKBS, SS, EK, EC and Mangiferin**

A) At 254 nm
B) At 366 nm, Derivatized

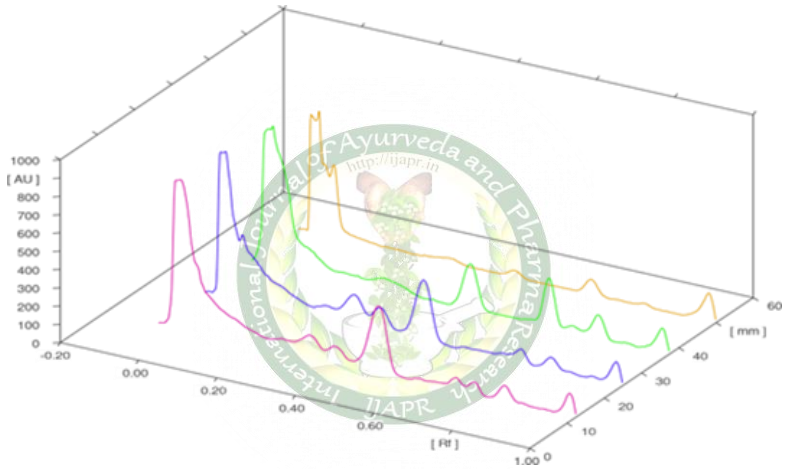
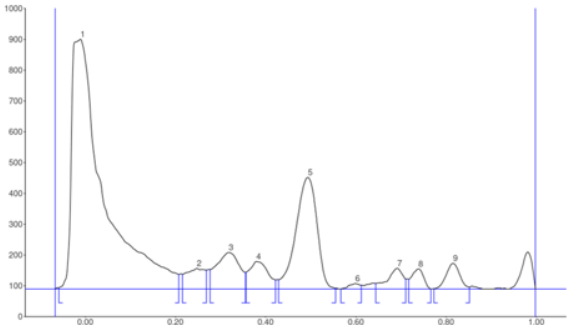
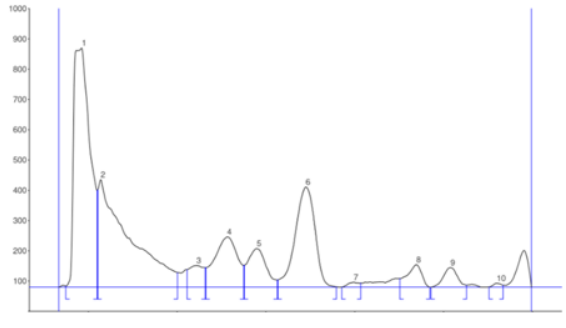



**Fig.12: Densitogram- All Tracks (EKBS, SS, EK, EC) at 254 nm**



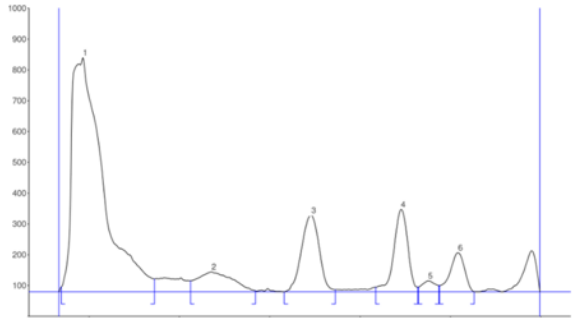
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.06 | 4.4 | -0.01 | 810.6 | 48.19 | 0.21 | 47.4 | 49862.4 | 61.05 | unknown * |
| 2 | 0.22 | 48.2 | 0.25 | 67.1 | 3.99 | 0.27 | 61.5 | 2432.6 | 2.98 | unknown * |
| 3 | 0.28 | 63.6 | 0.32 | 118.7 | 7.05 | 0.36 | 54.2 | 5320.4 | 6.51 | unknown * |
| 4 | 0.36 | 54.8 | 0.38 | 89.5 | 5.32 | 0.42 | 29.8 | 3241.9 | 3.97 | unknown * |
| 5 | 0.43 | 30.2 | 0.49 | 362.3 | 21.54 | 0.56 | 2.9 | 14777.6 | 18.09 | unknown * |
| 6 | 0.57 | 0.9 | 0.60 | 18.0 | 1.07 | 0.61 | 12.4 | 374.3 | 0.46 | unknown * |
| 7 | 0.65 | 18.8 | 0.69 | 67.2 | 3.99 | 0.71 | 32.5 | 1979.8 | 2.42 | unknown * |
| 8 | 0.72 | 32.2 | 0.74 | 65.2 | 3.88 | 0.77 | 0.2 | 1484.2 | 1.82 | unknown * |
| 9 | 0.77 | 1.3 | 0.82 | 83.6 | 4.97 | 0.85 | 6.0 | 2199.3 | 2.69 | unknown * |

