

# Comparative HPLC analysis of different samples of *Tribhuvankirti Rasa*

## Research Article

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## Abstract

**Background:** There are different methods of *Vatsanabha shodhana* but it is not mentioned in literature that which method should be used in preparation of *Tribhuvankirti rasa* which will make the drug safer. **Objectives:** To acquire knowledge regarding toxic principle in self prepared and market sample of *Tribhuvankirti Rasa* with respect to different *shodhana* methods of *Vatsanabha* and to develop a method of testing toxic principle in the formulation. **Material and methods:** Three samples were prepared out of which two samples were prepared using different *shodhana* media and one sample was prepared using impure ingredients and three samples of *Tribhuvankirti Rasa* were purchased from market. Physicochemical and phytochemical analysis of the final product was conducted and the observations were compared among six samples. **Results:** HPLC graphs indicate that the numbers of peaks are increased in the sample prepared with *Godugdha shodhita Vatsanabha* and all the market samples in comparison with *Ashodhita* and *Gomutra shodhita* samples of *Tribhuvankirti Rasa*. All samples contain alkaloids, however after purification there was decrease in the concentration of detected alkaloid. **Conclusion:** The toxic principle (Aconitine) in market and self prepared sample of *Tribhuvankirti Rasa* has been reduced by *shodhana*. In addition, flavonoids, glycosides and anti-oxidants are found which are beneficial for health.

**Key Words:** HPLC, *Tribhuvankirti rasa*, *Shodhana*, Toxic principle, Aconitine.

## Introduction

In Ayurveda, there are many formulation made up of herbs, minerals and herbo-mineral drugs. Herbo-mineral drugs are most efficacious and stable formulations in Ayurveda. *Tribhuvankirti rasa* is very popular formulation in Ayurved. It is used almost by every Ayurved physician because of its impactful effects on disease in which it is used. There are four different references for *Tribhuvanankirti Rasa* in the classical texts. All the four formulations have different ingredients, method of preparation, their doses, *anupana* (Vehicle) and indications. The first reference is described in Yoga Ratnakar, Rasayansara, Chikitsasara Sangraha, Rasa Chandanshu, Nighantu Ratnakar and Rasa Kaumudi. The second reference is described in Rasa Prakash Sudhakar and Rasa Chandanshu. The third reference is described in Rasaraj Shankar. The fourth is described in Rasamrutam. But out of these four, *Tribhuvankirti rasa* prepared by the first reference is available in market. *Tribhuvankirti rasa* is a *Kharaliya Rasayana* (prepared in mortar and pestle). Ingredients which are used to prepare *Tribhuvankirti*

*Rasa* are *Vatsanabha* (*Aconitum ferox* Linn), *Hingula* (Cinnabar), *Tankana* (Borax), *Sunthi* (*Zingiber officinalis* Linn), *Maricha* (*Piper nigrum* Linn), *Pippali* (*Piper longum* Linn), *Pippalimula* (*Piper longum* Linn), *Tulasi swaras* (juice of *Ocimum sanctum* Linn), *Ardrak swaras* (juice of *Zingiber officinalis* Linn) and *Dhattura patra swaras* (juice of *Datura metel* Linn). It is indicated in all types of *jwara* (Fever) especially *sannipatik jwara* (Typhoid), *yakruta* (Liver) and *pleeha vikara* (Spleen disorder) and to improve digestion in the dose of one *Gunja* (125mg) with *Ardrak Swarasa* (Juice of *Zinziber officinalis*) as *anupana* (vehicle). (1)

*Tribhuvankirti rasa* contains *Vatsanabha*, which is considered as main ingredients, also called as *Mahavisha* (Strong poison). *Visha dravya* (toxic drugs) are used in formulations for its potency for the therapeutic purposes. *Shodhana* (Purification) of *Vatsanabha* has been mentioned in many texts. *Shodhana* of *Vatsanabha* is usually done by immersing the roots in *Gomutra* (Cow's urine) for three days and *Gomutra* is changed every day. Second predominantly *shodhana* method used is *swedana* (boiling) in *dolayantra* filled with *Godugdha* (Cow's milk) for three hrs. Third method of *shodhana* is *swedana* (boiling) in *dolayantra* filled with *Ajadugdha* (Goat's milk) for three hrs. Fourth method of *shodhana* is *swedana* (boiling) in *dolayantra* filled with water and *Godugdha* (Cow's milk) for three hrs. The fifth method of *shodhana* is cooking of *Vatsanabha* in buffalo dung. (2) In preparation of *Tribhuvankirti Rasa*, it is not mentioned that which method of *Vatsanabha shodhana* is to be used out of these five methods. Even, it is not

