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Original Research Article (Experimental)

Preparation and physicochemical characterization of ingredients of Indian traditional medicine, *Mahamrutyunjaya Rasa*

Pallavi Rai [a, *], Sadhana J. Rajput [b]

[a] *RamEesh Institute of Vocational and Technical Education, Greater Noida, Uttar Pradesh, 201310, India*
[b] *Pharmacy Department, G. H. Patel Building, The Maharaja Sayajirao University of Baroda, Gujarat, 390 002, India*

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ABSTRACT

Background: *Mahamrutyunjaya rasa* is an ayurvedic formulation used in the treatment of cardiac disorders. It contains the purified roots of *Visa* (*Aconitum ferox*), *Brihati* (*Solanum indicum*), fruits of *Pippali Kana* (*Piper longum*), *Marica* (*Piper nigrum*), *Gandhaka* (Sulfur), *Hingula* (Cinnabar) and *Tankana* (Sodium metaborate) as per *Bhaishajya Ratnavali*. The purification (*shodhana*) process changes the physiochemical properties of the raw materials which need to be studied and understood.
Objective: The present work aims to perform a comprehensive physicochemical characterization of raw materials, intermediates and the final product obtained during purification, using modern analytical techniques.
Materials and methods: The standard methods as per traditional text were followed and the physicochemical changes were also investigated by collecting samples at different steps of purification. The samples were analysed using various techniques, viz. Fourier transform infra-red spectroscopic (FTIR), X-ray diffraction (XRD), Differential Scanning Calorimeter (DSC) and High Performance thin Layer chromatography (HPTLC).
Results: The FTIR and HPTLC analysis of the alkaloidal extracts of *Visa* showed loss of an ester group with shift in the peaks from 1720 cm^{-1} (C=O stretching of esters) to 1676 cm^{-1} (C=O stretching of Ketone) which signifies the conversion of alkaloid Aconitine (LD_{50} – 0.08 mg/kg) to Benzoylaconine (LD_{50} – 24 mg/kg) improving its safety. The analysis of *gandhaka* by XRD and DSC showed that purification brought about transformation of orthorhombic sulphur into monoclinic sulphur and it reverted back to original form with higher purity. The treatments given to *gandhaka* and *hingula* with organic compounds made them homologous to the body tissues. Analysis of purified *tankana* showed that the processing led to loss of water and slight change in the crystal structure with the shift in the endothermic peak from 110.6 °C to 104.2 °C.
Conclusion: Thus, the present study provides a scientific backing to the methodologies used by Ayurvedic practitioners. The study also provides physicochemical fingerprints for the standardization as well as characterization of raw materials and forms a technical platform for manufacturers to develop quality control standards.

1. Introduction

Ayurvedic medicine originated in India more than 2000 years ago and it makes use of herbs, metals and minerals for curative effects. Therapeutic effectiveness of the Ayurvedic drugs has been established and well documented in the form of classics attributed to them. However, lots of changes have occurred since the time these classics were written and the impact of these changes on the therapeutic efficacy of the preparations formulated has not been ascertained [2]. Further the art of preparing the formulations requires certain amount of expertise and no information is documented about the likely impact of changes in the manufacture techniques or improper preparation on the expression of biological activity including possibility of production of undesirable effects [15].

Ayurveda does not use heavy metals or minerals without extensive processes, like *shodhana* or purification to render them fit

* Corresponding author.
E-mail address: raipallav@gmail.com (P. Rai).
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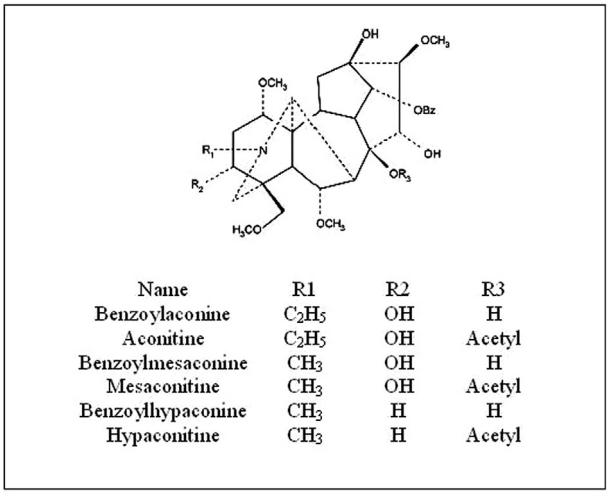
for human consumption. In this regard, it uses drugs medicinally but in a careful, complex and safe manner [13]. In this way, Ayurveda can employ the great healing power of minerals while avoiding their side effects [1]. It is believed that the *shodhana* process converts the metal into its specially desired chemical compound which eliminates the toxicity of the metal and has the necessary medicinal benefits [14].

Mahamrutyunjaya Rasa (MHR) is a compound herbal-mineral formulation often used to treat cardiac disorders. *Bhaishajya ratnavali* records the formula of MHR tablet as 1 part each of processed *Visa* (*Aconitum ferox*), *Brihati* (*Solanum indicum*), *Pippali Kana* (*Piper longum*), *Marica* (*Piper nigrum*), powdered and sieved through 100 mesh sieve. It is then mixed with 1 part purified *Gandhaka* (Sulfur), 1 part purified *Tankana* (Sodium metaborate) and 2 parts of purified *Hingula* (Cinnabar) [4].

The ingredients like *Visa*, *Gandhaka*, *Hingula* and *Tankana* have to be processed before internal administration as per the ayurvedic literature [17]. The principal active ingredients in *Visa* are C19-diterpenoid alkaloids, including aconitine, mesaconitine and hypaconitine (Fig. 1). However, these alkaloids are toxic with a very narrow safety range, because they easily induce ventricular tachycardia and fibrillation even at therapeutic dose levels. Down the ages, various processing methods were developed to reduce their toxicity during which the drug still retains pharmacological properties while their toxicity is reduced about a hundred-fold [8]. Hingula (HgS) is another ingredient in MHR. Hingula is insoluble, has very low bioavailability and its long-term use is major cause of mercury intoxication, but at the therapeutic doses, the adverse effects hingula-containing traditional medicines seem to be tolerable and reversible [6]. Gandhaka is also one of the ingredients. Gandhaka is mild laxative, detoxifying. Improperly purified gandhaka medicine if consumed over a long period causes toxic effects like dyspepsia, flatulence. Tankana is also added in MHR. In ayurveda, it is given internally in acidity of the stomach, amenorrhoea and to promote uterine pains during labour. Tankana is not acutely toxic with the LD_{50} 2.66 g/kg in rats. Consumption over a long duration of time may cause gastrointestinal distress including nausea and diarrhoea [23].