**A**



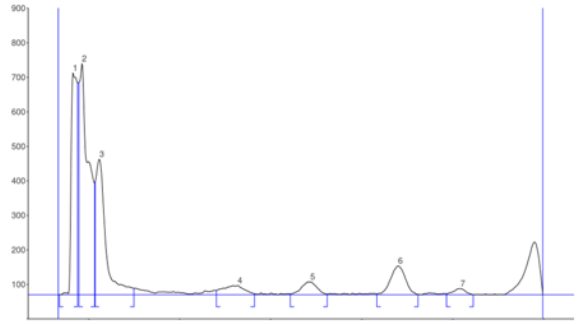
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.05 | 4.3 | -0.01 | 790.8 | 39.30 | 0.02 | 320.2 | 25540.0 | 32.53 | unknown * |
| 2 | 0.02 | 321.9 | 0.03 | 355.8 | 17.68 | 0.20 | 48.7 | 21438.5 | 27.31 | unknown * |
| 3 | 0.22 | 58.2 | 0.24 | 71.8 | 3.57 | 0.26 | 64.9 | 2139.3 | 2.73 | unknown * |
| 4 | 0.27 | 65.0 | 0.31 | 166.4 | 8.27 | 0.35 | 72.5 | 7458.2 | 9.50 | unknown * |
| 5 | 0.35 | 72.9 | 0.38 | 128.0 | 6.36 | 0.43 | 23.9 | 4604.0 | 5.86 | unknown * |
| 6 | 0.43 | 24.4 | 0.49 | 330.7 | 16.44 | 0.56 | 1.1 | 12840.6 | 16.36 | unknown * |
| 7 | 0.57 | 0.8 | 0.60 | 16.4 | 0.82 | 0.61 | 13.9 | 364.7 | 0.46 | unknown * |
| 8 | 0.70 | 29.1 | 0.74 | 74.5 | 3.70 | 0.77 | 0.2 | 2121.1 | 2.70 | unknown * |
| 9 | 0.77 | 0.2 | 0.82 | 64.8 | 3.22 | 0.85 | 6.9 | 1785.6 | 2.27 | unknown * |
| 10 | 0.90 | 0.9 | 0.92 | 13.0 | 0.65 | 0.94 | 6.8 | 209.8 | 0.27 | unknown * |

**B**



| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.06 | 16.5 | -0.01 | 759.9 | 50.62 | 0.15 | 41.5 | 45644.8 | 64.97 | unknown * |
| 2 | 0.23 | 36.0 | 0.27 | 64.7 | 4.31 | 0.37 | 3.2 | 4390.3 | 6.25 | unknown * |
| 3 | 0.43 | 0.0 | 0.49 | 247.8 | 16.51 | 0.55 | 6.7 | 8456.1 | 12.04 | unknown * |
| 4 | 0.64 | 15.0 | 0.69 | 266.9 | 17.78 | 0.73 | 15.7 | 7267.0 | 10.34 | unknown * |
| 5 | 0.73 | 15.7 | 0.75 | 35.4 | 2.36 | 0.78 | 20.2 | 939.8 | 1.34 | unknown * |
| 6 | 0.78 | 20.2 | 0.82 | 126.4 | 8.42 | 0.85 | 0.0 | 3561.3 | 5.07 | unknown * |

