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Research Article

ASSESSING THE EFFECT OF *SHODHANA* (PURIFICATION) PROCESS BY *TRIPHALA* DECOCTION ON *SHILAJIT* USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Standardization of *Shodhit Shilajit* is a major challenge. **Objective:** Objective of the present study was to assessing the effect of *Shodhana* purification process on *Shilajit* as well as assessing the difference in fingerprint of raw *Shilajit* and *Shodhit Shilajit* by HPTLC and physicochemical tests. **Methods:** The standardization parameters such as appearance, identification of active marker compound Gallic acid and Fingerprint comparison by HPTLC, Physicochemical test parameters like loss on drying, pH value, ash value, water soluble extractive, ethanol soluble extractive and assay of humic acid and fulvic acid were evaluated during the study. Chromatographic separation was achieved by using Toluene: Ethyl acetate: Formic acid (5.0:4.0:1.0 v/v/v) as mobile phase followed by comparing the Rf values. **Results:** The presence of active marker compound gallic acid in *Shodhit Shilajit* was confirmed by comparing the Rf value with standard. HPTLC fingerprint also confirmed that constituents from raw *Shilajit* transferring to the *Shodhit Shilajit* after the process of *Shodhana*. Total Ash value and Acid insoluble ash value reduced from 52.95% to 21.67% and 2.35% to 0.39% respectively while Water soluble extractive value increased from 59.33% to 72.13% after *Shodhana* process. There is also an increase in percentages of humic acid and fulvic acid from 7.10% to 8.23% and 36.91% to 50.12 % in *Shodhit Shilajit.* **Conclusion:** All these results showed the significance and effectiveness of *Shodhana* process in reducing the impurities and ultimately enhancing the therapeutic activity of *Shodhit Shilajit*. These evaluation parameters can be used for the standardization of *Shodhit Shilajit* and also can be routinely employed for analysis in Quality Control Lab.

INTRODUCTION

Shilajit is an exudation of pale-brown to blackish-brown with variable consistency, found in many mountain ranges of the world, especially the Himalayan ranges of the Indian subcontinent. [1]

In herbal formulations *Shilajit* has been used from hundreds of years.[2] According to the *Charaka* there is hardly any curable disease which cannot be controlled or cured with the aid of *Shilajit*.[3-5] So looking into the benefits of *Shilajit* it is widely used in the herbal formulations for curing of disease and to develop immunity. [6]

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But as the source of *Shilajit* is natural exudation from rocks it is exposed to the soil and rocks of the mountain ranges, it is highly susceptible to be incorporated with the adulterants and toxic elements like rock pieces, heavy metal ions, vegetable compounds, reactive free radicals, toxins and soil particles etc. [7-8]

Without proper purification, the use of *Shilajit* may lead to adverse health effects. Hence, the Ayurvedic texts emphasize the use of purified, or *Shodhit, Shilajit* for medicinal purposes.[9-12] Traditional purification methods involve decocting *Shilajit* with *Triphala*, a blend of three medicinal fruits- *Emblica officinalis*, *Terminalia chebula*, and *Terminalia belerica*- in water.[13-19]

Pharmacopoeial book references like Indian Pharmacopoeia, Ayurvedic Pharmacopoeia of India does not contain the monograph of *Shilajit* and being *Shiajit* of herbo-mineral origin the content in *Shilajit*

differ from region to region, and depending on the place from which it was extracted, eventually makes it is complex to standardize each and every content present in *Shilajit*. In this study an attempt has made to assess the difference in physicochemical parameters and HPTLC fingerprint by analysing the raw *Shilajit* and *Shodhit Shilajit*. [20-22]

Keeping the challenges in mind for assessment of *Shodhit Shilajit*, Siddhayu Ayurvedic Research Foundation Pvt. Ltd. has formulated *Shodhit Shialjit* by *Triphala* decoction process mentioned in Ayurveda and evaluated it with respect to identification of Gallic acid marker compound of *Triphala* by HPTLC. Fulvic and humic acid content were also quantified in both the *Shilajit*. [23]

MATERIALS AND METHODS

Material used for *Shodan* of *Shilajit*

Shodhit Shilajit was formulated using raw *Shilajit*, *Amalaki* (*Embelica officinalis), Haritaki (Terminalia chebula)* and *Bibhitaki (Terminalia belerica)*. All these raw materials were screened for identity, purity and strength before *Shodhan*.