Extensive literature is available regarding the pharmacological actions of all these components. The purification procedures are also well documented but none of the methods have been studied in detail to determine the structural and chemical changes taking place in the ingredients, which is essential requirement to discuss the non-toxicity and therapeutic value of such formulations. Since these preparations are sustaining themselves since centuries in clinical use, therefore one cannot exclude its use [11]. In this report, an attempt was made to derive certain standard data which may form the basis of quality control of the raw materials present in the formulation. The standard methods as per traditional text were followed and the physicochemical changes were also investigated by collecting samples at different steps of purification. The samples were analysed using various techniques, viz. Fourier transform infra-red spectroscopy (FTIR), X-ray diffraction (XRD), Differential Scanning Calorimeter (DSC) and High Performance Thin Layer Chromatography (HPTLC).



Name	R1	R2	R3
Benzoylaconine	C_2H_5	OH	H
Aconitine	C_2H_5	OH	Acetyl
Benzoylmesaconine	CH_3	OH	H
Mesaconitine	CH_3	OH	Acetyl
Benzoylhypaconine	CH_3	H	H
Hypaconitine	CH_3	H	Acetyl

Fig. 1. Chemical structures of benzoylaconine, aconitine, benzoylmesaconine, mesaconitine, benzoylhypaconine and hypaconitine.

2. Experimental

2.1. Materials

Dried roots of *Visa*, were purchased from the local store and identified by NISCAIR, Delhi. Hingula was purchased from a local ayurvedic store. Gandhaka, Tankana, Chloroform, Toluene, and all other solvents of analytical grade were purchased from Qualigens (Mumbai).

2.2. Method of preparation

The following methods of purification were used as per the reported methods [14] and samples were withdrawn at intermediate and final steps for analysis.

2.3. Purification of aconite alkaloids

The aconite roots were washed with water and soaked in cow urine for 48 h. It was then washed with water and boiled in milk. The drug was again washed with water and dried. The samples of crude drugs were collected and the alkaloid fraction was subjected to HPTLC and IR studies. The samples were collected at following steps (i) Pure Drug (A-1), (ii) Washed with water (A-2), (iii) Soaked in cow urine for 24 h (A-3), (iv) Soaked in cow urine for 48 h (A-4), (v) Washed with water and boiled with milk (A-5), (vi)Washed with water and dried (A-6).

2.4. Purification of Gandhaka

Gandhaka mixed with ghee (a cow milk preparation) in an iron vessel was heated up to its melting temperature and the resulting liquid was poured through a filter into a vessel containing boiled milk. The final product was taken out, washed with hot water and dried. The samples at following steps were collected and subjected to XRD and DSC studies. (i) Crude Gandhaka (S-1), (ii) Gandhaka treated with cow ghee (S-2). (iii) Melted mixture in iron vessel (S-3), (iv) Mixture washed with cow milk (S-4). (v) The mixture washed with water and dried (S-5).

2.5. Purification of Hingula

Crude hingula was soaked in lemon juice until the colour of the powder became dark red. It was then washed with water and the same process was repeated seven times. After final treatment hingula was dried. The samples were collected at following steps and subjected to XRD. (i) Crude hingula (H-1), (ii) Hingula treated

with lemon juice-three times (H-2), (iii) Hingula treated with lemon juice-seven times (H-3).

2.6. Purification of Tankana

The purification of *Tankana* was done by heating till constant weight was obtained. The moisture free *Tankana* was stored in air tight container. The samples were collected at the following steps and analysed using XRD and DSC. (i) Crude *tankana* (B-1), (ii) *Tankana* heated for 1 h at 80 °C (B-2), (iii) *Tankana* heated for 2 h at 80 °C (B-3) (iv) Sample weight was constant in three consecutive weighing (B-4).

2.7. Characterization

For powder X-ray diffraction (XRD) a Philips 1710 X-ray diffractometer with CuKα radiation (λ = 1.5418 **Å**) operating at 30 KV and 20 mA was used. Pattern was recorded for the angle (2θ) ranging from 5 to 80° at a scanning rate of 3°/second. The results were compared with the values of standard substances [7].

IR spectra in the region (4000–450 cm^{-1}) were recorded on Perkin Elmer FTIR spectrophotometer (Perkin Elmer – 377) in KBr pellets.

Thermograms were obtained using DSC. Mettler Toledo DSC 821° module was used. 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 Aluminium crucibles. The system was purged with nitrogen gas to maintain inert atmosphere.

For the HPTLC studies, the alkaloid fraction of each sample was used. It was prepared by treating 1 gm sample with ammonia and extracting with ethyl acetate. The extract was concentrated and evaporated under vacuum. A 10 mg/ml solution of alkaloid fraction was prepared in chloroform. A Camag microlitre sample (Hamilton, Bonaduz, Switzerland) syringe was used for sample application on pre-coated silica gel aluminium plate 60F-254, 20 cm × 10 cm with 0.2 mm thickness, (E. Merck, Darmstadt, Germany) using a Camag Linomat-V (Switzerland). The linear ascending development was carried out in Toluene: Ethyl Acetate: Diethyl amine (7:2:1 v/v). Densitometric scanning was performed on Camag TLC scanner III in the reflectance–absorbance mode for all measurements and operated by CATS software (V1.4.3 Camag). The plate was scanned at 235 nm. The plate was sprayed with Dragendorffs reagent immediately scanned at 500 nm and data of peak area and height of each band were recorded.