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described anywhere which method of *Vatsanabha shodhana* is used by pharmaceutical companies to prepare *Tribhuvanankirti Rasa*. In a previous study, with the help of thin layer chromatography only the presence of *Vatsanabha* was detected before and after *shodhana*. (3) Hence, analytical study was undertaken to acquire knowledge regarding toxic principle in self made and marketed sample of *Tribhuvankirti Rasa* with respect to different *shodhana* methods of *Vatsanabha* and to develop a method of testing toxic principle in the formulation. Total three samples were self made out of which two sample were prepared using different *shodhana* media and one sample was prepared using impure ingredients and three sample of *Tribhuvankirti Rasa* was purchased from market.

## Materials and Methods

After receiving the approval of Institutional Ethics Committee the study was conducted in two phases: 1) Pharmaceutical study, 2) Analytical study. Pharmaceutical study included *shodhana* of *Vatsanabha*, *Tankana*, *Hingula*, preparation of *Tribhuvanankirti Rasa* by three methods. Analytical study included physicochemical and phytochemical analysis of all the six samples.

### Materials

**Table No.1: Showing ingredients of Tribhuvankirti Rasa:**

SN	Ingredients	Useful parts	Quantity
1	<i>Vatsanabha</i> ( <i>Aconitum Ferox</i> Linn)	Root	80gms
2	<i>Hingula</i> (Cinnabar)	-----	80 gms
3	<i>Tankan</i> (Borax)	-----	80gms
4	<i>Sunthi</i> ( <i>Zingiber officinalis</i> Linn)	Rhizome	80gms
5	<i>Maricha</i> ( <i>Piper nigrum</i> Linn)	Seeds	80gms
6	<i>Pippali</i> ( <i>Piper longum</i> Linn)	Stamen	80gms
7	<i>Pippalimula</i> ( <i>Piper longum</i> Linn)	Root	80gms
8	<i>Tulasi swarasa</i> ( <i>Ocimum sanctum</i> Linn)	Leaves	2 kg
9	<i>Ardrak swarasa</i> ( <i>Zingiber officinalis</i> Linn)	Rhizome	1 kg
10	<i>Dhatra patra swarasa</i> ( <i>Dhatra metal</i> Linn )	Leaves	1 kg
11	Market preparation of <i>Tribhuvankirti Rasa</i>	-----	03 samples from different pharmaceuticals

### Methods

Ingredients of *Tribhuvankirti Rasa* were collected from local market of Nagpur. The herbal drugs were identified and authenticated from department of Dravyaguna, MGACH &RC, Salod (H), Wardha,

Maharashtra. Market samples of *Tribhuvankirti Rasa* of three different companies were purchased and coded as MRSTK-A, MRSTK-B and MRSTK-C. The ingredients and other details mentioned on the label were noted.

