Preparation, method of optimization and physicochemical evaluation of traditional formulation, *Triphala Mashi*

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*Triphala Mashi* was mentioned in *Bharat Bhaishhya Ratnakar* (2522) and *Sharangdhar Samhita-uttar khanda*. *Triphala Mashi* is prepared by using muffle furnace and silica crucible. Physical evaluation of *Triphala* and *Triphala Mashi* was done by using DSC and chemical profile of *Triphala* and *Mashi* was obtained by preliminary phytochemical screening, total organic carbon content, total inorganic content, ascorbic acid content, HPTLC, and IR. DSC thermograms of *Triphala* and *Triphala Mashi*, all are reproducible and can be used as a promising tool for the quality control of the process development. Spectroscopic and chromatographic techniques are proved to be useful in obtaining chemical profile of both *Triphala* and *Triphala Mashi*. These techniques are also useful in studying qualitative and quantitative differences in inorganic as well as organic chemical constituents, thermal degradation and conversion of chemical constituents.

**Keywords:** *Triphala Mashi*, Ayurvedic formulation, *Triphala*

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Current advancements in drug discovery technology and search for novel chemical diversity have intensified the efforts for exploring leads from the Indian System of Medicine, Ayurveda. Ayurveda offers knowledge about herbal and herbomineral formulations and there is current need for scientific validation utilizing modern techniques. The present study is an attempt in this direction and involves scientific investigations based on traditional claims mentioned in texts. *Mashi* means any substance, when burnt, gets blackened and forms amorphous black mass *Mashi*, followed by *Colyrium* and *Bhasma*. The study uses *Triphala Mashi* as prototype traditional formulation. *Triphala Mashi* is mentioned in classical Ayurveda texts, *Bharat Bhaishya Ratnakar* and *Sharangdhar Samhita-uttar khanda*. Study involves physical, chemical profiling utilizing modern techniques. The study also compares the effect of heat treatment on physical and chemical properties as compared to *Triphala* formulation. *Triphala Mashi* is obtained by thermal stress and contain organic as well as bioinorganic constituents and hence utilizes DSC as part of physical profiling. Chemical profiling includes chromatographic, spectroscopic characterization.

*Triphala* (=three fruits) is a traditional Ayurvedic herbal formulation consisting of the dried fruits of three medicinal plants *Terminalia chebula* (Garten.) Retz., *Terminalia belerica* (Garten.) Roxb. and *Phyllanthus emblica* Garten also known as three myrobalan\(^1\). *Triphala* has been described in the ancient Ayurvedic text as a *Tridoshic Rasayana*, an therapeutic agent with balancing and rejuvenating effects on the three humours or constitutional elements in Ayurveda, *vata*, *pitta* and *kapha*. *Triphala* is used in Ayurvedic medicine in the treatment of a variety of conditions and also forms part of many other Ayurvedic formulations. Conditions for which *Triphala* is employed include headache, dyspepsia, constipation, liver conditions, ascites and leucorrhoea. It is also used as a blood purifier, to improve the mental facilities and is reported to posses antiinflammatory, analgesic, antiarthritic, hypoglycaemic and antiaging properties\(^2-6\).

When any natural product from vegetable or animal source is heated slowly, at lower temperature (below 450°C) *Mashi* (Black ash) is obtained. If heating is continued further at higher temperatures (above 450°C), it forms *Bhasma* (white ash). This indicates *Mashi* is nothing but an intermediate product *Bhasma*, where unlike *Bhasma*, both organic and inorganic types of constituents are present.
**Mashi** is dosage form in which bulk of raw material is reduced to a greater extent by application of certain quantum of energy. Due to this treatment hidden chemical constituent become prominent and/or new chemical moieties are formed which are therapeutically active. Also due to thermal degradation or decomposition thermo labile constituent are lost. Thus without application of any costly method for extraction using organic solvents, we can get therapeutic active organic and inorganic chemical constituents in the form of black mass known as **Mashi**, by simple heat treatment in controlled manner. If the **Mashi** is heated further, the burning of carbon particles starts and **Mashi** gradually is converted to **Bhasma**, which is undesirable here. The black colour indicates high percentage of carbon and oxides. Non-specific odour and charcoal like taste may be attributed to oxides, inorganic elements and carbon$^1$.

References about **Triphala** Mashi were found in *Bhaisajyaratnavali* and *Bharat Bhaishya Ratnakar* (Figs 1 and 2). *Bhaisajyaratnavali* mentions harada, behada and amla in the same proportion the iron pot, cover it and heat it$^6$. After converting it to the **Bhasma**, keep it into the glass container. Make its cream in honey and apply it daily, it would heal wound.

