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PHARMACEUTICO-ANALYTICAL AND ANTIFUNGAL STUDY OF TRIPURABHAIRAVA RASA

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ABSTRACT

Tripurabhairava Rasa[1] is one among the *Kharaleeya Rasayana Yoga* found in our classics. The primary reference is from *Rasendra Chintamani* in the context of *Jwara Chikitsa*. A Pharmaceutical and analytical study was conducted by preparing *Tripurabhairava Rasa* in 3 batches. Various analyses were conducted including Physico-chemical, Instrumental analysis and Anti-fungal study on *Mucor* and *Rhizopus* fungal species. The present article describes the details of *Tripurabhairava Rasa.*

KEYWORDS: *Tripurabhairava Rasa*, Mucormycosis, *Dantimoola, Visha, Upavisha,* Antifungal.

INTRODUCTION

Rasoushadhis proved to be a major tool in the treatment of Covid-19 pandemic and Post Covid-19 complications. Many formulations were explored and researched on their Antiviral and Antifungal activity to combat the pandemic. In this purview, Classical preparation which can treat the *Jwara* as well as *Krimi* in the situation of Covid-19 Pandemic was looked for throughout the Ayurvedic literature in order to find a single yoga which can be used to treat many conditions.

Any disease left untreated for a longer duration may lead to complications leading to bad prognosis of the condition. Covid-19, similarly led to the complication of Mucormycosis

making the condition more fatal in few cases causing a major concern during the second wave of Covid-19 pandemic.

Hence an attempt was made to explore the scope of *Ayurveda* in this aspect to reduce the occurrence of Mucormycosis and treating the disease prior to its progression. *Tripurabhairava Rasa* is multifaceted formulation having combination of drugs which targets multiple symptoms at the same time.

OBJECTIVES OF THE STUDY

1. To identify, collect and carry out the *Shodhana* of ingredients of *Tripurabhairava Rasa.*
2. To carry out Pharmaceutical study of *Tripurabhairava Rasa.*
3. To carry out Analytical study of *Tripurabhairava Rasa.*
4. To carry out Antifungal study of *Tripurabhairava Rasa* on the fungal species *Mucor racemosus* and *Rhizopus oryzae* responsible for Mucormycosis.

MATERIALS AND METHODS

Literary source: Information was collected from Ayurvedic classical literature, relevant contemporary literature and relevant research works.

Drug source

- Required raw materials- *Vatsanabha, Tankana, Gandhaka, Hingula, Jayapala, Pippali, Madhuka, and Kusha* were procured from Amruth Kesari depot at K. R Market, Bengaluru.
- *Dantimoola* was procured from herbal garden of Sri Sri College of Ayurvedic Science and Research, Bengaluru.
- Fresh drug *Ardraka* was procured from reliable market source of Bengaluru.
- *Shodhana Dravyas- Godugdha* and *Goghrita* were procured from reliable source of Nandini milk dairy, Goshala of Sri Sri College of Ayurvedic Science and Research, Bengaluru and O.B Chudanahalli.

Drugs were authenticated by the Department of Dravyaguna and Department of Rasashastra & Bhaishajya Kalpana, Sri Sri College of Ayurvedic Science and Research, Bengaluru.

Pharmaceutical source: The preparation was carried out at teaching pharmacy of Department of Rasashastra & Bhaishajya Kalpana, Sri Sri College of Ayurvedic Science and Research, Bengaluru.

Pharmaceutical study

a) **Shodhana of *Vatsanabha*** [2] was conducted as per the reference of Rasatarangini (**Method: *Plavana* in *gomootra* for 3 days)**
b) **Shodhana of *Tankana*** [3] was conducted as per the reference of Rasatarangini (**Method: *Nirjaleekarana*)**
c) **Shodhana of *Gandhaka*** [4] was conducted as per the reference of Ayurveda Prakasha / Rasamanjari (**Method: *Dalana* in *godugdha* for 7 times)**
d) **Shodhana of *Hingula*** [5] was conducted as per the reference of Rasatarangini (**Method: 7 *Bhavana* with *ardraka swarasa*)**
e) **Shodhana of *Jayapala*** [6] was conducted as per the reference of Rasatarangini (**Method: 3 *Swedana* in *goksheera* using *dolayantra*)**
f) **Preparation of *Pippali choorna*** [7] was conducted as per the reference of Sharngadhara Samhita (**Method: Pulverizing)**
g) **Preparation of Madhuka/ Yastimadhu choorna** [7] was conducted as per the reference of Sharngadhara Samhita.(**Method: Pulverizing)**
h) **Shodhana of *Dantimoola*** [8] was conducted as per the reference of Charaka Samhita (**Method: *Agni-Sweda-Aatapa shoshana*)**