### Pharmaceutical study

#### Method 1: Preparation of Tribhuvankirti Rasa using impure Vatsanabha (Sample 1- ASVTK)

Ingredients Sr.no 1 to 7 (Table No 1) were made into fine powder separately and they were mixed together in a dried *Khalva Yantra* (mortar) in equal quantity (10gms each). In this, *Vatsanabha*, *Hingula* and *Tankana* were used without *shodhana*. Fresh juice of *Tulasi* leaves was prepared and 85ml *tulasi swarasa* added to the mixture which was then triturated well for three hours until it becomes thick paste. It was then allowed to dry overnight at room temperature. Next day, dry paste was again triturated with 75ml of fresh *Ardrak swarasa* for three hours till it becomes thick. It was then allowed to dry overnight at room temperature. On 3<sup>rd</sup> day, it was again triturated with 65ml of *Dhatra patra swarasa* for three hours and allowed it to dry. On 4<sup>th</sup> day, the mixture was triturated with 55ml of *tulasi swarasa* until the mixture becomes thick. On 5<sup>th</sup> day the mixture was again triturated with 45 ml *Ardrak swarasa* till it becomes thick. On 6<sup>th</sup> day, mixture was triturated with 35 ml of *dhatra patra swarasa* till it becomes thick. On 7<sup>th</sup> day, the mixture was triturated with 30 ml of *tulasi swarasa*. On 8<sup>th</sup> day, the mixture was triturated with 25ml *Ardrak swarasa* and on the 9<sup>th</sup> day *bhavana* was given with 20ml *dhatra patra swarasa* and it was then allowed it to dry at room temperature. In this way, three *bhavana* (trituration) of fresh *swarasa* of *tulasi*, *Ardrak* and *Dhatra* was given to prepare *Tribhuvankirti rasa*.

#### Preparation of Tribhuvankirti Rasa using Ashuddha Vatsanabha and Hingula (Sample 1:- ASVTK)

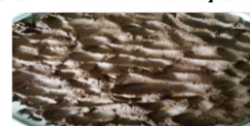
Fig. 1- 3<sup>rd</sup> Bhavana of Tulasi swarasa



Fig. 2 - 3<sup>rd</sup> Bhavana of Ardrak swarasa



Fig.3- 3<sup>rd</sup> Bhavana of Dhatra patra swarasa



#### Method 2: Preparation of Tribhuvankirti Rasa using Gomutra Shoddhita Vatsanabha, Shuddha Hingula and Shuddha Tankana (Sample 2- GMSVTK)

##### Purification of Vatsanabha with Gomutra: (2)

Thirty gms *Ashuddha Vatsanabha* was cut into pieces and soaked in 200ml *Gomutra*. Daily *Gomutra* was replaced by fresh 200ml of *Gomutra*. After 3 days, *Vatsanabha* was taken out and its upper layer of skin was removed with knife. The *Vatsanabha* was kept in sunlight and then made into fine powder and stored as *Shuddha Vatsanabha*. 12.7gms of *Shuddha Vatsanabha* was obtained after *shodhana* and 10gms was used in

preparation of *Tribhuvankirti rasa* in sample 2 GMSVTK.

#### Purification of *Hingula*: (4)

Raw *hingula* 30gms was taken in clean *khalva yantra* and was made into fine powder and then triturated with 45ml of *Ardrak swarasa* till it becomes dry. This process was repeated for 7 times. Everyday fresh *Ardrak swarasa* was taken for trituration. Then it was dried in sun light. 20gms of *Shuddha hingula* was obtained after *shodhana* and 10 gms were used in sample 2 GMSVTK and 10 gms was used in sample 3 GDSVTK.

#### Purification of *Tankan*: (5)

Thirty gms of *Tankan* was taken in a clean wide mouth iron vessel. The vessel was placed over fire and heated with regular stirring. When the drug loses all its moisture and becomes light and brittle, the heating was stopped and the drug was stored in a suitable container as *Shuddha Tankan*. 10gms was used in sample 2 GMSVTK and 10gms was used in sample 3 GDSVTK.



Fig. 4 *Shuddha Vatsanabha*

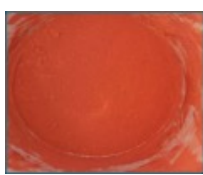


Fig. 5 *Shuddha Hingula*



Fig. 6 *Shuddha Tankana*

#### Method of Preparation of *Tribhuvankirti rasa*: (1)

Ingredients like *Shuddha Vatsanabha*, *Shuddha Tankan*, *Shuddha Hingula*, *Shunthi*, *Maricha*, *Pippali* and *Pippalimoola* were taken and made into fine powder separately. 10gms of each ingredient was taken in *Khalva yantra* and triturated well to form a homogenous mixture. Three *bhavana* each of *tulasi swarasa*, *ardraka swarasa* and *dhatu swarasa* was given and then dried in sunlight.