C



| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.06 | 0.4 | -0.03 | 642.7 | 34.36 | -0.02 | 613.5 | 7462.5 | 22.98 | unknown * |
| 2 | -0.02 | 615.9 | -0.01 | 669.9 | 35.81 | 0.01 | 324.5 | 12461.6 | 38.37 | unknown * |
| 3 | 0.01 | 328.4 | 0.02 | 392.7 | 20.99 | 0.10 | 18.6 | 7530.4 | 23.18 | unknown * |
| 4 | 0.28 | 12.3 | 0.33 | 26.8 | 1.43 | 0.37 | 2.8 | 1114.4 | 3.43 | unknown * |
| 5 | 0.44 | 2.7 | 0.49 | 37.2 | 1.99 | 0.53 | 1.9 | 1117.1 | 3.44 | unknown * |
| 6 | 0.63 | 2.4 | 0.68 | 83.6 | 4.47 | 0.73 | 1.2 | 2377.6 | 7.32 | unknown * |
| 7 | 0.79 | 2.9 | 0.82 | 17.7 | 0.95 | 0.85 | 0.1 | 416.2 | 1.28 | unknown * |

D

**Fig.13: HPTLC Peaks at 254 nm: A) Track1, ID: EKBS, B) Track 2, ID: SS, C) Track 3, ID: EK, D) Track 4, ID: EC**

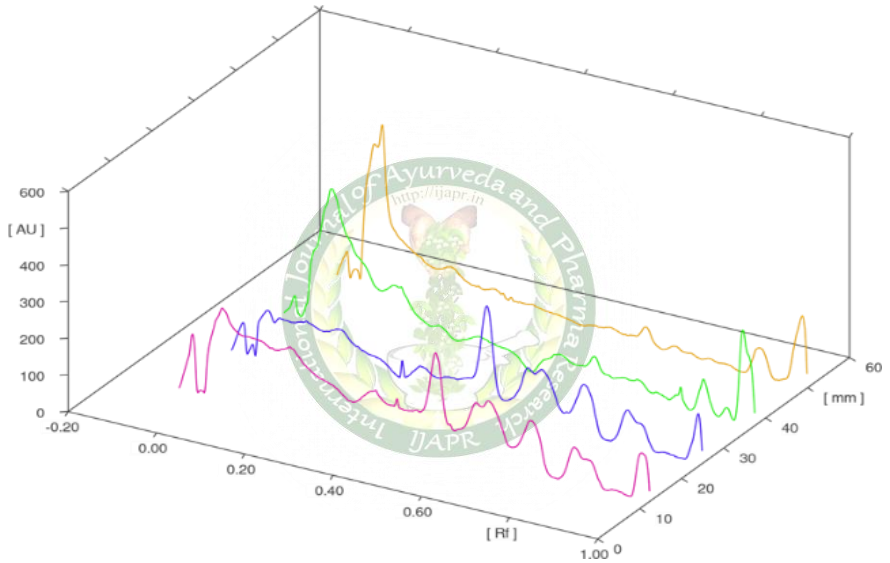
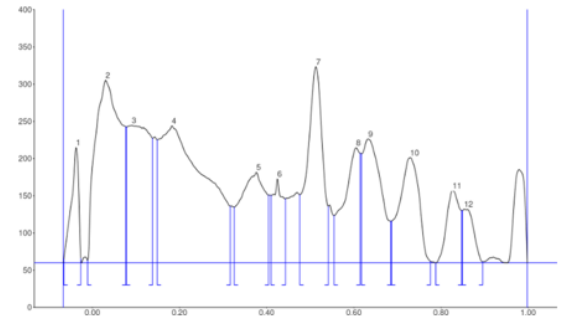
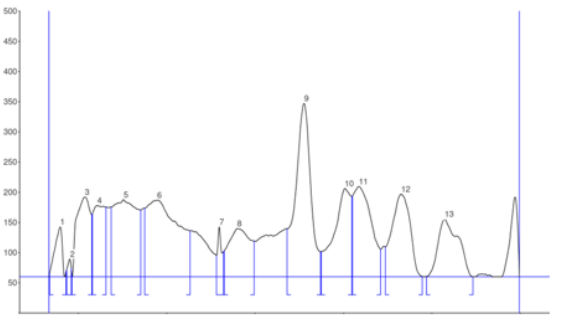