Dantimoola was smeared with the paste of *Pippali* and *Madhuka/ Yastimadhu* and wrapped with *Kusha*. Later it was coated with mud and subjected to *Swedana*. After fomentation, the roots were dried under sun

Preparation of ingredients of Tripurabhairava Rasa was done judiciously by taking precautions to avoid loss of the drug.

Table 01: Quantity of ingredients Before and After shodhana.

Ingredient	***Ashuddha dravya***	***Shuddha dravya***
Vatsanabha	200g	98g
Tankana	152g	81.7g
Gandhaka	248.2g	217.4g
Hingula	195g	202.3 g
Dantibeeja/ jayapala	293g	144.4 g
Dantimoola	110g	106g

i) Preparation of *Dantimoola kashaya* [9] as per the method mentioned for *Kashaya* used for *Bhavana* purpose in Bhaishajya Ratnavali

j) Preparation of *Ardraka swarasa* [10] was conducted as per the reference of Sharngadhara Samhita. (**Method: Extraction as per the classical method of pounding)**

k) Preparation of *Tripurabhairava rasa* [1] according to the reference of *Rasendra Chintamani*.

Shuddha vatsanabha**-** 1part

Shuddha tankana**-** 2 parts

Shuddha gandhaka**-** 3 parts

Shuddha hingula**-** 4 parts

Shuddha jayapala**-** 5 parts

Dantimoola Kwatha* and *Ardraka swarasa**-** Quantity sufficient

Each batch/ Sample was prepared with the quantity of ingredients as stated in the reference: (*Kramavruddhi pramana* -Sequentially increasing in quantity)

Preparation of *Tripurabhairava Rasa* was carried out in 2 Batches- *Vati* and *Choorna* as the dosage form is not specifically mentioned in the reference and well as to fix the standard dose as per the reference.

Table 02: Ingredients taken for preparation of tripurabhairava rasa.

Vati sample 1		**Vati sample 2**		**Vati sample 3**	
Shuddha vatsanabha Shuddha tankana Shuddha gandhaka Shuddha hingula Shuddha jayapala Shuddha dantimoola kwatha	2.5g 5g 7.5g 10g 12.5g Q.s	Shuddha vatsanabha Shuddha tankana Shuddha gandhaka Shuddha hingula Shuddha jayapala Shuddha dantimoola kwatha Ardraka swarasa	2.5g 5g 7.5g 10g 12.5g Q.s Q.s	Shuddha vatsanabha Shuddha tankana Shuddha gandhaka Shuddha hingula Shuddha jayapala Ashuddha dantimoola kwatha	3g 6g 9g 12g 15g Q.s
Choorna sample 1		**Choorna sample 2**		**Choorna sample 3**	
Shuddha vatsanabha Shuddha tankana Shuddha gandhaka Shuddha hingula Shuddha jayapala Shuddha dantimoola kwatha	7.5g 15g 22.5g 30g 37.5g Q.s	Shuddha vatsanabha Shuddha tankana Shuddha gandhaka Shuddha hingula Shuddha jayapala Shuddha dantimoola kwatha Ardraka swarasa	7.5g 15g 22.5g 30g 37.5g Q.s Q.s	Shuddha vatsanabha Shuddha tankana Shuddha gandhaka Shuddha hingula Shuddha jayapala Ashuddha dantimoola kwatha	3g 6g 9g 12g 15g Q.s

i. The fine powder of ingredients were weighed and added to *Khalwayantra*.

ii. *Shuddha Hingula* and *Shuddha Gandhaka* were added at the beginning and homogenous mixture was made.

iii. The other drugs were added to it and trichurated well to obtain homogenous mixture.