Fig. 7: 3rd *bhavana* of *Tulasi Swarasa*



Fig. 8: 3rd *bhavana* of *Ardraka Swarasa*



Fig. 9: 3rd *bhavana* of *Dhatu Swarasa*



Fig. 10: Final product



Fig. 11: Tablets

**Preparation of *Tribhuvankirti Rasa* using *Gomutra Shoddhita Vatsanabha*, *Shuddha Hingula* and *Shuddha Tankana* (Sample 2- GMSVTK)**

#### Method 3: Preparation of *Tribhuvankirti Rasa* using *Godugdha shoddhita Vatsanabha* (Sample 3 GDSVTK)

##### Purification of *Vatsanabha* with *Godugdha*: (2)

Thirty gms of *Ashuddha Vatsanabha* was cut into pieces and were tied in a cloth as *pottali* and immersed in vessel having one litre of *Godugdha* (Cow milk) as liquid media. The vessel was placed over moderate fire and the drug was subjected for *swedana* for one *yama* i.e. three hrs. After cooling the pieces of *Vatsanabha* were washed with warm water, dried under sunlight and made into fine powder.

##### Method of Preparation of *Tribhuvankirti rasa*: (1)

Ingredients like *Shuddha Vatsanabha*, *Shuddha Tankan*, *Shuddha Hingula*, *Shunthi*, *Maricha*, *Pippali* and *Pippalimoola* were taken and made into fine powder separately. 10gms of each ingredient was taken in *Khalva yantra* and triturated well to form a homogenous mixture. Three *bhavana* each of *tulasi swarasa*, *ardraka swarasa* and *dhatu swarasa* was given and then dried in sunlight.



Fig. 12: *Godugdha Shodhita Vatsanabha*



Fig. 13: 3rd *bhavana* of *Tulasi Swarasa*



Fig. 14: 3rd *bhavana* of *Ardraka Swarasa*



Fig. 15: 3rd *bhavana* of *Dhatu Swarasa*



Fig. 10: Final product

#### Preparation of *Tribhuvankirti Rasa* using *Godugdha Shoddhita Vatsanabha* (Sample 3:- GDSVTK)

Physicochemical and phytochemical analysis of all the three self prepared samples and three market six samples of *Tribhuvankirti Rasa* was performed. Physicochemical analysis was performed according to the methods mentioned in *Ayurvedic Pharmacopoea of India*. All the six samples were labeled as:

- 1.ASVTK- *Tribhuvankirti rasa* prepared from *Ashodhita Vatsanabha*, *Hingula* and *Tankana*
- 2.GMSVTK- *Tribhuvankirti rasa* prepared from *Gomutra shodhita Vatsanabha*, *shodhita Hingula* and *Tankana*
- 3.GDSVTK- *Tribhuvankirti rasa* prepared from *Godugdha shodhita Vatsanabha*, *shodhita Hingula* and *Tankana*
- 4.MRSTK-A- Market sample of *Tribhuvankirti rasa*
- 5.MRSTK-B- Market sample of *Tribhuvankirti rasa*
- 6.MRSTK-C - Market sample of *Tribhuvankirti rasa*



## Phytochemical analysis

### HPLC (High Pressure Liquid Chromatography)

Chromatographic analysis was performed with Shimadzu Prominence HPLC instrument. It is equipped with quaternary pump, (LC20 -80) degasser (DGU-20As) column oven (CTO-10As) Auto sample (SIL-20 AC) Diode-Array-Detector (UVSPD-M20). Phytochemical were analyzed with Prime SIL C<sub>18</sub> column (250\*4.6 mm. ID).

### Chemicals

HPLC grade methanol and water were used for HPLC analysis. They were purchased from Merck (Mumbai MH, India), HPLC grade ammonium acetate was purchased from Sigma-Aldrich (Mumbai MH, India). 0.2μ sample filters and 0.45μ nylon solvent filters were purchased from Milipore, India. Sample sonication was performed in Labman sonicator, purchased from Multilab, Ltd, Chennai, India. Marker was not used in this study.

## Methodology

### Extraction of Sample

Exactly 20g of weighted sample 1 ASVTK in powder form was dissolved in 20ml of ethanol. Same procedure was carried out with the sample 2, 3, 4, 5 and 6. All the six samples were kept for 24 hrs at room temperature to improve the release of all potential components. Furthermore, all six samples were ultra sonicated for 30 minutes and filtered through 0.2 μ nylon sample filters.