3. Results and discussion

3.1. HPTLC studies of Visa alkaloids

The contents of *Visa* alkaloids in different extracts of processed aconite roots and unprocessed aconite roots were compared using HPTLC. The representative HPTLC chromatograms are shown in Fig. 2(a and b). The tracks from 1 to 6 show that there was a gradual chemical degradation with decrease in the concentration of two alkaloids, and increase in concentration of other alkaloids (Table 1).

3.2. IR studies of V alkaloids

The IR spectra of the six samples have been shown in Fig. 3. IR (KBr): 3435 (O–H stretch), 2929 and 2819 (Alkyl C–H stretch), 1720 (C=O stretching of esters), 1676 (C=O stretching of Ketone), 1601 (C=C stretching of ring), 1514 (N–H bending vibrations), 1462 (C–H Symmetrical bending vibrations of cycloalkanes), 1362 (C–H Asymmetrical bending vibrations of cycloalkanes), 1294 (C–O or O–H stretch), 1271 (Asymmetrical C–O–C stretch), 1224 (C–O stretch, 1177 (C–C(=O)–O saturated esters)), 1096 (Symmetrical C–O–C stretch), 1024 (Aromatic ethers ($O–CH_2$)), 985 and 765 (Out of plane –C–H bend). The peak at 1720 cm^{-1} disappears gradually till A-6 and is replaced with a peak at 1676 cm^{-1} depicting the conversion ester to keto-group. The peak at 1294 cm^{-1} is also lost which depicts the C–O or O–H stretch. Thus, there is an alteration in the ester group of the alkaloids.

3.3. XRD studies of gandhaka

Fig. 4 shows the XRD patterns of gandhaka samples. The d-spacing values of gandhaka samples were compared with coinciding values of the reference standards of various allotropes of gandhaka. The pattern of S-1 shows that the raw material *gandhaka* (sulphur) has a number of peaks coinciding with the reference orthorhombic sulphur in the Fddd space group (Table 2). The diffraction pattern of S-2 shows that the number of peaks coinciding with orthorhombic sulphur increase, depicting the increase in that form of gandhaka. The pattern of S-4 shows the presence of only two intense peaks which are found to be present in monoclinic type of gandhaka crystal. Again in S-5, the peaks coincide with the orthorhombic sulphur. However, the peaks are even sharper which reflect higher purity of the final product.

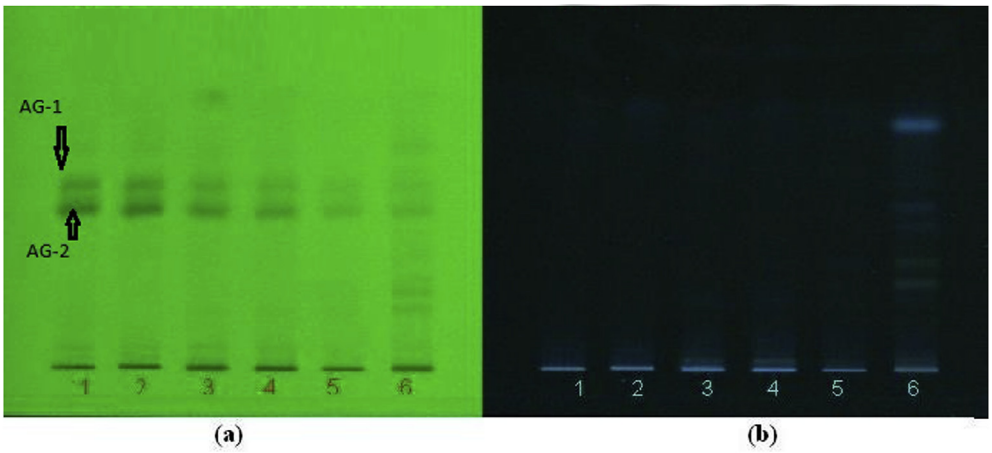


Fig. 2. HPTLC chromatograms of *Visa* alkaloids (a) Image at 254 nm and (b) Image at 366 nm.

Table 1
Results of the finger printing analysis of Aconitum alkaloids.

Sample	Concentration µg per spot	Peak	Rf value	Height	Area	% Height	% Area
A-1	100	AG-1	0.42	210.3	6295.1	44.77	41.28
	100	AG-2	0.48	149.7	5618.0	31.86	38.58
A-2	100	AG-1	0.42	160.6	6092.4	41.12	40.21
	100	AG-2	0.48	144.3	5294.0	29.26	32.79
A-3	100	AG-1	0.42	148.9	5748.1	30.00	35.40
	100	AG-2	0.48	135.3	5070.5	26.25	33.28
A-4	100	AG-1	0.42	129.9	5309.5	24.88	28.74
	100	AG-2	0.48	126.8	5142.2	24.01	32.98
A-5	100	AG-1	0.42	120.2	5018.5	20.30	25.22
	100	AG-2	0.48	116.5	5093.7	21.04	29.14
A-6	100	AG-1	0.42	114.6	4974.6	16.39	22.06
	100	AG-2	0.48	100.3	5325.6	14.89	24.72

3.4. Thermal studies of Gandhaka

All the thermograms (Fig. 5, Table 3) show two sharp peaks up to the temperature of 122 °C. The peaks displayed for S-1, S-2 and S-5 are similar with changes in the number and sharpness of the peaks. A small endothermic peak is observed at 115.61 °C in the S-5 sample which may be due to a different type of allotrope of gandhaka formed during the heating procedure.

3.5. XRD studies of hingula

The pattern of H-1(Fig. 6) when compared with the reference XRD pattern shows that hingula is present along with other components. In the intermediate sample (H-2), the peaks of other components are reduced. Further in the final XRD spectra of H-3, a number of peaks are absent and the intensity of remaining peaks is increased. The d-spacing values of H-3 matched with the reference data showing high purity of hingula in the trigonal trapezohedral crystalline form. (Table 4)

3.6. XRD studies of Tankana

The XRD spectra of tankana (Fig. 7) depicts that there was a gradual loss in the sharpness and number of peaks from B-1 to B-4.