**Fig.14: Densitogram- All tracks (EKBS, SS, EK, EC) At 366 nm**



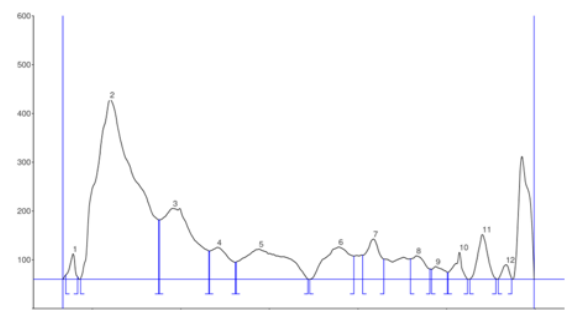
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.07 | 4.5 | -0.04 | 155.0 | 8.16 | -0.03 | 5.9 | 2290.4 | 2.96 | unknown * |
| 2 | -0.01 | 3.2 | 0.03 | 245.4 | 12.92 | 0.08 | 182.9 | 12406.1 | 16.01 | unknown * |
| 3 | 0.08 | 182.6 | 0.09 | 184.6 | 9.72 | 0.14 | 167.5 | 8297.1 | 10.71 | unknown * |
| 4 | 0.15 | 165.5 | 0.18 | 184.0 | 9.69 | 0.32 | 76.2 | 17102.0 | 22.07 | unknown * |
| 5 | 0.33 | 74.8 | 0.38 | 121.9 | 6.42 | 0.41 | 91.1 | 6016.2 | 7.77 | unknown * |
| 6 | 0.41 | 90.8 | 0.43 | 112.8 | 5.94 | 0.44 | 86.1 | 2426.3 | 3.13 | unknown * |
| 7 | 0.48 | 91.6 | 0.51 | 263.4 | 13.87 | 0.54 | 75.9 | 8058.7 | 10.40 | unknown * |
| 8 | 0.56 | 63.8 | 0.61 | 154.6 | 8.14 | 0.62 | 147.3 | 4876.5 | 6.29 | unknown * |
| 9 | 0.62 | 146.5 | 0.63 | 166.4 | 8.77 | 0.69 | 56.1 | 6321.4 | 8.16 | unknown * |
| 10 | 0.69 | 56.2 | 0.73 | 141.3 | 7.44 | 0.78 | 2.1 | 5533.6 | 7.14 | unknown * |
| 11 | 0.79 | 0.0 | 0.83 | 97.0 | 5.11 | 0.85 | 70.2 | 2538.8 | 3.28 | unknown * |
| 12 | 0.85 | 70.4 | 0.85 | 72.2 | 3.80 | 0.90 | 1.5 | 1607.3 | 2.07 | unknown * |