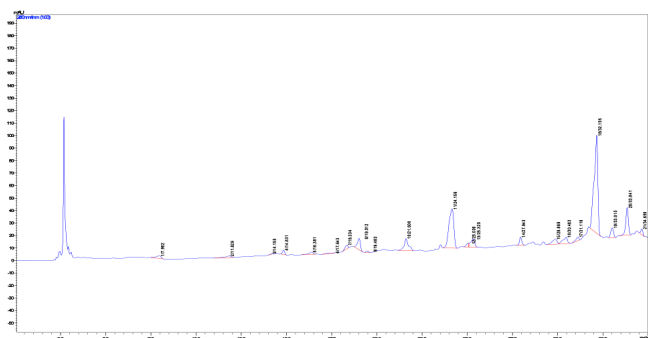
### HPLC Analysis

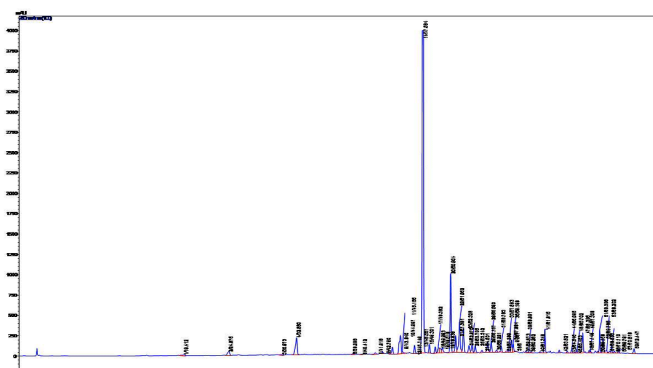
20ul of freshly prepared stock solution of sample 1 ASVTK was injected to the C<sub>18</sub> column (200\* 46 mm) and eluted at the flow rate of 1.5 ml. min<sup>-1</sup>. Binary composition of methanol and water including ammonium acetate (50 Mm) were employed throughout the HPLC analysis. 254 nm UV wavelength and ambient temperature were considered for achieving better peak shape and peak area. The same procedure was carried out with the sample 2, 3, 4, 5 and 6. All the samples were separately analyzed and graphic analysis report was obtained.

## Observations and Results

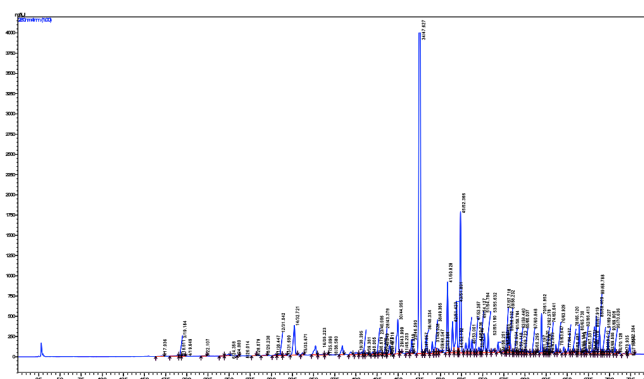
Table No 2: Physico-chemical observations of different samples of Tribhuvankirti rasa.

Sr. No	Test Parameters	ASVTK	GMSVTK	GDSVTK	MRSTK-A	MRSTK-B	MRSTK-C
1	Loss on drying at 105°C	15.5%	12.5%	12.5%	8.40%	8.25%	8.35%
2	Total Ash value	40.0%	13.0%	36.0%	20.5%	25.2%	23.7%
3	Acid insoluble ash	15.0%	10.0%	8.75%	8.20%	7.25%	8.55%
4	Water soluble extractive	45.0%	39.0	40.0%	35.72%	38.5 %	31.0%
5	Alcohol soluble extractive	55.0%	51.0	50.0%	30.0%	35.7%	40.0%
6	pH	9.0%	7.33	8.60%	8.20%	8.25%	8.30%