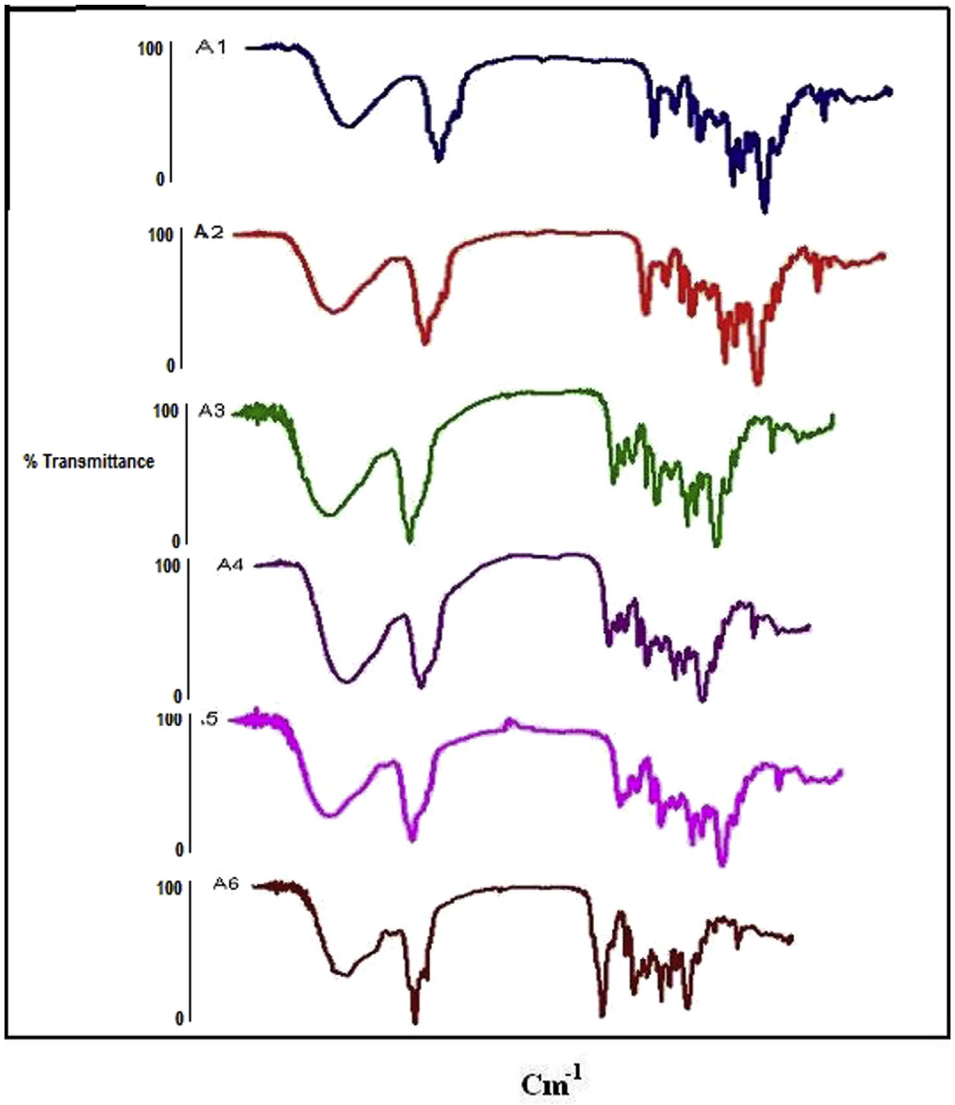


Fig. 3. IR spectra of samples of *Visa* extracts.