A



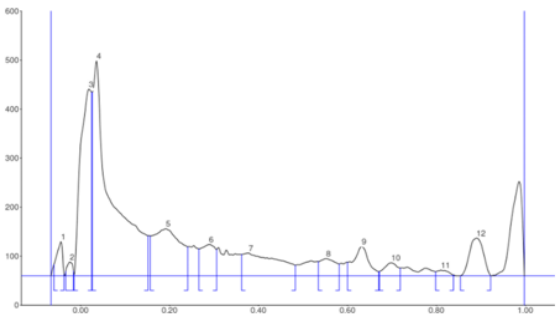
| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.07 | 4.7 | -0.04 | 82.8 | 5.19 | -0.03 | 8.5 | 1188.8 | 2.22 | unknown * |
| 2 | -0.03 | 12.1 | -0.02 | 29.2 | 1.83 | -0.02 | 14.2 | 191.1 | 0.36 | unknown * |
| 3 | -0.01 | 3.3 | 0.01 | 132.1 | 8.28 | 0.03 | 103.1 | 3545.7 | 6.64 | unknown * |
| 4 | 0.03 | 104.6 | 0.04 | 118.1 | 7.41 | 0.06 | 115.6 | 2765.9 | 5.18 | unknown * |
| 5 | 0.07 | 115.6 | 0.10 | 127.5 | 8.00 | 0.14 | 110.8 | 6080.5 | 11.38 | unknown * |
| 6 | 0.15 | 113.8 | 0.18 | 126.9 | 7.96 | 0.25 | 76.2 | 8032.5 | 15.03 | unknown * |
| 7 | 0.31 | 35.1 | 0.32 | 82.4 | 5.17 | 0.33 | 41.8 | 681.8 | 1.28 | unknown * |
| 8 | 0.33 | 43.1 | 0.36 | 79.8 | 5.01 | 0.40 | 59.4 | 3436.2 | 6.43 | unknown * |
| 9 | 0.47 | 79.6 | 0.51 | 287.3 | 18.02 | 0.55 | 41.5 | 8551.1 | 16.00 | unknown * |
| 10 | 0.55 | 41.6 | 0.60 | 146.1 | 9.17 | 0.62 | 133.3 | 4857.7 | 9.09 | unknown * |
| 11 | 0.62 | 133.5 | 0.64 | 149.4 | 9.37 | 0.69 | 45.7 | 5386.0 | 10.08 | unknown * |
| 12 | 0.70 | 49.7 | 0.73 | 136.7 | 8.58 | 0.78 | 0.7 | 4922.6 | 9.21 | unknown * |
| 13 | 0.79 | 0.6 | 0.83 | 95.7 | 6.00 | 0.89 | 0.1 | 3790.1 | 7.09 | unknown * |

B



| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.06 | 9.3 | -0.04 | 51.9 | 4.75 | -0.03 | 5.7 | 535.1 | 0.95 | unknown * |
| 2 | -0.03 | 1.4 | 0.04 | 368.2 | 33.66 | 0.15 | 122.1 | 28384.1 | 50.33 | unknown * |
| 3 | 0.15 | 122.1 | 0.18 | 145.4 | 13.29 | 0.26 | 58.2 | 9235.7 | 16.38 | unknown * |
| 4 | 0.27 | 58.3 | 0.28 | 65.4 | 5.98 | 0.32 | 35.1 | 2399.1 | 4.25 | unknown * |
| 5 | 0.33 | 35.5 | 0.38 | 61.9 | 5.66 | 0.49 | 0.7 | 5335.4 | 9.46 | unknown * |
| 6 | 0.49 | 0.2 | 0.56 | 66.4 | 6.07 | 0.59 | 47.8 | 3342.7 | 5.93 | unknown * |
| 7 | 0.61 | 49.5 | 0.64 | 82.7 | 7.56 | 0.66 | 41.3 | 2285.7 | 4.05 | unknown * |
| 8 | 0.72 | 42.2 | 0.73 | 48.3 | 4.41 | 0.76 | 21.6 | 1310.3 | 2.32 | unknown * |
| 9 | 0.77 | 20.8 | 0.78 | 26.2 | 2.40 | 0.80 | 14.2 | 594.7 | 1.05 | unknown * |
| 10 | 0.81 | 14.3 | 0.83 | 55.4 | 5.07 | 0.85 | 0.4 | 813.3 | 1.44 | unknown * |
| 11 | 0.85 | 0.5 | 0.88 | 92.4 | 8.44 | 0.91 | 1.8 | 1761.1 | 3.12 | unknown * |
| 12 | 0.92 | 0.8 | 0.94 | 29.7 | 2.71 | 0.95 | 1.6 | 398.6 | 0.71 | unknown * |