**Fig. 5:- HPLC graph of MRSTK-B (Market sample of *Tribhuvankirti Rasa*)**



**Fig. 6:- HPLC graph of MRSTK-C (Market sample of *Tribhuvankirti Rasa*)**

## Discussion

*Vatsanabha* is the only available *kandavisha* (tuberose poison) and *kandavisha* are most virulent among *Sthavara visha*. Poisoning occurs when it is used in crude form in medicine or due to the overdose of medicinal formulations. In ancient times, the crude plant extracts were used by Acharyas to prepare the herbo-mineral medicines. However, due to lack of methods and techniques of component characterization and exact quantification, they did not mention the specific activities along with their adverse effects. Moreover, they did not mention their limitation or contradiction. Even, they did not ascertain which specific component exhibits the pharmacological effects in crude extract after administration. Hence, in recent few decades, to understand the exact mode of action of Ayurvedic preparation and its chemical characterization, phytochemical analysis is being used. There are many analytical techniques such as thin layer chromatography (TLC), paper chromatography (PC), high performance thin layer chromatography (HPTLC), gas chromatography (GC) etc. However, all these techniques are not applicable to separate the Ayurvedic medicines since it includes various components of different classification. Moreover, these techniques are not retentive enough to separate all components. High performance liquid chromatography (HPLC) nowadays is more popular technique since it separates almost all nature of compounds. Even, it is used in chemical separation, isolation, purification and characterization. Considering all these benefits, RP-HPLC technique has been comprehensively used to analyze the different market and self prepared samples of *Tribhuvankirti Rasa*.

*Vatsanabha* (*Aconitum ferox*) is a cardiac poison which is a potent constituent of *Tribhuvankirti rasa* and hence to determine its toxicity, it is important to investigate its concentration in *Tribhuvankirti rasa*. Therefore, three marketed formulations of *Tribhuvankirti rasa* were compared with three self-prepared formulation of same *Tribhuvankirti rasa* among which one sample was prepared by using *Ashuddha Vatsanabha* and remaining two formulations were prepared by using *Gomutra Shoddhita Vatsanabha* and *Godugdha Shoddhita Vatsanabha*. In a previous study of *Vatsanabha shodhana* it is reported that after *shodhana*, the total alkaloid content decreases (6) but the contents of less toxic substances such as aconine, hypoaconine, and benzyl hypoaconine increases

possibly due to conversion of the toxic aconitine into aconine or hydrolysis of the alkaloids to their respective amino alcohol.(7) It has been reported that *Gomutra* converts Aconite to a compound with cardiac stimulant property, whereas, raw Aconite showed cardiac depressant properties.(8,9) *Shodhana* by both *Gomutra* and *Godugdha* makes Aconite devoid of cardiac and neuro- muscular toxic effects without affecting its antipyretic activity.(6)

In the present study, there is appreciable difference in the physicochemical values of the samples of *Tribhuvankirti Rasa*. Loss on drying at 105° C indicates presence of moisture content. If moisture content is more then the formulation is more likely to get infected by fungal growth. Moreover unwanted changes can also occur due to presence of more moisture. In self prepared samples moisture is more than rest of the samples; hence market sample is more stable than the self prepared sample. This may be due to moisture and fat content of *shodhana* media in self prepared samples. Market samples and the raw drugs used for it are stored for many days. Whereas, self prepared sample is prepared in small quantity and by using fresh ingredients and analysis is done as soon as the formulation is prepared.

Total ash value was found to be higher in sample ASVTK (40%) in comparison with all other samples which indicates that it is due to inorganic content. The acid insoluble ash is a part of the total ash that is insoluble in dilute hydrochloric acid. The higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is higher. Acid insoluble ash represents presence of inorganic content which is not expected in pure herbal formulation. The obtained value of acid insoluble ash is more in sample ASVTK which indicates that the inorganic content is more in it.

The extractive values namely water soluble and alcohol soluble indicates the amount of active constituents in given amount of plant material when extracted with respective solvent, a lower value compared to the rest of the samples indicates presence of exhausted material. Both the values indicate that all the samples are soluble in water as well as alcohol. pH value indicates that all samples of *Tribhuvankirti Rasa* are basic in nature.

