

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

Yogesh S Biradar, Piush Sharma & K R Khandelwal *

Department of Pharmacognosy, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandwane,
Pune 411 038, Maharashtra
E-mail: khandelwal_kr53@rediffmail.com

Received 21 July 2005; revised 30 October 2006

Triphala Mashi was mentioned in *Bharat Bhaishjya 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*

IPC Int. Cl.⁸: C01, A61K36/00

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 Bhaishjya 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 myrobalan¹. *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 possess antiinflammatory, analgesic antiarthritic, hypoglycaemic and antiaging properties²⁻⁶.

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.

* Corresponding author

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 carbon⁷.

References about *Triphala Mashi* were found in *Bhaisajyaratnavali* and *Bharat Bhaisjya Ratnakar* (Figs 1 and 2). *Bhaisajyaratnavali* mentions harada, behada and amla in the same proportion the iron pot, cover it and heat it⁸. 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 Bhaisjya Ratnakar mentions *Triphala* and silk cloth in earthen pot and makes its *Bhasma*⁹. Mix it in sesamum 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^e 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 groups¹¹. 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% HNO₃ (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 % FeCl₃. 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 Bhaishjya Ratnakar* and *Sharangdhar Samhita-uttar*

khand. For scientific study it is necessary to develop method of preparation of *Triphala Mashī*. 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 *Mashī*. 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.

Mashī—a dosage form is obtained as a result of thermal stress. So, temperature optimization is the most important step in the study of *Mashī*. 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 *Mashī* preparations at temperatures 450°C, 500°C, 550°C, and 600°C were selected for the study. While preparing *Triphala Mashī*, 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 Mashī*, alcoholic extract of *Triphala* and *Triphala Mashī*, which may be due to presence of more saponins in aqueous extract of *Triphala* than *Triphala Mashī*. Colour of different extracts of *Mashī* was blackish indicating presence of organic carbon in the *Mashī* form. Difference in the extractive values of *Triphala* at 450°C and 600°C *Mashī* indicates that there was more chemical degradation in 600°C *Mashī*. 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 *Mashī* because these constituents may be lost during formation of *Mashī* due to thermal stress. Degradation pattern above 350°C is not prominent in *Triphala* but it was seen in *Triphala Mashī* because there may be transformation of constituents in this temperature range during formation of *Triphala Mashī*. DSC pattern of gallic acid was taken as marker to study the degradation of the gallic acid in *Triphala Mashī*. 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 Mashī* 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 *Mashī* 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 Mashī* form (Table 4). Study reveals that degradation/decomposition of

Table 1—Yield % of *Triphala Mashī*, aqueous extractive value, alcoholic extractive value

Sample	Yield % of <i>Triphala</i> <i>Mashī</i> (% w/w)	Aqueous extractive value (% w/w)	Alcoholic extractive value (% w/w)
<i>Triphala</i>		44.76	33.7
450 C <i>Mashī</i>	77	13.16	6.04
600 C <i>Mashī</i>	47	0.08	0.03

Table 2—Preliminary phytochemical screening

Chemical Test	<i>Triphala</i> aqueous	<i>Triphala Mashi</i> aqueous	<i>Triphala</i> alcoholic	<i>Triphala Mashi</i> alcoholic
Alkoids – Dragendorff's reagent test	–	–	–	–
Anthraquinone glycosides – Borntrager test	+	–	+	–
Saponins – Foam test	++	+	–	–
Steroids and Triterpenoids	–	–	–	–
Phenolic compounds Tannins	++	++	++	++
Proteins	–	–	–	–
Ascorbic acid	+	+	+	+

Table 3—Total organic carbon content

Sample	Total Organic Carbon (% w/w)
<i>Triphala</i>	50.005
450 °C <i>Mashi</i>	47.792
600 °C <i>Mashi</i>	2.443

Table 4—Inorganic content

Inorganic element	<i>Triphala</i> (%w/w)	450°C <i>Mashi</i> (%w/w)	Method
Calcium	0.416	0.5937	AAS
Magnesium	0.381	0.5428	AAS
Potassium	1.56	2.2276	Flame photometer
Zinc	0.0027	0.00398	AAS
Iron	0.049	0.07234	AAS
Phosphates	0.231	0.33	Visible spectrophotometer (690 nm)
Sodium	0.035	0.05452	Flame photometre
Sulphates	0.105	0.0759	Visible spectrophotometer (420 nm)
Chlorides	0.126	0.1835	Volumetry
Hydroxides	Nil	Nil	Volumetry
Lead	Nil	Nil	AAS
Aresnic	Nil	Nil	AAS
Mercury	Nil	Nil	AAS

Table 5—Ascorbic acid determination

Sample	Ascorbic acid content (% w/w)
<i>Triphala</i>	0.3337
450 °C <i>Mashi</i>	0.3187
600 °C <i>Mashi</i>	0.04485

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: acetone: 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.