iv. **Preparation of Sample 1 and 3 of Batch 1:** The mixture was then added with the liquid media till the mixture was completely soaked in the liquid and trichurated for 1 *Yama* (3 hours) (*Shuddha Dantimoola Kwatha* in case of Sample 1) (*Ashuddha Dantimoola Kwatha* in case of Sample 3)

v. After 3 hours, *Vati* (pills)[11] were rolled into sufficient size as stated in the reference and shade dried.

vi. **Preparation of Sample 2 of Batch 1:** After the mixture of ingredients were shade dried after 3 hours trituration, it was added with *Ardraka Swarasa*[12] and *Mardana* was done for around 12 minutes and *Vati* were prepared as earlier.

vii. **Preparation of Sample 1,2,3 of Batch 2:** The procedure was similar to that of Sample 1, 2, 3 of Batch 1 respectively but the mixture was not rolled into pills and was preserved as *Choorna*.

viii. The dried samples were packed, sealed and preserved in a clean airtight container.

Table 03: Quantity of bhavana dravya used.

Vati sample 1	**Vati sample 2**	**Vati sample 3**
Shuddha dantimoola kwatha-35 ml	**Shuddha dantimoola kwatha**-35ml **Ardraka swarasa**-20ml	**Ashuddha dantimoola kwatha-** 40ml
Choorna sample 1	**Choorna sample 2**	**Choorna sample 3**
Shuddha dantimoola kwatha-100ml	**Shuddha dantimoola kwatha**-100ml **Ardraka swarasa**-70ml	**Ashuddha dantimoola kwatha-** 40ml

Table 04: Samples of tripurabhairava rasa.

	Sample 1	**Sample 2**	**Sample 3**
Batch 1(vati)	110 tablets(41g)	110 tablets(41g)	120 tablets(46.5g)
Batch2(choorna)	114g	113.5g	46.5g

Analytical study[13]

- **Physico-chemical and Phytochemical analysis** of the samples were conducted at Central Research Laboratory of Sri Sri College of Ayurvedic Science and Research, Bengaluru.
- **Instrumental analysis (Sem edax)** was conducted at Material Characterization and Research Lab (ISO Labs), Bengaluru.

- **Testing of tablets** was conducted at Sri Sri Tattva Factory, Bengaluru.
- **Antifungal study** of samples was conducted at Radiant Research Laboratory, Bengaluru.

The tests were conducted as per the API guidelines and the mean value of 3 readings was recorded and the mean value was considered as the final test value.

Antifungal study on *mucor* and *rhizopus* species[14] [15]

Determination of the Antimicrobial activity of test samples against *Mucor racemosus* and *Rhizopus oryzae* by microdilution method

a. Experiment was performed in triplicates under aseptic conditions.
b. A volume of 100µl broth was added to all 96 wells except first three wells (A1 B1 C1) of the Microtitre plate. RPMI broth was used for *Mucor racemosus* and *Rhizopus oryzae.*
c. In first three wells (A1 B1 C1) of plate, 200µl of the test sample was added and double diluted till A12 B12 C12 to get the desired concentration.
d. To all the wells containing test sample, 10µl of *Mucor racemosus* and *Rhizopus oryzae* suspension of 104 CFU/ml was added.
e. A growth control (10µl of fungal culture + 100µl broth medium) from G1 to G12 and broth control (only 100µl broth medium) from H1 to H12 was kept as negative control.
f. In separate micro dilution plate, 200µl of Standard Antibiotic Ketoconazole solution was added to the three wells (D1 E1 F1) of plate and double diluted till D12 E12 F12 to get the desired concentration.
g. Then the test microtiter plate was incubated at 25 ±2°C for 5 days.
h. After incubation, 20µl of working solution of resazurin was added to all wells.
i. The plate was wrapped with aluminum film and incubated for 1hour.
j. The color change was then assessed visually. Any color change from purple to pink or colorless was recorded as positive (growth).
k. The lowest concentration at which there is no color change occurred was taken as the MIC value.

SUMMARY

The Sample 1 and 2 were evaluated for Antimicrobial activity against *Mucor racemosus* and *Rhizopus oryzae* by Microdilution method at different concentrations. The MIC values of test substance were compared with the activity of standard antibiotic and the results were noted.