One important characteristic is tapped bulk density or simply tapped density that is the maximum packing density of a powder (or blend of powder)

achieved under the influence of well defined, externally applied forces. The minimum packed volume thus achieved depends on a number of factors including particle size distribution, true density, particle shape and cohesiveness due to surface force including moisture. Therefore, the tap density of a material can be used to predict both its flow properties and its compressibility. Finer the particle size more will be the solubility and thus more will be the gastrointestinal absorption. The particles of all the samples were little crude to pass through 60 mesh size.

After performing the reverse phase chromatography (RP-HPLC) of all selected samples of *Tribhuvankirti rasa* which included 3 market and 3 self-prepared sample, it was revealed that all samples contain alkaloids, however, their quantities were significantly less. Importantly, comparing all of them, highest amount of the same alkaloid was seen in sample ASVTK (Fig.1). ASVTK is prepared from raw drugs without *shodhana* for comparison whereas rest of all other samples were prepared after proper *shodhana* according to classical text. It represents that after purification there was decrease in the concentration of detected alkaloid. Moreover, it could be predicted that the displayed alkaloids at retention time pk-11/24.15 & pk-18/32.15 does not have any expected effects.

More importantly, as displayed in sample GMSVTK (Fig. 2), one basic nitrogenous component was identified at RT value 25.52 and several polyphenols were detected. They might have antioxidant property. As observed in all sample, their concentration were similar. In sample GDSVTK (Fig. 3), in *Tribhuvankirti Rasa* very few minute quantity of ionic components like amines and acids were detected. Almost all components separated are belonging to flavonoids/ polyphenols or anti-oxidants. Apart from this, many other important class of compound were identified between 60-72 min RT. Maximum concentration of fraction no. 15; RT value 56.101 min was observed which accounted about 34% of total concentration of *Tribhuvankirti Rasa*. Furthermore, in sample MRTK-A (Fig 4) very few ionic components were identified and more number of flavonoids / anti-oxidant were detected and maximum concentration accounted for fraction no.33 having the RT value 47.04 min. Whereas in sample MRTK-B (Fig 5) few nitrogenous components were detected and more of flavonoids/ anti-oxidants were identified and maximum concentration accounted for fraction no.33 having the RT value 47.04 min. In sample MRTK-B (Fig 6), few nitrogenous components and more of flavonoids/ anti-oxidants and glycosides were also detected. Glycosides are the steroidal derivatives which most often conjugated with sugar molecules to increase its water solubility and easy to transport sugar molecules. Beside these components, few tocopherols were also observed. Presumably, the anti- pyretic activities of *Tribhuvankirti rasa* could be associated with these separated components.

HPLC graphs indicate that the numbers of peaks are increased in the sample GDSTK and all the

market samples in comparison with *Ashodhita* and *Gomutra shodhita* samples of *Tribhuvankirti Rasa* (GMSVTK). This may be due to the effect of *shodhana* procedure, *shodhana* media and drugs used for *bhavana* (trituration). The drugs used for *shodhana* and *bhavana* may exert antidotal effect on the toxic principle in *Vatsanabha* (Aconitine) and mercury. From the phytochemical analysis of all the samples, it can be said that the market samples of *Tribhuvankirti Rasa* may be from *Godugdha shodhita Vatsanabha*.

## Conclusion

In the references for preparation of *Tribhuvankirti Rasa*, it is advised to use *Shuddha Vatsanabha* but media of *Shodhana* of *Vatsanabha* is not mentioned in the preparation. The HPLC graphs of *Tribhuvankirti Rasa* prepared with *Godugdha shodhita Vatsanabha* are nearly similar to the HPLC graphs of market sample of *Tribhuvankirti Rasa*, hence it can be said that the market samples might be prepared from *Godugdha shodhita Vatsanabha*.

HPLC has separated the phytoconstituents very well. With the help of HPLC, it is concluded that some constituents are decreased and some are added with the effect of *shodhana* and *shodhana* media. In addition, flavonoids, glycosides and anti-oxidants are found which are beneficial for health. Thus, HPLC is a sophisticated method of identifying, separating and testing the phytoconstituents in the formulation from analytical perspective.

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