*Bharat Bhaishya Ratnakar* mentions **Triphala** and silk cloth in earthen pot and makes its **Bhasma$^7$**. Mix it in sesame oil and use it as external application for wound healing.

### Methodology

All chemicals and solvents used in the experiments were of analytical grade. **Triphala** was obtained from local market. **Triphala** is procured from the local pharmacy, which is prepared by mixing 1:1:1 proportion of dried fruit pulp **Terminalia chebula** (Garten.) Retz., **Terminalia belerica** (Garten.) Roxb. and **Phyllanthus emblica** Garten. 10 gm of **Triphala** powder at a time is used for the preparation of **Triphala Mashi**. Steps involved in optimization of method of preparation included heating in muffle furnace in closed crucibles and heating from 30°C to desired higher temperature by continuously increasing temperature by 10°C/min for about 30 min.

Aqueous and alcoholic extracts of **Triphala** and **Triphala Mashi** were prepared in pure dry powder form. Aqueous extracts (1:6) of **Triphala** and **Triphala Mashi** were prepared by hot maceration method and methanol extracts (1:6) of both were prepared using soxhlet apparatus. The extracts were filtered and the solvent was removed using rotary evaporator. The extracts were stored in airtight glass bottle in refrigerator. In order to observe the thermal pathway of **Triphala** and its **Mashi** form (obtained by heating at 450°C and 600°C), study of **Triphala** and **Triphala Mashi** with reference to thermal stress was carried out on Differential Scanning Calorimeter (DSC)$^{10}$. Thermal pathway of Gallic acid was used as marker to study degradation of chemical constituents. Pattern and details of transitions (exotherms and endotherms) can serve a fingerprint tool in the evaluation. Thermo grams were obtained using DSC. Mettler Toledo DSC 821° module and DSC Star® software were used. Heating program: Heating range: 30°C-550°C; Rate of heating: 10°C/min; Rate of nitrogen flow: 100 ml/min.

The DSC of the selected samples was done by using Aluminum crucibles. The system was purged with nitrogen gas to maintain inert atmosphere.

Chemical profile of **Triphala** and **Mashi** was obtained by preliminary phytochemical screening, total organic carbon content, total inorganic content, HPTLC, and IR. Phytochemical evaluation of aqueous and ethanol extracts of **Triphala** and **Triphala Mashi** were prepared prepared by dissolving 500 mg extract in 20 ml of solvent and subjected to preliminary phytochemical testing for the detection of major chemical groups$^{11}$. The organic matter present in the **Triphala** and **Triphala Mashi** was digested with excess of potassium dichromate and sulphuric acid and the residual unutilized dichromate is then titrated with ferrous ammonium sulphate. For detection of the inorganic constituents in the **Triphala** and **Mashi** they were dissolved in 50% HCL (v/v) and 50% HNO$_3$ (v/v) for an hour or more, then filtered through ash less filter paper. Finally, selective tests were made for detection of the elements. To confirm the presence of gallic acid, plate was developed in a system comprised of Toluene: Acetone: Glacial acetic acid (3:1:2). It was scanned at 254 nM and derivatised using 5 % FeCl$_3$. IR spectras of **Triphala**, **Triphala Mashi**, and Gallic acid were obtained in potassium bromide pellet on FT-IR JASCO 5300 and were expressed in terms of wave number (cm$^{-1}$).

### Results and discussion

In Ayurveda, preparation of **Triphala Mashi** is mentioned by Anter-Dhum padhati method in *Bharat Bhaishya Ratnakar* and Sharangdhar Samhita-uttar
khand. For scientific study it is necessary to develop method of preparation of Triphala Mashi. Three steps are involved in the optimization of method of preparation, viz. equipment optimization, optimization of heating pattern, and temperature optimization. For the optimization of equipment, following trials were done: heating in open air in the earthen pot, heating in oven in silica crucible and earthen pot, and heating in muffle furnace in silica crucible, earthen pot and steel container. Due to certain limitations like temperature control, feasibility, applicability in large scale of other methods, heating in muffle furnace in closed crucible was selected as method of preparation of Mashi. Heating pattern was optimized by heating the Triphala powder from 30°C to desirable higher temperature by continuously increasing temperature at a rate of 10°C/min and heating the Triphala at fixed higher temperature for fixed time. Due to the threat of charring of chemical constituents it is practicable to heat the raw material from low temperature to desirable higher temperature.