RESULTS

Organoleptic characters

Table 05: Organoleptic characteristics of samples of *tripurabhairava rasa.*

	Sample 1	Sample 2	Sample 3
Colour	Reddish brown		
Odour	Pungent, teekshna	Pungent, teekshna	Pungent, ati teekshna
Taste	Characteristic, katu	Characteristic, katu	Characteristic, ati katu
Touch/ Consistency	**Vati batch**- semisolid after bhavana (hard pills in dry form)		
	Choorna batch -powder, soft on touch		

Testing of tablets

Table 06: Analysis of tablets of samples of *tripurabhairava rasa.*

	Sample 1	Sample 2	Sample 3
Hardness	2.5kg/cm^2	2.5kg/cm^2	3.5kg/cm^2
Disintegration	30min 16sec	25min 15sec	38min 16sec
Friability	0.16%	0.72%	0.16%
Uniform weight of tablets	375±5mg (%)	375±5mg (%)	375±5mg (%)
Thickness and diameter	0.64cm	0.66cm	0.68cm

Physico-chemical parameters

Table 07: Physico-chemical parameters of samples of *tripurabhairava rasa.*

	Sample 1	Sample 2	Sample 3
Loss on drying	23.17%	17.1%	16.58%
Moisture content	23.17%	17.1%	16.58%
Total ash	14.5%	14.5%	15.25%
Acid insoluble ash	2.5%	2.5%	2%
Water soluble ash	12%	12.5%	13%
Ph	8.56	8.57	8.59
Water soluble extractive	23.2%	17.6%	29.6%
Alcohol soluble extractive	20%	22.4%	19.2%

Sem-edax analysis of samples

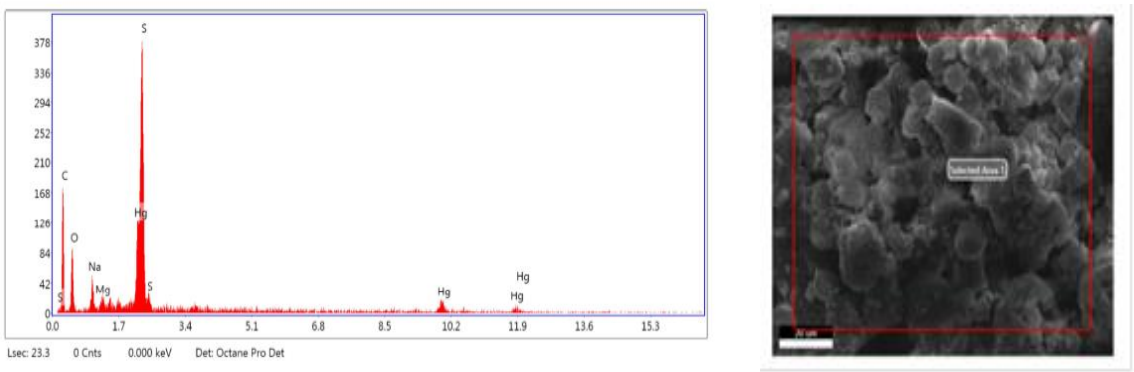

Figure 1: SEM Graph and Photograph of sample 1.

Summary of the report: The above scanning electron microscopy results indicates the morphology is irregular shape (Formation of layer) and amorphous nature (Porous nature).

Summary of the report: EDAX test report indicates that sample one contains Carbon, Oxygen, Sodium, Magnesium, Sulphur and Mercury.

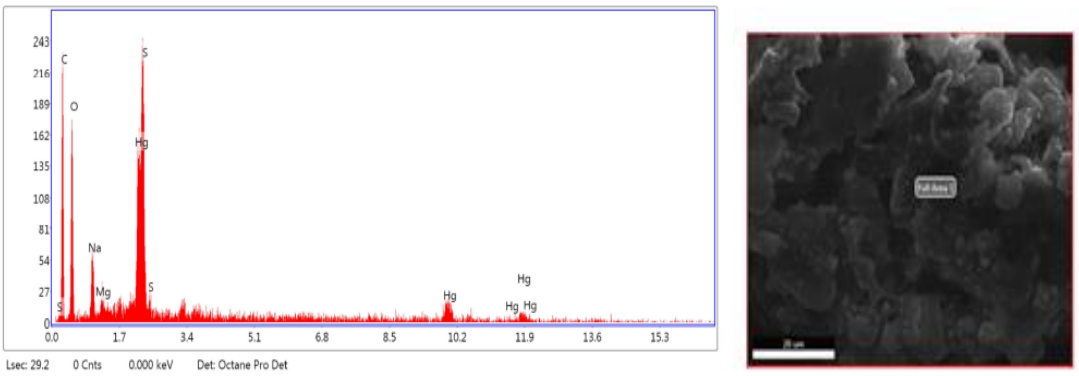

Figure 2: SEM Graph and Photograph of Sample 2.