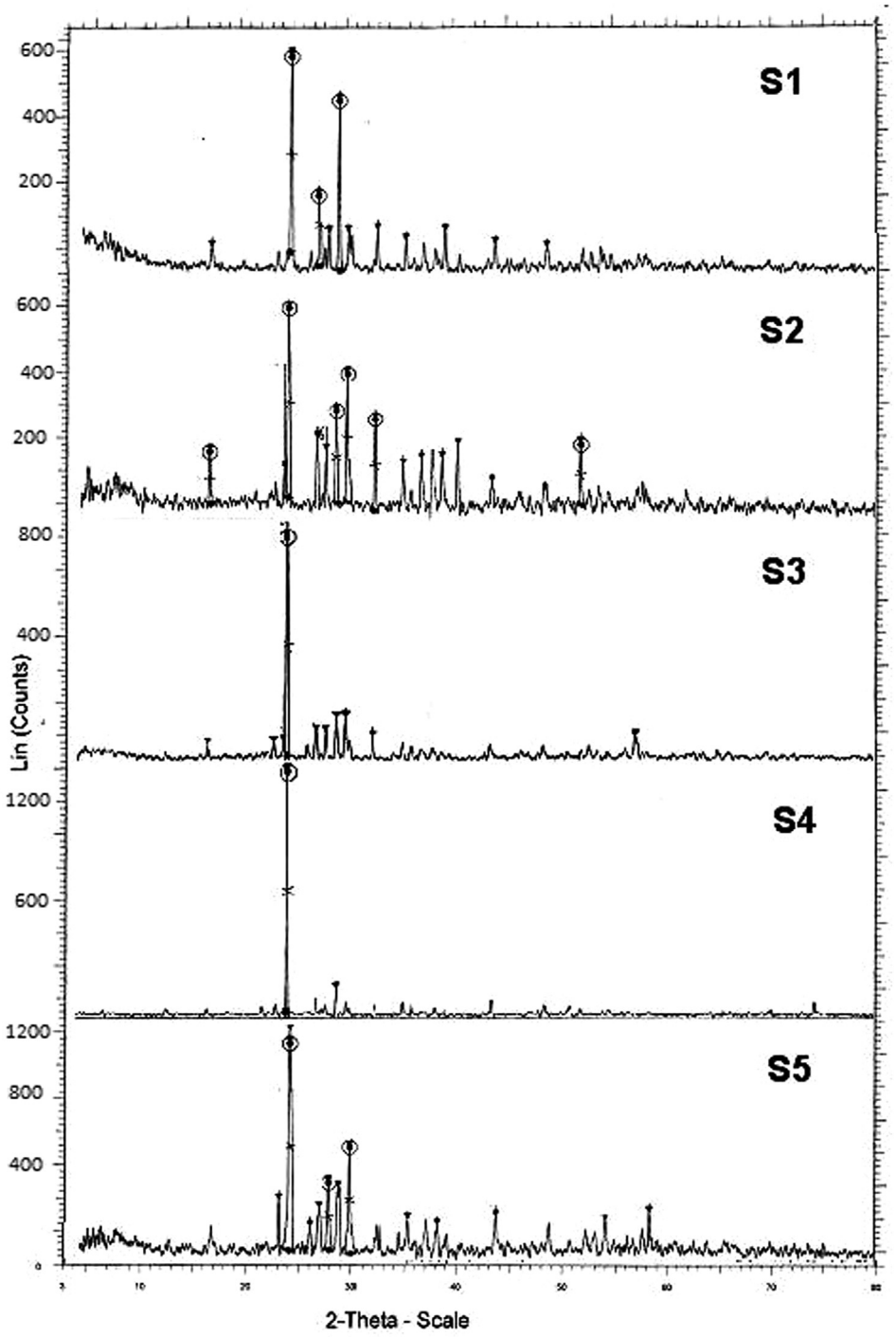


Fig. 4. X-ray diffraction spectra of Gandhaka samples.

Table 2
d-Spacing values of samples and standard sulphur.

S-1 d(Å)	S-2 d(Å)	S-3 d(Å)	S-4 d(Å)	S-5 d(Å)	Orthorhombic sulphur d(Å)	Monoclinic sulphur d(Å)
3.825 (a = 23.235)	3.844 (a = 23.114)	3.834 (a = 23.178)	3.781 (a = 23.505)	3.853 (a = 23.174)	3.85 (a = 23.083)	3.803 (a = 23.372)
3.198 (a = 27.871)	3.102 (a = 28.757)	3.099 (a = 28.77)	3.166 (a = 28.156)	3.084 (a = 28.927)	3.113 (a = 28.653)	3.168 (a = 28.145)
	3.215 (a = 27.721)	3.321 (a = 26.823)		3.312 (a = 26.891)	3.219 (a = 27.690)	
	3.331 (a = 26.743)			3.205 (a = 27.81)	3.336 (a = 26.315)	
	2.838 (a = 31.499)				2.848 (a = 31.385)	

a = 2 Theta (deg).

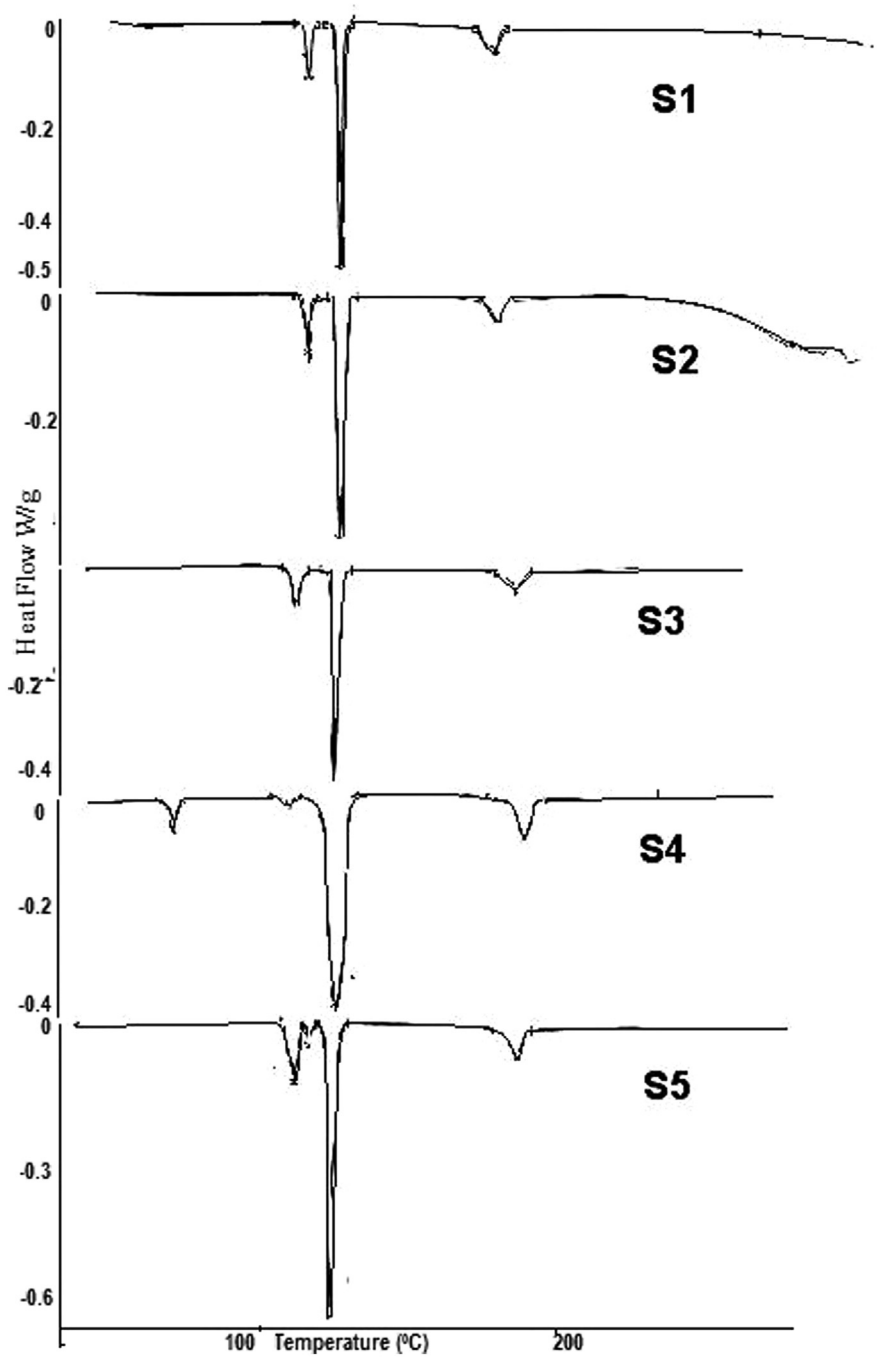


Fig. 5. Differential thermograms of Gandhaka samples.