Mashi—a dosage form is obtained as a result of thermal stress. So, temperature optimization is the most important step in the study of Mashi. Determination of optimum temperature, at which it maintains its physical, chemical characteristics, is of prime importance. Triphala turns black at around 450°C temperature; hence Mashi preparations at temperatures 450°C, 500°C, 550°C, and 600°C were selected for the study. While preparing Triphala Mashi, free flowing powder of Triphala gets converted into the hard mass. This may be attributed to the changes occurring in the physical and chemical properties of the drug due to thermal stress. During extracts preparation more amount of frothing was observed in Triphala aqueous extract than aqueous extract of Triphala Mashi, alcoholic extract of Triphala and Triphala Mashi, which may be due to presence of more saponins in aqueous extract of Triphala than Triphala Mashi. Colour of different extracts of Mashi was blackish indicating presence of organic carbon in the Mashi form. Difference in the extractive values of Triphala at 450°C and 600°C Mashi indicates that there was more chemical degradation in 600°C Mashi. There was temperature dependent degradation of chemical constituents (Table 1).

All the four samples showed first endothermic peak below 150°C indicating loss of moisture content or volatile matter. All the four samples were having totally different DSC pattern (Figs 3-6). In Triphala, second peak was observed between 161.09°C-212.31°C and third peak between 286.58°C-339.53°C. These two peaks were not observed in the 450°C and 600°C Mashi because these constituents may be lost during formation of Mashi due to thermal stress. Degradation pattern above 350°C is not prominent in Triphala but it was seen in Triphala Mashi because there may be transformation of constituents in this temperature range during formation of Triphala Mashi. DSC pattern of gallic acid was taken as marker to study the degradation of the gallic acid in Triphala Mashi. There was no matching of gallic acid peak with other samples that means there is no degradation of gallic acid when it is present in complex form with other constituents. DSC pattern of gallic acid showed sharp endothermic peak between 266.78°C and 273.73°C, where degradation / decomposition takes place but such transition couldn’t be seen in any other sample like Triphala, Triphala Mashi samples prepared at 450°C and 600°C indicating that degradation / decomposition of gallic acid is not taking place in those samples. This protection may be due to its chemical complex in which it is present in these samples. Further study is required for exploring the correct answer to this. Chromatographic study also supports to the conclusion that decomposition / degradation of gallic acid doesn’t take place up to a temperature 450°C.

Total organic carbon content of Triphala, 450°C and 600°C Mashi was 50.005 %, 47.792 %, 2.443 %, respectively (Table 3). Inorganic content is determined by AAS, and it was observed that there is increase in (% w/w) content of inorganic elements as the temperature increases. This was due to loss of some organic matter from the formulation due to their thermal degradation. Potassium is the major inorganic element present in the Triphala Mashi form (Table 4). Study reveals that degradation/decomposition of

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield % of Triphala Mashi (%w/w)</th>
<th>Aqueous extractive value (%w/w)</th>
<th>Alcoholic extractive value (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triphala</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>450 C Mashi</td>
<td>77</td>
<td>13.16</td>
<td>6.04</td>
</tr>
<tr>
<td>600 C Mashi</td>
<td>47</td>
<td>0.08</td>
<td>0.03</td>
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anthraquinone takes place as temperature increases while phenolic compounds like tannins; flavonoids and ascorbic acid remain stable even at higher temp (Table 2). Ascorbic acid content for *Triphala*, 450°C and 600°C *Mashi* were 0.3337, 0.3187 and 0.04485, respectively (Table 7). Using gallic acid as marker HPTLC analysis of *Triphala* and *Triphala Mashi* was done (Fig. 7). Mobile phase Toluene: Glacial acetic acid (3:1:2) was used for the study and sprayed with 5 % FeCl₃. In this system, alcoholic extracts of *Triphala* and 450°C *Mashi* were compared with the marketed *Triphala* for gallic acid. All the samples showed sharp peaks at RF value 0.44 like authentic sample of gallic acid. This confirms that gallic acid remains present at 450°C.
Fig. 1 Verse from Bhaisajyaratnavali

Fig. 2 Verse from Bharat Bhaisjya Ratnakar

Fig. 3 HPTLC fingerprint using gallic acid as marker

Fig. 4 IR Spectra
acid is protected from degradation if it is present in complex with other constituents.

**Conclusion**

After evaluating the different samples of Mashi as a whole and their extracts, from the above results 450°C was regarded as the optimum temperature for the preparation of Triphala Mashi. Further study is necessary to explore and justify the biological claims of Triphala Mashi, which are mentioned in Ayurvedic texts. Also during TLC studies, some new bands were observed in Triphala Mashi, which were not present in Triphala that may be due to reaction due to heat treatment. So, further fractionation and studying biological effect is necessary to get new lead molecule from Triphala Mashi. There is need to pursue the characterization of active principles, to optimize the observed activities.

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