Summary of the report: The above scanning electron microscopy results indicates the morphology is irregular shape. Sample-2 material is formed a layer. The layer is embedded some other material.

Summary of the report: EDAX test report indicates that sample two contains Carbon, Oxygen, Sodium, Magnesium, Sulphur and Mercury.

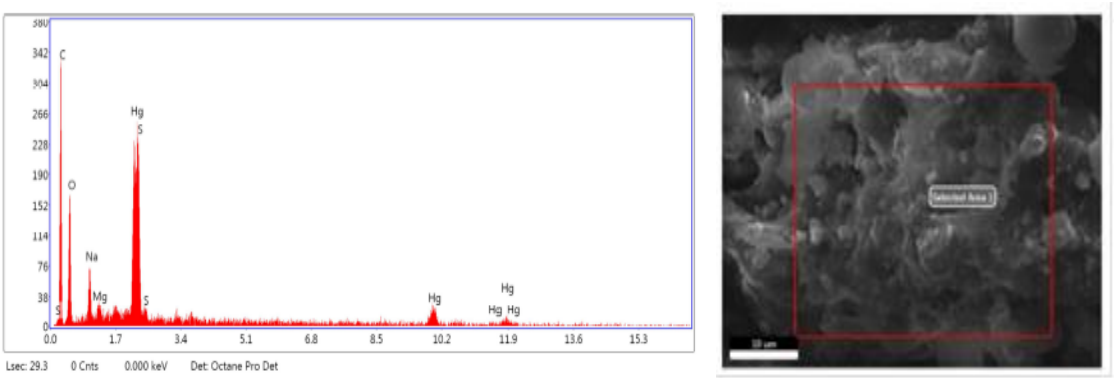

Figure 3: SEM Graph and Photograph of Sample 3.

Summary of the report: The above scanning electron microscopy results indicates the morphology is irregular shape. Sample-3 material is clearly shows a layer. The layer is embedded with material (irregular shape of the embedded material). Porous in nature.

Summary of the report: EDAX test report indicates that sample three contains Carbon, Oxygen, Sodium, Magnesium, Sulphur and Mercury.

Table 08: EDAX Report of 3 Samples of tripurabhairava Rasa.

	Sample 1	Sample 2	Sample 3
Element	Weight %	Weight %	Weight %
C k	41.66	44.11	49.02
O k	22.30	31.18	28.21
Nak	3.01	4.08	4.01
Mgk	0.74	0.65	0.75
S k	20.59	4.87	3.79
Hgl	11.71	15.10	14.22

Antifungal study on Mucor and Rhizopus species

Table 09: MIC of Sample 1 against *Mucor Racemosus* and *Rhizopus oryzae.*

Sample name	Concentration tested	Mic	
		Mucor racemosus	*Rhizopus oryzae*
Sample 1	1mg to 0.00048mg (w/v)	0.0625mg	0.125mg
Ketaconazole	1mg to 0.00048mg (w/v)	0.00048mg	0.00048mg

Table 10: MIC of Sample 2 against *Mucor Racemosus* and *Rhizopus oryzae.*

Sample name	Concentration tested	Mic	
		Mucor racemosus	*Rhizopus oryzae*
Sample 2	1mg to 0.00048mg (w/v)	0.125mg	0.125mg
Ketaconazole	1mg to 0.00048mg (w/v)	0.00048mg	0.00048mg