Table 3
Values of endothermic peaks of sulphur in DSC.

Sr. no.	S-1	S-2	S-3	S-4	S-5
1	–	–	–	75.97 °C	–
2	108.60 °C	110.93 °C	110.04 °C	108.49 °C	111.55 °C
3	–	–	–	–	115.61 °C
4	119.97 °C	121.10 °C	121.29 °C	121.01 °C	121.31 °C
5	186.83 °C	174.17 °C	174.73 °C	173.96 °C	174.74 °C
6	410.87 °C	–	–	–	–

3.7. Thermal studies of Tankana

The thermograms (Fig. 8) of B-1 show an extra peak at 74.71 °C which may be due to the presence of solvates of water, while the peak is missing in the differential thermogram of B-2. Further, the range of peak onset and endset is narrow in the B-3 thermogram as compared to B-1 and B-2. A gradual increase in the sharpness of an endothermic peak at 137 °C is observed. The peak at 110.62 °C of B-2 is slightly shifted to 104.22 °C in B-3, which may be due to some change in the crystal structure of the final product (Table 5).

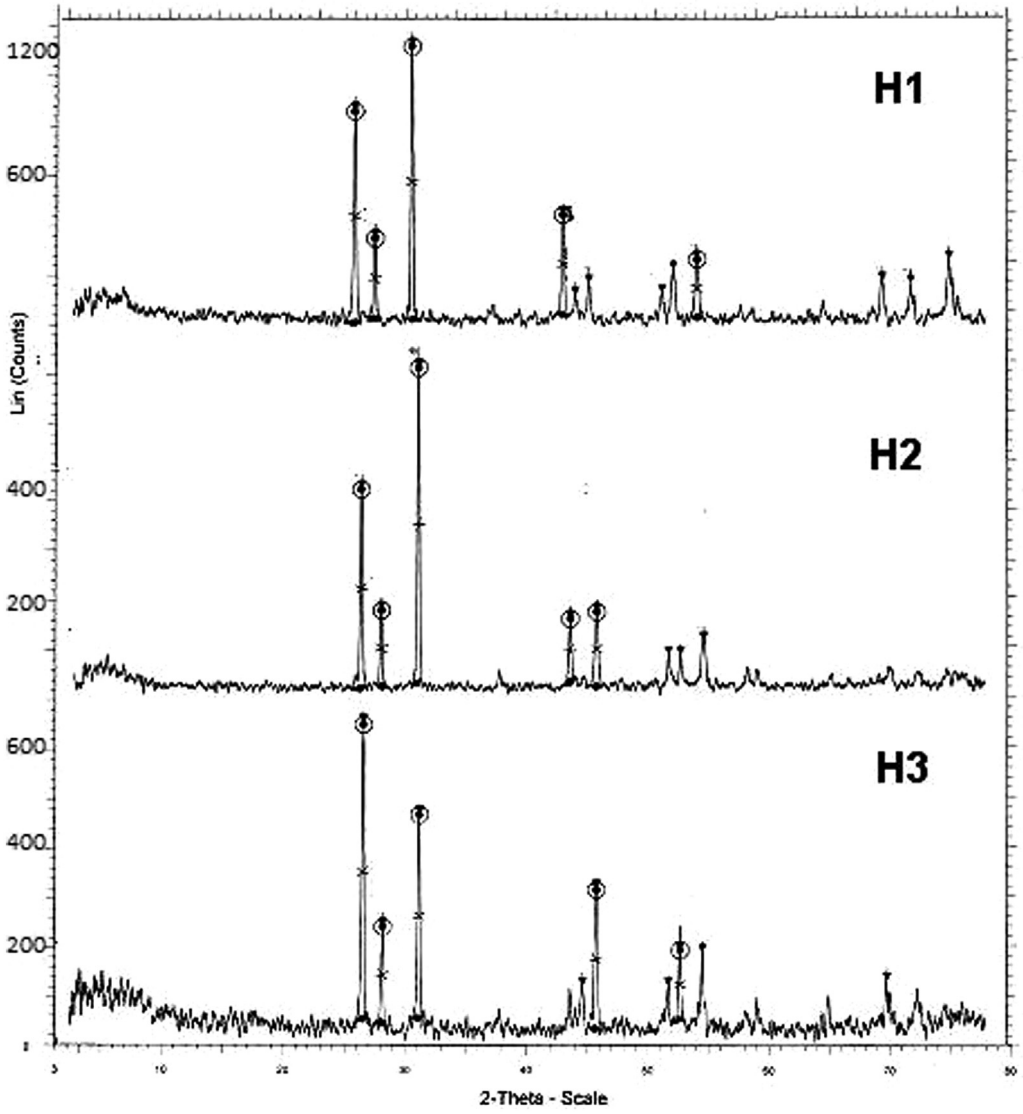


Fig. 6. X-ray diffraction spectra of Hingula samples.

Table 4
d-Spacing values of samples and standard Cinnabar.

d-Spacing values	
H-1 d(Å)	**HgS d(Å)**
3.337 (a = 26.363)	3.36 (a = 26.507)
3.181 (a = 28.024)	3.181 (a = 28.218)
2.877 (a = 31.060)	2.85 (a = 31.362)
2.078 (a = 43.50)	2.06 (a = 43.917)
1.682 (a = 54.508)	1.67 (a = 54.937)
H-2 d(Å)	**HgS d(Å)**
3.370 (a = 26.422)	3.36 (a = 26.507)
3.171 (a = 28.109)	3.181 (a = 28.218)
2.874 (a = 31.089)	2.85 (a = 31.362)
2.030 (a = 44.593)	2.06 (a = 43.917)
1.682 (a = 54.490)	1.67 (a = 54.937)
H-3 d(Å)	**HgS d(Å)**
3.358 (a = 26.517)	3.36 (a = 26.507)
3.162 (a = 28.199)	3.181 (a = 28.218)
2.862 (a = 31.224)	2.85 (a = 31.362)
2.071 (a = 43.665)	2.06 (a = 43.917)
1.677 (a = 54.677)	1.67 (a = 54.937)

a = 2 Theta (deg).