Reference standards

Reference standard gallic acid (99%) was purchased from Natural Remedies, Bangalore, India.

Chemicals and reagents

All chemicals used throughout this work were of analytical grade or HPLC grade purchased from Merck Chemicals, India. Stationary phase was pre-coated silica gel aluminium plate 60 F_{254} was obtained from Merck, Germany.

Shodhana (Purification) of *Shilajit* by Tiphala decoction

Awala (*Embelica officinalis*), *Hirda* (*Terminalia chebula*) and *Baheda* (*Terminalia belerica*) dried and cleaned herb material was pulverised and accurately weighed 2.5kg of each material was transferred into a container. About 16 times (120 litres) purified water was added into the materials and heated, till 1/4th volume of decoction remains. The decoction was cooled and filtered. The filtrate (*Triphala* decoction) was transferred in a separate iron vessel. About 50kg of small pieces of cleaned raw *Shilajit* was added to the *Triphala* decoction and allowed to soak for 24 hours. Impurities from raw *Shilajit* like stones and soil etc. was settled at the bottom of the vessel. Upper layer of supernatant suspension was decanted in separate iron vessel and evaporated to obtain a concentrated thick paste having moisture content not more than 20%.The yield of *Shodhit Shilajit* was obtained about 40.4%.

Evaluation of *Shilajit* and *Shodhit Shilajit*

Appearance

Organoleptic characters of the material such as color, odor, and taste were performed.

Physicochemical analysis

Physicochemical test parameters like loss on drying, pH value, ash value, water soluble extractive and ethanol soluble extractive were evaluated as per standard methods mentioned in the Pharmacopoeia. Assay of humic acid and fulvic acid by Gravimetric method was carried out as per standard test procedure.

Identification of Gallic acid, marker compound of *Triphala* decoction in *Shodhit Shilajit* as well as assessing the difference in fingerprint of Raw *Shilajit*, *Shodhit Shilajit* (prepared in-house) and *Shodhit Shilajit* (marketed) by HPTLC.

Instrumentation details are mentioned in below table 1.

Table 1: Instrumentation details

Instruments	Specification
HPTLC instrument	Camag, Switzerland
Sample applicator	Camag Linomat 5
Detection by	Camag TLC scanner 4
Visualizer	TLC Visualizer
Heating by	TLC Plate Heater III
Syringe	Hamilton (100µl)
Software	Win CATS (ver.1.4.9)
TLC Plates	Pre-coated Silica Gel 60 F_{254} TLC Plate

Preparation of Standard solutions

The standard solution of gallic acid was prepared by dissolving 1.0mg in 10ml volumetric flask containing HPLC grade methanol.

Preparation of *Shodhit Shilajit* (In-house) Solution:

1.0g of *Shodhit Shilajit* sample was weighed accurately into a 100ml beaker. 10ml HPLC grade methanol was added to it and sonicated and warmed on water bath for 15 minutes. The solution was filtered

through Whatman filter paper No. 41. This procedure was repeated twice on the remaining residue. All the methanolic extractions were combined and evaporated on water bath. The residue was reconstituted to 20 ml by HPLC grade methanol and filtered through Whatman filter paper. The resulting solution was used as sample solution for the study.

Preparation of *Shodhit Shilajit* (Marketed Sample) Solution

1.0g of *Shodhit Shilajit* marketed sample was weighed accurately into a 100ml beaker. 10ml HPLC grade methanol was added to it and sonicated and warmed on water bath for 15 minutes. The solution was filtered through Whatman filter paper No. 41. This procedure was repeated twice on the remaining residue. All the methanolic extractions were combined and evaporated on water bath. The residue was reconstituted to 20ml by HPLC grade methanol and filtered through Whatman filter paper. The resulting solution was used as sample solution for the study.