C



| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.06 | 23.0 | -0.04 | 69.8 | 5.23 | -0.04 | 3.2 | 798.0 | 1.93 | unknown * |
| 2 | -0.03 | 9.4 | -0.02 | 28.2 | 2.11 | -0.02 | 10.2 | 305.2 | 0.74 | unknown * |
| 3 | -0.01 | 2.5 | 0.02 | 380.9 | 28.55 | 0.03 | 374.2 | 8063.7 | 19.53 | unknown * |
| 4 | 0.03 | 374.6 | 0.04 | 438.6 | 32.88 | 0.15 | 81.6 | 15889.5 | 38.48 | unknown * |
| 5 | 0.16 | 81.5 | 0.19 | 96.1 | 7.21 | 0.24 | 59.3 | 5192.5 | 12.58 | unknown * |
| 6 | 0.27 | 55.2 | 0.29 | 63.9 | 4.79 | 0.31 | 53.8 | 1842.8 | 4.46 | unknown * |
| 7 | 0.36 | 43.8 | 0.38 | 46.3 | 3.47 | 0.48 | 21.6 | 3276.2 | 7.93 | unknown * |
| 8 | 0.54 | 29.2 | 0.55 | 35.1 | 2.63 | 0.58 | 24.1 | 1106.4 | 2.68 | unknown * |
| 9 | 0.60 | 27.8 | 0.63 | 59.9 | 4.49 | 0.67 | 8.6 | 1774.2 | 4.30 | unknown * |
| 10 | 0.67 | 9.0 | 0.70 | 26.7 | 2.00 | 0.72 | 16.0 | 695.9 | 1.69 | unknown * |
| 11 | 0.80 | 8.6 | 0.81 | 11.7 | 0.88 | 0.84 | 1.6 | 261.5 | 0.63 | unknown * |
| 12 | 0.86 | 0.5 | 0.89 | 76.9 | 5.76 | 0.92 | 0.3 | 2082.3 | 5.04 | unknown * |