3.8. Discussion

The above study was performed in order to understand the physicochemical changes taking place in the raw materials due to the purification methods reported. The HPTLC and IR studies for *Visa* alkaloids show that the alkaloids undergo degradation on being processed for purification. The results show that there were significant differences in alkaloid contents between the processed and unprocessed aconite roots. It is observed from the IR spectra that during the purification there was loss of an ester group (peak at 1720 cm^{-1}), which may have been replaced with a Keto-group (1676 cm^{-1}). However, due to the presence of a number of alkaloids in the alkaloid fraction, the exact chemical changes cannot be predicted. These findings are in accordance with the earlier reports [5,10,19,21] that the di-ester alkaloids of *Visa* are prone to hydrolysis. The purification procedure may thus be responsible for the chemical degradation of the diester alkaloids. It has also been reported that the di-ester alkaloids (aconitine, hypaconitine and mesaconitine) are toxic as compared to the monoester alkaloids (benzoylaconine, benzoylhypaconine and benzoylmesaconine)

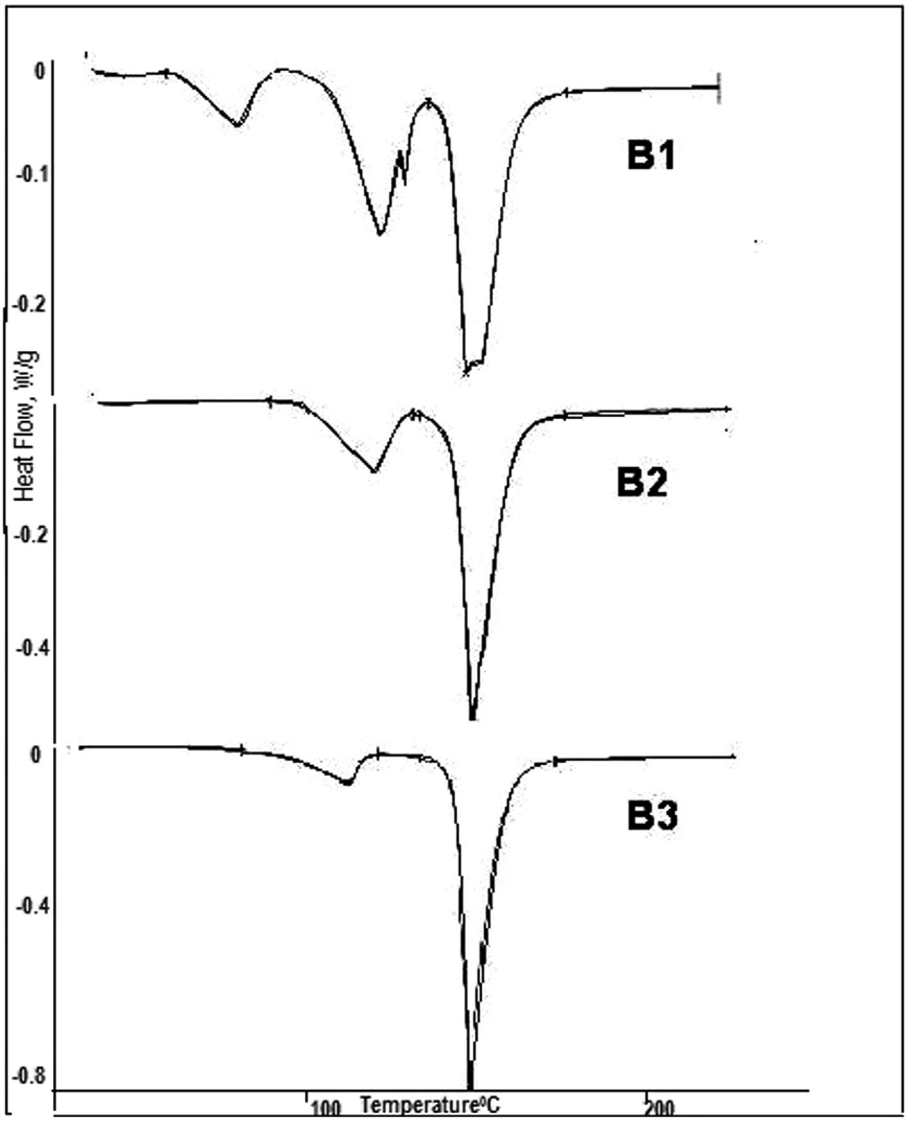


Fig. 7. X-ray diffraction spectra of Tankana samples.

[12]. The monoester alkaloids are significantly effective in inflammation and pain [8]. Toxicological studies have demonstrated that the toxicity of diester alkaloids is almost the same with LD_{50} values of about 0.08 mg/kg body weight, while the hydrolysed monoester alkaloids show much lower toxicity (LD_{50} – 24 mg/kg) in rats [22].

Studies were also performed on gandhaka using XRD and DSC. In nature, Gandhaka atoms aggregate, with each other forming long chains. In any sample of gandhaka, thermal agitation is constantly breaking and reassembling these chains. The S_8 molecule is stable and their two crystalline forms compete, orthorhombic α-sulphur and monoclinic β-sulphur. Below 96 °C, the orthorhombic form is more stable and the conversions between the two forms are slow [18]. If the liquid is cooled slowly, needle-like monoclinic crystals form. When the temperature falls below 96 °C, these crystals slowly change to orthorhombic microcrystal [3].