Preparation of *Triphala Kadha* (Decoction) Solution

20.0g of *Triphala Kadha* was weighed accurately into a 100ml beaker and evaporated on water bath till dry residue was obtained. The residue was reconstituted to 20ml by HPLC grade methanol and filtered through Whatman filter paper. The resulting solution was used as reference solution for the study.

Preparation of Reference *Awala* herb Solution

500mg herb powder was transferred to 25ml volumetric flask containing 20ml HPLC grade methanol and sonicated for 15 min. The solution was diluted up to the mark with HPLC grade methanol and filtered through Whatman filter paper. The resulting solution was used as reference sample solution for the study.

Preparation of Reference Hirda herb Solution

500 mg herb powder was transferred to 25ml volumetric flask containing 20ml HPLC grade methanol and sonicated for 15 min. The solution was diluted up to the mark with HPLC grade methanol and filtered through Whatman filter paper. The resulting solution was used as reference sample solution for the study.

Preparation of Reference *Baheda* herb Solution

500mg herb powder was transferred to 25ml volumetric flask containing 20ml HPLC grade methanol and sonicated for 15 min. The solution was diluted up to the mark with HPLC grade methanol and filtered through Whatman filter paper. The resulting solution was used as reference sample solution for the study.

Preparation of Reference *Shilajit* (Raw) Solution

1000mg Raw *Shilajit* powder was transferred to 25ml volumetric flask containing 20ml HPLC grade methanol and sonicated for 15 min. The solution was diluted up to the mark with HPLC grade methanol and filtered through Whatman filter paper. The resulting solution was used as reference sample solution for the study.

Chromatographic conditions

Stationary Phase: Silica Gel 60 F_{254} TLC Plate

Mobile phase: Toluene: Ethyl acetate: formic acid (5.0:4.0:1.0 v/v/v)

Application: Apply 10µl of reference and test sample solutions on TLC plate.

Developing distance: 7 cm

Saturation Time: 20 min.

Photograph: At 254 nm

RESULT AND DISCUSSION

Appearance

Raw *Shilajit* stone was appeared as blackish brown coloured hard rocky substance with characteristic odour whereas after purification *Shodhit Shilajit* was appeared as brownish-black coloured semi-solid mass with characteristic odour.

Physicochemical Analysis

Values of physicochemical parameters are useful tools. pH of *Shodhit Shilajit* was reduced by 2.49, which indicates the *Shodhit Shilajit* is slight acidic in nature, which might be obtained because of purification with *Triphala* decoction which is acidic in nature.

Loss on drying is an important parameter for the determination of moisture content. 4.97% loss was found on drying of raw *Shilajit* and 18.62% in *Shodhit Shilajit*.

Total ash value and acid insoluble ash value reduced by 31.28% and 1.96% respectively after purification, which indicates there is almost elimination of contamination from raw *Shilajit* which lead to increase its therapeutic activity in *Shodhit Shilajit*.

Water soluble extractive value increased by 12.80% after *Shodhana* process. It is clearly seen that solubility in water is increased after *Shodhana*. Solubility of *Shodhit Shilajit* in alcohol was not observed as pure *Shilajit* is practically insoluble in alcohol.

Assay of humic acid and fulvic acid both are important active constituents contributing to its potential health benefits in *Shilajit*. Total humic acid assay increased by 1.13%. Similarly the fulvic acid assay showed a notable increase of 13.21% after purification. All these results are tabulated in Table 2.

Table 2: Comparative Result of Physicochemical Parameter of Raw *Shilajit* and *Shodhit Shilajit*

S.No.	Parameters	Raw *Shilajit*	*Shodhit Shilajit*
1.	Description	Brown coloured hard rocky substance, with characteristics odour.	Brownish-black coloured semi-solid mass; odour characteristic.
2.	pH (5 % aqueous solution)	8.63	6.14
3.	Loss on Drying (% w/w)	4.97%	18.62%
4.	Total Ash (% w/w)	52.95%	21.67%
5.	Acid Insoluble Ash (% w/w)	2.35%	0.39%
6.	Water soluble extractive (%w/w)	59.33%	72.13%
7.	Alcohol soluble extractive (%w/w)	12.37%	-
8.	Assay of humic acid by Gravimetry (%w/w) on dried basis	7.10%	8.23%
9.	Assay of fulvic acid by Gravimetry (%w/w) on dried basis	36.91%	50.12%