D

**Fig.15: HPTLC peaks at 366 nm: A) Track 1, ID: EKBS, B) Track 2, ID: SS, C) Track 3, ID: EK, D) Track 4, ID: EC**

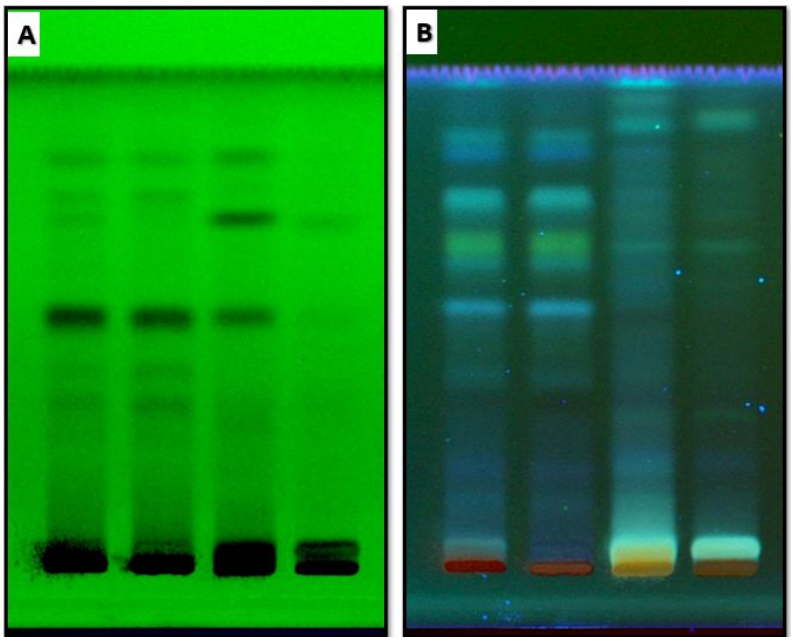



**Fig.16: HPTLC profile of EKBS, SS, EK and EC**

A) At 254 nm
B) At 366 nm

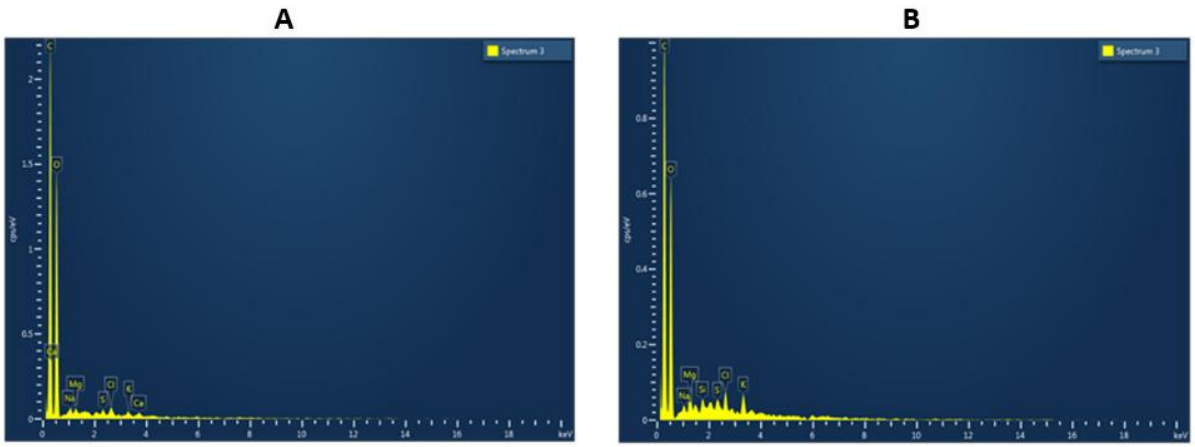



**Fig 17: Elemental Composition by EDAX :** A) SS, B) EK

## DISCUSSION

This study explores the use of *Ekanayaka*, a single, underutilised drug with documented anti-diabetic properties, for *Shilajathu Bhavana*, unlike traditional formulations like *Siva gudika,* which employ multiple drugs. Streamlining the approach promotes drug conservation and minimal effective treatment, tailoring therapy to specific conditions based on the drug's pharmacological action. *Ekanayaka* is widely available in Southern India, particularly in coastal and monsoon forests, likely contributing to its prominence in regional medicine.[15] The *Suryatapi* method might have preserved volatile principles, rendering it therapeutically superior to *Agnitapi*, although further research is needed to confirm this. Conducting *Shodhana* in the summer season (in the month of July) as specified in the ancient literature facilitated rapid scum formation and efficient drying.