The gandhaka used as the crude raw material in our study as is evident from XRD, is largely of orthorhombic crystalline nature and is probably the mixture of α-Sulphur and small amounts of β-sulphur, displayed by two sharp endothermic peaks at about 109 °C and 122 °C in the differential thermogram. There is no significant difference between the DSC as well as XRD of S-1 and S-2, suggesting no interaction between gandhaka and ghee. This also suggests amorphous nature of cow ghee, with absence of any additional peak in XRD pattern of sample S-2. XRD and DSC patterns of sample S-3 and S-4 is more or less same as that of S-2. Significant change is observed in DSC of S-4 wherein additional endothermic peak is observed at 76 °C which may be due to the removal of the hydrates. The hydrates may have been formed during the washing of the gandhaka with hot water. All endotherms of S-4 are with lesser energy changes. As also seen in XRD patterns, the sample tends to display the presence of monoclinic sulphur. The XRD and DSC patterns of S-5, display that the structure of S-4 reverts back to the S_8 orthorhombic sulphur. Further, the unwanted components are also reduced which may be observed by the sharpness of the peaks in the X-ray diffraction pattern of S-5. Thus, from the above study it can be concluded that the processing of gandhaka brings about purification, reducing the toxic nature of gandhaka. By impregnating with organic material, like ghee, gandhaka is made homologous to the tissue cells and their toxicity is reduced and acceptability to the cell is increased.

One of the toxic components in MHR is hingula containing mercury. Mercury's toxicity and bioavailability are reduced in the

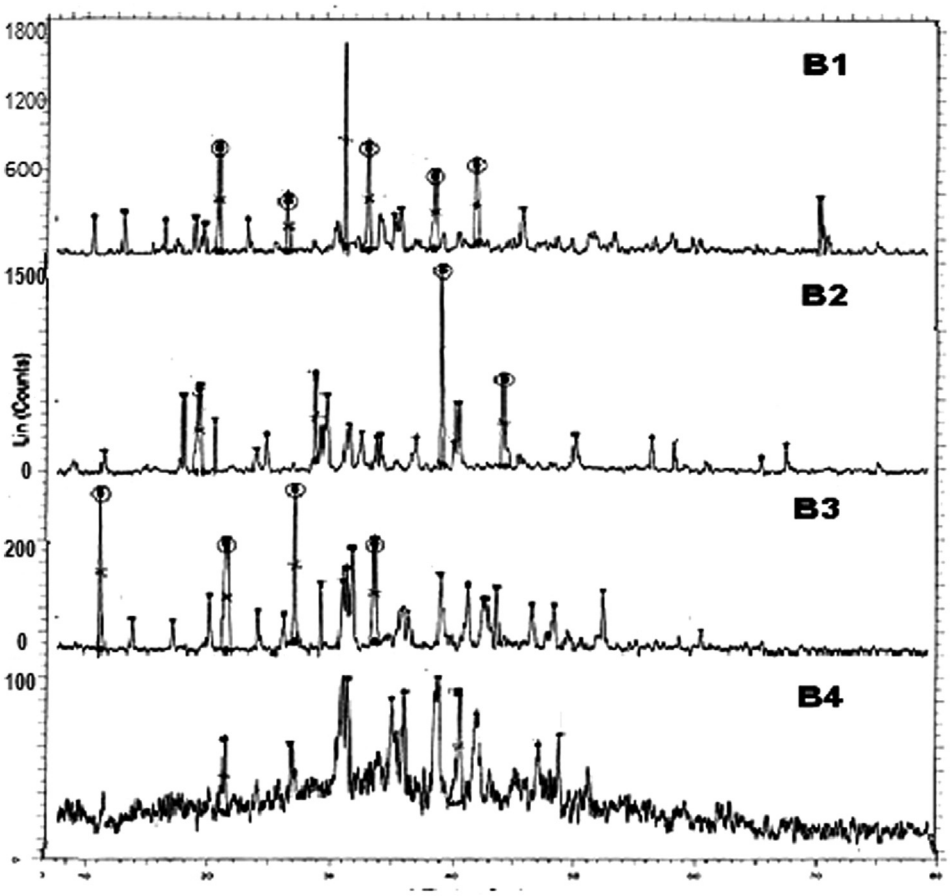


Fig. 8. Differential thermograms of Tankana samples.

form of hingula because it is relatively insoluble. The dissolution of hingula, however, is significantly enhanced in the presence of dissolved organic matter. In nature hingula is seldom available in its physically and chemically pure form. The contamination takes place naturally in the mine during the process of extraction with extraneous material which should be removed to avoid toxicity [16]. Metals, even in their physically and chemically pure form might produce adverse effects because they are inorganic in nature and they are heterogeneous to the body tissue [20]. By impregnating and triturating with organic material, like juices, decoctions of herbs etc., they are made homologous to the tissue cells and their toxicity is reduced and acceptability to the cell is increased. During this process certain organic materials are added to mercury, which help to increase its medicinal efficacy and safety. From the XRD pattern of hingula it is evident that there is no chemical change taking place due to the processing done. However, when compared with reference values of trigonal trapezohedral crystalline form of hingula, it is clear that the final product is of very pure nature. Further, on treatment with lemon juice the organic material make hingula more homologous to the body for its assimilation and the therapeutic effect.

Table 5
Values of endothermic peaks of sodium metaborate in DSC.

Sr. no.	B-1	B-2	B-2
1	74.71 °C	–	–
2	114.65 °C	110.62 °C	104.22 °C
3	138.67 °C	138.39 °C	137.54 °C

In case of Tankana study, marked changes are observed in the XRD and DSC patterns. The purity of the raw material can be ascertained from the DSC study as the sharpness of the endothermic peak at 137 °C increased markedly even when low concentration of sample was analysed. However, the slight shift in the endothermic peak at 104.22 °C in final product shows certain changes taking place in the crystal structure which further needs to be studied.

The data obtained certainly proves that all the procedures had marked effect on the nature of the raw materials. It indicates that the traditional methods of purification are responsible for making the formulation therapeutically useful [8,9] with less toxicity and thus should be followed very carefully. Even after all, the actual biological role of the metal present in such drugs is not very clear. In order to accept such kind of herbo-metallic drugs especially containing heavy metals, an extensive research is needed for the complete pharmacokinetic study on the animal system regarding its safety and efficacy.

4. Conclusion

The characterization techniques like FTIR, XRPD, DSC, HPTLC which have been used in the present studies can be used as a physico-chemical fingerprint for characterization of the raw materials in industry not only to check uniformity but also to ensure that each step is been followed as per the standard text. A routine use of such scientific techniques will lead to standardization of the product to a certain extent and would definitely help in building confidence in use of such products for medication.

Conflict of interest

None declared.

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