DISCUSSION

Pharmaceutical study

i. Shodhana of vatsanabha

- This method of *Shodhana* was opted as ***Gomootra*** possess *Vishaghna Prabhava* thereby balancing/ reducing the toxic alkaloids of *Vatsanabha.*
- Aconite and Pseudoaconite present in *Ashuddha Vatsanabha* might have been converted to less toxic substances- Benzyl Aconine and Veratroyl Pseudoaconine in *Shuddha Vatsanabha* owing to the purpose of *Shodhana*.[16]

ii. Shodhana of tankana

- This method of *Shodhana* was opted as it is convenient to remove the water molecules of *Tankana.*
- Purified Tankana contains more Boron (13.48%) compared to raw Tankana (10.08%).[17]

iii. Shodhana of gandhaka

- This method of *Shodhana* was opted as the reference indicates its utility as *Jaramrutyurujapaha, Agnisandeepaka* and *Veeryavruddhikara.*[18]

iv. Shodhana of hingula

- This method of *Shodhana* was opted as *Bhavana*[19] facilitates reduction of particle size.
- ***Ardraka*** helps in removal of impurities; acidic pH of *Ardraka Swarasa* may act upon releasing the bonds of HgS in *Hingula* and ease the removal of impurities.

v. Shodhana of jayapala

- This method of *Shodhana* was opted as ***Goksheera*** and *Swedana*/ heat application helps in the removal / reduction of toxic components.
- The change in the colour of the milk from white to brownish pink suggests the presence of, β-sitosterol, Hemolytic protein, Fats present in Croton oil.[20]
- Changes in the pH of *Goksheera* were noted before and after the procedure may be attributed to presence of chemical constituents of *Jayapala Beeja* such as Tiglinic acid, Crotonic acid Glycerides of Stearic, Palmitic, Myristic and Lauric acids and volatile acids.[20]

vi. Shodhana of dantimoola

- Use of herbal drugs like *Pippali, Yastimadhu* helps in modifying the levels of *Teekshnata* in *Dantimoola*.
- *Pippali* helps in breaking the bonds of Terpenoids, Glycosides present in *Ashuddha Dantimoola* so as to facilitate removal of excess contents during *Shodhana*.
- *Yastimadhu/ Madhuka* helps in reducing the *Teekshna Guna* by virtue of its *Madhura, Snigdha Guna.*
- *Kusha- Mritlepa* helps in facilitating Swedana and sustaining the heat within the bolus subjected for heat.

vii. Preparation of dantimoola kashaya

- Difference in the organoleptic characters of *Ashuddha* and *Shuddha Dantimoola Kwatha* attributed to the changes before and after *Shodhana* of *Dantimoola.*
- Changes in pH and Total Solids were supportive proofs suggesting the reduction in the Terpenoids, Glycosides content of *Dantimoola*.

viii. Preparation of tripurabhairava rasa

- The *Gomootragandha* of *Vatsanabha* was more during Sample 3 preparation than other 2 samples. This may be attributed to the additive effect of *Teekshnagandha* of *Ashuddha Dantimoola Kwatha* in Sample 3.
- Change in the consistency of mixture for every 1 hour *Bhavana* was observed suggesting the absorption of *Dantimoola Kwatha* into the homogenous mixture.
- The ingredients of the formulation possess *Deepana-Pachana- Srotoshodhaka* property thereby helping in the *Sampraptivighatana.*
- In addition to it, they also possess *Jwaraghna, Krimighna, Shothaghna* properties, which due to the synergetic effect with *Dantimoola* help in *Vyadhishamana* and *Shodhana* of *Srotas*.
- With specific *Anupana* and *Pathya* mentioned in the classical reference, the effect of the *Yoga* can be well appreciated and probability of side effects, if any, due to the *Teekshna-Ushna- Vikasi Guna* of *Dravyas* might become less which can be concluded only after clinical study.

Analytical study

Organoleptic characters: Differences between 3 Samples were noted due to change in the composition.

Disintegration time

- Change in the time of disintegration of tablets may be attributed to change in the formulation and processing.
- Sample 2 took lesser time to disintegrate owing to its minuteness of particles due to 2 *Bhavana*. This may be helpful in Pharmacokinetic aspect of drug.
- Sample 1 took lesser time compared to Sample 3 which may be attributed to the use of *Shuddha* and *Ashuddha Dantimoola Kwatha* suggesting that *Shuddha Dantimoola*

Kwatha has properties of making the drug minute and facilitates quicker disintegration, a helpful characteristics in Pharmacokinetics.

Hardness test

- Difference in hardness of tablets of 3 samples is indicative of effect of *Bhavana* on the ingredients.
- Increase in hardness of tablets of Sample 3 implies the adhesion of particles on to *Ashuddha Dantimoola Kwatha.*

Uniform weight of tablets

- Weight of tablets was weighed before and after drying it.
- The uniformity of weight of tablets is indicative of Standardization, suggesting that each Vati consists of prescribed amount of drug.