*Ekanayaka Kwatha Bhavitha Shilajathu Gutika* was prepared through a multi-step process, including raw drug collection, quality assessment, *Sodhana* of *Shilajathu* using *Triphala Kwatha* and hot water. Notably, *Shilajathu* processed with *Triphala Kashaya* demonstrated exceptional antioxidant properties previously, making it the preferred medium for *Sodhana.*[16] The preparation of *Ekanayaka Kwatha Bhavitha Shilajathu* was successfully accomplished through a seven-fold *Bhavana* process, with a gradual increase in the weight of *Shilajathu* after each step, indicating efficient absorption of *Ekanayaka Kwatha*. The seventh *Bhavana* process was stopped when it yielded a partially dried and moldable consistency, suitable for tablet or pill formation. Pills were prepared according to Rastarangini's dosage guidelines. The pills displayed minor sticking issues, highlighting the necessity of incorporating *Prakshepa choornas* to prevent sticking and enhance efficacy. Ayurvedic texts emphasize *Bhavana*'s role in enhancing *Shilajathu*'s potency and *Rasayana* properties. Repeated soaking/drying may have enhanced the absorption of *Ekanayaka Kwatha*'s bioactive compounds into *Shilajathu* which may be further potentiated by *Shilajathu*'s *Yogavahi* attribute.[17]

The macroscopic evaluation of *Ekanayaka* root confirmed its authenticity and quality, revealing distinctive features such as a bright yellowish outer layer, brown inner surface, and seven concentric circles likely representing annual growth rings. Microscopic analysis of the powder showed calcium oxalate crystals, lignified stone cells, and other anatomical features consistent with *Salacia chinensis and Salacia reticulata*.[18] Phytochemical screening of *Ekanayaka Kwatha Bhavitha Shilajathu* revealed a diverse profile of bioactive compounds, including carbohydrates, flavonoids, phenol, and tannins. However, the *Bhavana* process possibly transformed or degraded glycosides, maintaining the presence of major phytochemicals exhibiting a promising pharmacological profile.[19-20]

HPTLC analysis confirmed the presence of Mangiferin in EKBS, EK, and EC, generating a characteristic chromatographic profile. This profile can serve as a benchmark for quality assurance and verification. Mangiferin is considered a standard biomarker for *Ekanayaka* (*S. chinensis*).[15] Salacia's bioactive compounds, including salacinol, kotalanol, mangiferin, and 13-MRT, exhibit therapeutic potential. Mangiferin, the most studied compound, demonstrates a multifaceted pharmacological profile, showing promise as an antihyperglycemic, alpha-glucosidase inhibitor, and antioxidant agent, among other properties.[18] HPTLC analysis revealed distinct differences between *Bhavitha Shilajathu* and *Sodhitha Shilajathu. Bhavitha Shilajathu* contained mangiferin and additional bioactive compounds matching *Ekanayaka Kwatha*'s Rf values, absent in *Sodhitha Shilajathu.* This suggests unique phytochemicals, potentially greater therapeutic potency or synergistic effects.

Energy Dispersive X-ray Analysis revealed increased weight% of C, Na, Mg, Cl, and K in EKBS compared to that of *Sodhitha Shilajathu*. The EDS data of SS suggests the presence of carbonate minerals, while EKBS indicates the presence of oxide, silicate, chloride, and potassium-containing minerals. The findings suggest that the *Shilajathu* sample may have formed in a sedimentary or hydrothermally altered environment, or possibly from an altered igneous rock. Notably, heavy metal levels were within permissible limits as per API, ensuring the sample's safety for consumption and minimal risk of heavy metal toxicity.

## CONCLUSION

In this study, *Ekanayaka Kwatha Bhavitha Shilajathu* was prepared and its qualitative phytochemical profile and elemental composition in comparison to *Shilajathu* prior to *Bhavana* was evaluated, providing insights into the Ayurvedic concept of *Bhavana*, providing insights into the Ayurvedic concept of *Bhavana.* The findings demonstrate the presence of diverse bioactive compounds, including mangiferin, and reveal enhanced absorption of *Ekanayaka Kwatha's* active principles into *Shilajathu* during the *Bhavana* process. The formulation's safety was confirmed by the detection of heavy metal levels within permissible limits. This research contributes to the development of a standardized, safe, and potentially efficacious

Ayurvedic formulation for managing *Madhumeha,* warranting further research and clinical investigation.

**ACKNOWLEDGEMENT**

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