IR spectral of *Triphala* and *Triphala Mashi* were taken to study functional group degradation due to thermal stress (Fig. 8). All the three constituents of *Triphala churna* known to contain gallic acid and therefore IR spectra of gallic acid along with the spectra of *Triphala*, *Triphala Mashi* and gallic acid heated at 450°C were recorded to study gallic acid degradation. The IR spectra of gallic acid showed typical frequencies due to -OH stretching vibrations of both phenolic as well as hydroxyl group of carboxylic acid and carbonyl stretching frequencies¹². Therefore, the bands in the region 3390-3200 cm⁻¹ can be assigned to -OH stretching vibrations. The bands in the region 1725-1600 cm⁻¹ may be assigned to C=O stretching vibrations. All these bands were also observed in *Triphala*, 450°C *Mashi*, 600°C *Mashi* with slight shifting of bands while these bands were not observed in 700°C *Mashi* and gallic acid heated up to 450°C. In all the spectra, there was a band of low intensity at 2360 cm⁻¹, which could not be assigned to any of the frequencies attributable to gallic acid. One more interesting feature observed was the presence of the band of variable intensity in the region of 1250-1200 cm⁻¹ in gallic acid and *Triphala* and absent in thermally treated samples. From the IR spectra of *Triphala churna*, its *Mashi* form, gallic acid and gallic acid heated up to 450°C it is observed that gallic acid is also present in its *Mashi* form of *Triphala*. However, if pure gallic acid is heated to the same temperature it is degraded, it showed that gallic

प्रतिदिन त्रिफला काय स जपया ७००
 छित करते रहना चाहिये ॥ ७ ॥
 त्रिफलाभस्माद्विलेपाः—
 दहेत्कटाहे त्रिफलां समांशां मधुसंयुताम् ।
 उपदंशे प्रलेपोऽयं सद्यो रोपयति व्रणम् ॥ ८ ॥
 हरद, बहेवा तथा भाँवला इन तीनों को समान प्रमाण में
 लेकर छोड़े की कवाही में भर के सकोरे से ढक कर कवाही
 को पृष्ठे पर चढ़ाकर अग्नि प्रज्वलित कर दें। भस्म हो
 जाने पर त्रिफला के शीशी में भर दें। इस भस्म को साहब
 में मकड़म सा बनाकर उपदंश पर प्रतिदिन लेप करने से
 व्रण का रोपण हो जाता है ॥ ८ ॥
 रसाखनं शिरीषेण पच्यया वा समन्वितम् ।
 सञ्चोद्रे वा प्रलेपोऽयं सर्वलिङ्गनादापहः ॥ ९ ॥

Fig. 1 Verse from Bhaisajyaratnavali

(२५२२) त्रिफलासञ्चोद्रेण (२.भि.र.।भवित्.)
 अन्तर्ग्रहविदग्धं त्रिफलासञ्चोद्रेण विमिश्रितं तिलैः ।
 शीमेः शीमे जम्बवत्प्रतिव्रणमायुं लेपेन ॥
 त्रिफला और शीमी कचहरी के हाथीके नीले
 चन्द करनेके भस्म करें । इसे तिलके तिलमें मिलाकर
 लेप करनेके जम्बवत्प्रवण (जामके जलनेके होने
 वाला प्राय) जम्बवत् शीघ्र जप ही जाता है ।
 (शीमे—तिल इत्यादि मिलाकर चाहिये कि
 जिसके मन्त्रम सा बन जाय ।)
 (२५२३) त्रिफलासञ्चोद्रेणः
 (२. भि. र. । भू. सा. । उपदंशः, सा. भ. खं. २. अ. २२)
 दहेत् कटाहे त्रिफलां वा मर्षीं मधुसंयुताम् ।
 कर्वापदंशे जेषीऽये सद्यो रोपयति व्रणम् ॥
 त्रिफलाकी कटाहीमें जलानेकर बीसकर साहबमें
 मिलाकर लेप करनेके उपदंश (जलानेका) के प्राय
 जप हीता है ।
 इति तपान्तरादिलेखनकारणम् ॥