High-performance thin-layer liquid chromatography analysis

Identification of gallic acid, marker compound of *Triphala* decoction in *Shodhit Shilajit* as well as comparative fingerprinting of raw *Shilajit*, *Shodhit Shilajit* (prepared in-house) and *Shodhit Shilajit* (marketed) by HPTLC was not studied before.

TLC method using Toluene**:** Ethyl acetate: Formic acid (5.0:4.0:1.0 v/v/v) as mobile phase showed the presence of gallic at 254nm in standard gallic acid, *Triphala* decoction, *Awala* (*Emblica officinalis*), *Hirda* (*Terminalia chebula*), *Baheda* (*Terminalia belerica*) reference herbs and *Shodhit Shilajit* (In-House) at Rf 0.32. Gallic acid band was not observed in the *Shodhit Shilajit* (marketed) which clearly indicates that the marketed *Shodhit Shilajit* was not undergone the treatment of *Shodhana* (purification) process.

In comparative fingerprinting of raw *Shilajit*, *Shodhit Shilajit* (prepared in-house) and *Shodhit Shilajit* (marketed) by HPTLC it was seen that four principle bands were observed (at Rf: 0.20, 0.38, 0.56, 0.70) in Raw *Shilajit* which were also observed in the *Shodhit Shilajit* (In-House) confirming that all these constituents from raw *Shilajit* transferring to the *Shodhit Shilajit* after the process of *Shodhana* (purification). Not only these active constituents of raw *Shilajit* are transferring to the *Shodhit Shilajit* but also the active constituents of *Triphala* decoction along with Gallic acid are observed in the *Shodhit Shilajit* which may enhance the therapeutic activity of *Shodhit Shilajit*. The results are shown in Figure 1-7.