Fig. 2 Verse from Bharat Bhaisjya Ratnakar

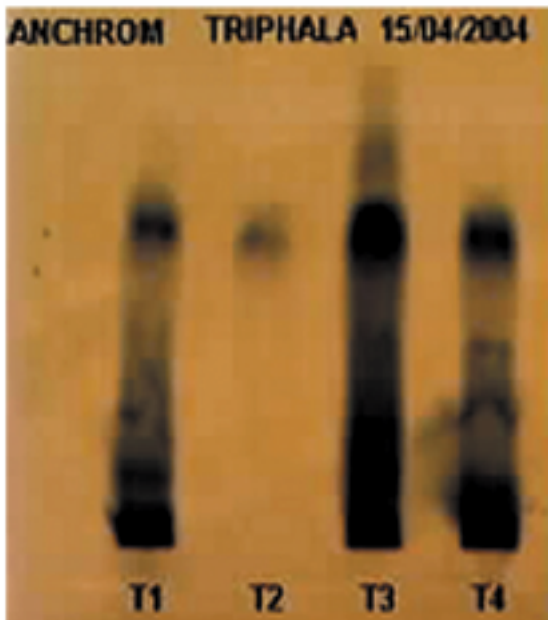
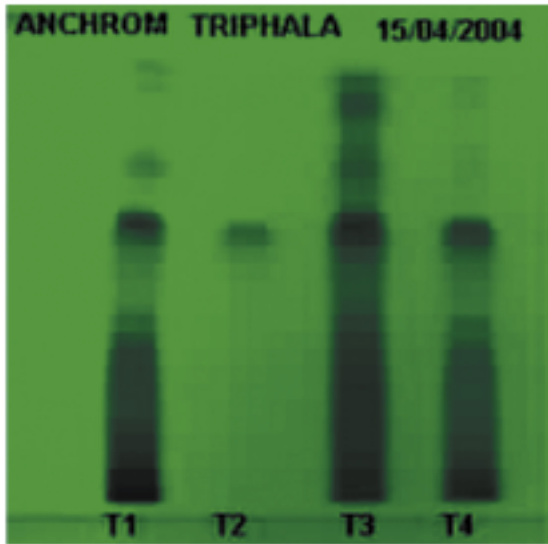


Fig. 3 HPTLC fingerprint using gallic acid as marker

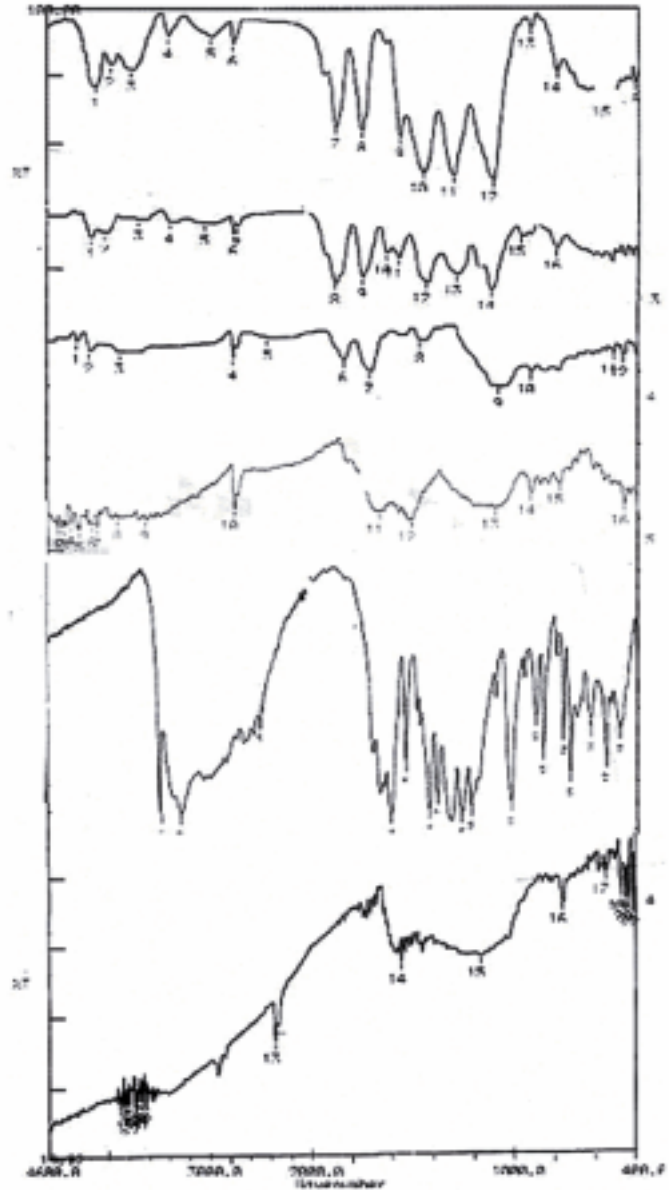


Fig. 4 IR Spectra

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.

Acknowledgement

Authors are thankful to AICTE for the fellowship to Mr Yogesh S Biradar. The generous help of Accurate Analytical Laboratory, Pune-53 (FDA Lic.No. PD/TL-9 dated 13.10.1997) by way of extending the laboratory facilities is gratefully acknowledged.

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