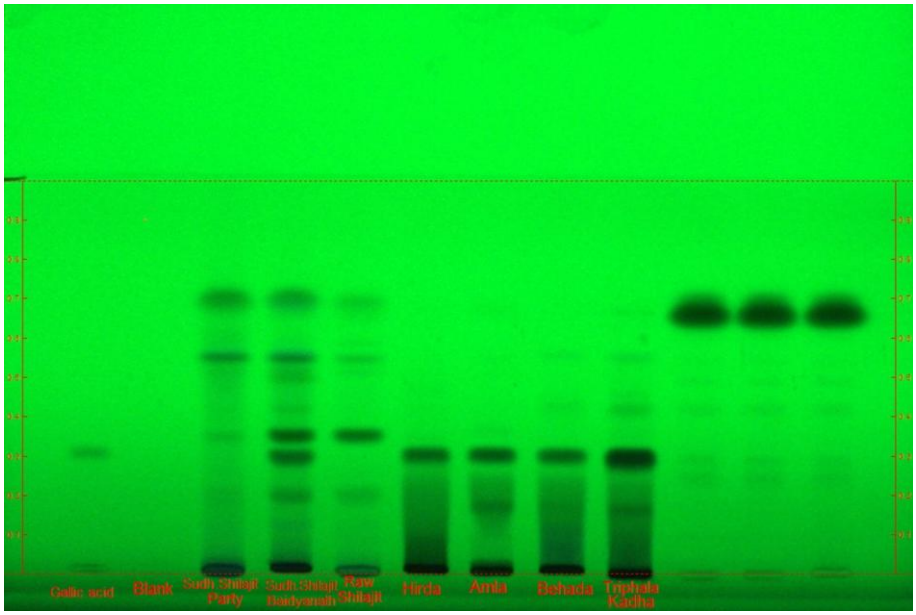

Figure 1: Photograph at 254 nm

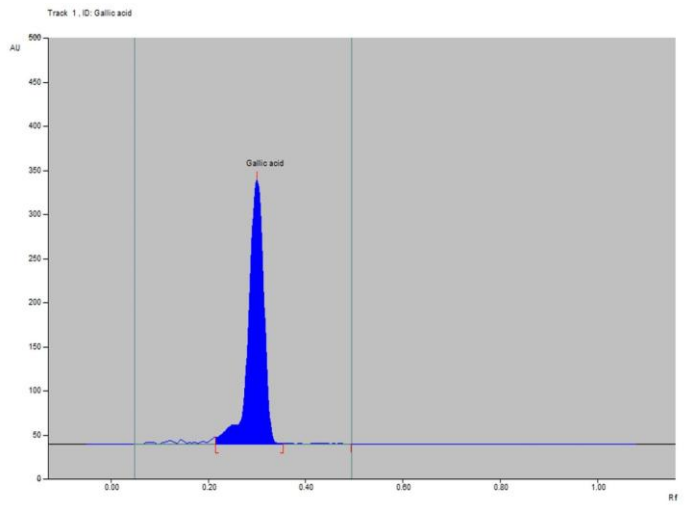

Figure 2: Chromatogram of Standard Gallic Acid

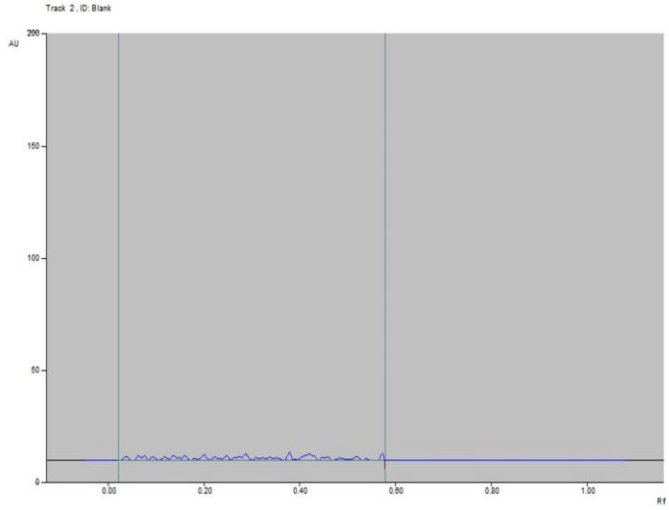

Figure 3: Chromatogram of Blank solution

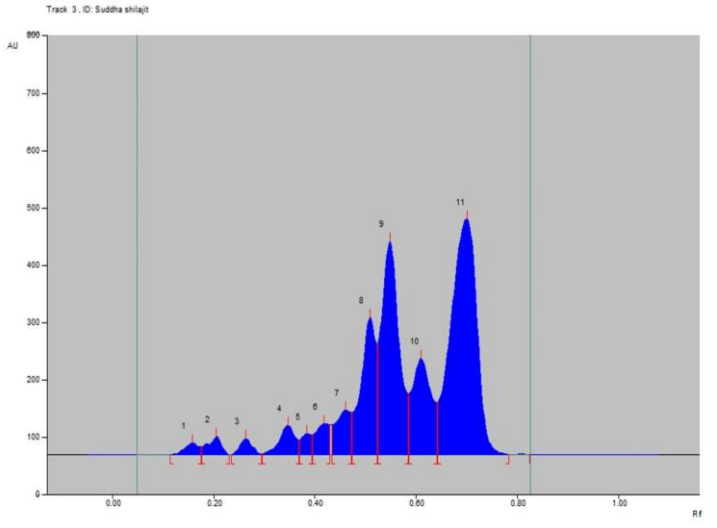

Figure 4: Chromatogram of *Shodhit Shilajit* (Marketed) solution

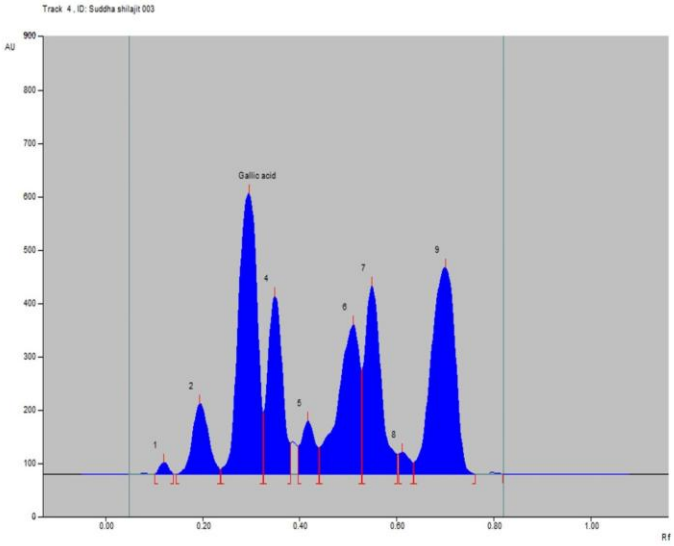

Figure 5: Chromatogram of *Shodhit Shilajit* (In-house) solution

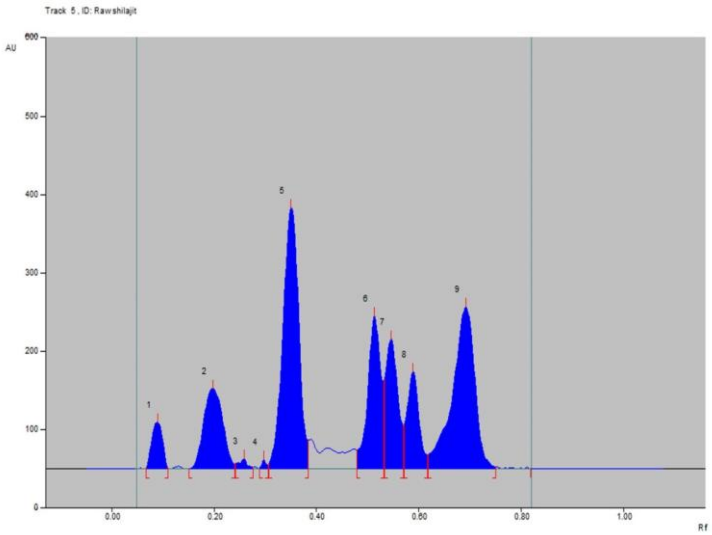

Figure 6: Chromatogram of Raw *Shilajit* solution

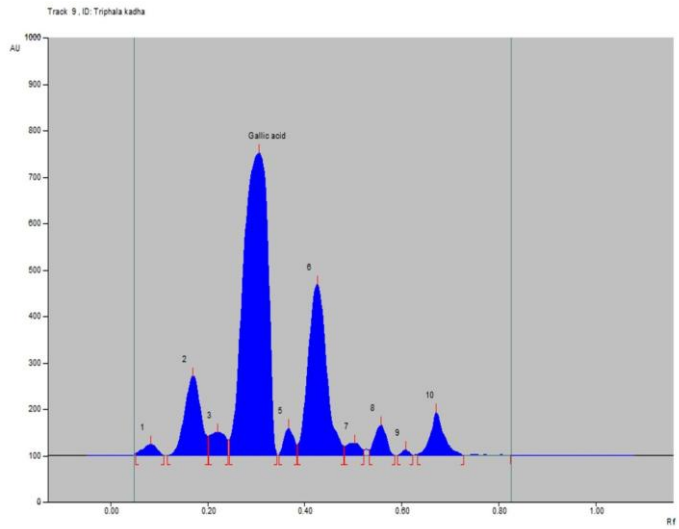

Figure 7: Chromatogram of *Triphala Kadha* (Decoction) solution

CONCLUSION

In the present work *Shodhit Shilajit* was standardized. A special approach was given to the identification of gallic acid, marker compound of *Triphala* decoction in *Shodhit Shilajit* as well as assessing the difference in fingerprint of raw *Shilajit* and *Shodhit Shilajit* by HPTLC.

The developed identification method was found to be simple, rapid, cost effective and able to identify the active constituents.

All these evaluation parameters can be used for the standardization of *Shodhit Shilajit* and also can be routinely employed for analysis in Quality Control Lab.

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