Thickness and Diamter of tablets

- Uniformity in the diameter was notes with 5% variation between 3 samples.

Friability

- Friability plays a major role in storage and transportation of tablets.
- The increase in Friability values of Sample 2 compared to the other samples might suggest the fineness of the product due to additional *Bhavana* of *Ardraka Swarasa* along with *Shuddha Dantimoola Kwatha.*
- The additional *Bhavana* might be contributory to the fact that the product is easily breakable or powdered compared to other samples.

Physico-chemical parameters

Loss on drying- moisture content

- Difference in the values can be attributed to the amount of moisture and volatile compounds in the samples.
- Sample 1 contains higher amount of moisture content which suggests the water content is more in *Shuddha Dantimoola Kwatha.*
- Sample 2 contains lesser moisture compared to Sample 1 which is suggestive of evaporation of moisture content and volatile compounds due to additional *Bhavana*.

- Sample 3 contains lesser moisture content compared to Sample 1 but similar to Sample 3 implying the lesser water molecules and higher concentration of chemical constituents in *Ashuddha Dantimoola Kwatha.*

pH value

- Though the pH values are in the same range, slight changes in the value can be suggestive of the content of the sample.
- pH of Sample 3 > pH of Sample 2 > pH of Sample 1 implies that the *Ashuddha Dantimoola* is slightly more alkaline compared to *Shuddha Dantimoola Kwatha* suggesting the removal of certain alkaloids from *Dantimoola* after *Shodhana.*
- Addition of *Ardraka Swarasa* to *Shuddha Dantimoola Kwatha* might have altered the pH.

TOTAL ASH, ACID INSOLUBLE ASH AND WATER SOLUBLE ASH

- Ash value signifies the ignition of organic substances and amount of inorganic substances in the sample.
- Compared to the 3 samples, Sample 3 has higher value suggestive of higher inorganic content in it which is more acid soluble and less water soluble comparatively.

Water soluble Extractive and Alcohol soluble extractive

- Extractive values of sample suggest the amount of content/ active ingredients/ phyto-constituents which can be extracted alone.
- Values suggest that constituents of Sample 1 is more Water soluble than Alcohol soluble. This may be due to presence of additional herbal drugs in *Shuddha Dantimoola Kwatha*- presence of *Pippali* and *Yastimadhu* contents.
- Sample 2 has better extraction in Alcohol than Water which is suggestive of interaction of ingredients with *Ardraka Swarasa* making it inclined towards Alcohol solubility.
- Sample 3 has better extractive value in Water indicative of presence of constituents in *Ashuddha Dantimoola Kwatha* which are Water soluble.

Instrumental parameters

Sem-edax reports

- **Sem analysis report**

Sample 1

- SEM results indicate that morphology is irregular shape (Formation of layer) and amorphous nature (Porous nature).
- The irregular shape might be due to presence of *Shuddha Dantimoola Kwatha*. Unlike other samples, embedded material is absent suggesting the utility of *Shodhana* of *Dantimoola.*
- The presence of *Shodhana Dravyas* might have contributed to smooth texture of particle thereby avoiding embedment of materials.
- Porous nature of the Sample may enable easy absorption and enhance bioavailability.(Pharmacokinetics and Pharmacodynamics)

Sample 2

- SEM results indicate that morphology is irregular shape. Sample-2 material formed a layer. The layer is embedded some other material.
- This might suggest that the embedded layer may be due to *Ardraka Swarasa* and particles adhered to it. Adherence may lead to slower absorption and distribution.

Sample 3

- SEM results indicate that morphology is irregular shape. Sample-3 material clearly showed a layer.
- The layer is embedded with material (irregular shape of the embedded material). Porous in nature
- The formation of embedded material might be due to presence of *Ashuddha Dantimoola Kwatha*. The *Kwatha* might have caused the adherence of materials onto it.
- Though the Sample is porous in nature the adherence of material may cause hindrance in absorption of drug.

- **Edax test report**

EDAX test reports of all the three samples indicate presence of Carbon, Oxygen, Sodium, Magnesium, Sulphur and Mercury. However the percentage of contents varied in each sample.

Antifungal study

- With respect to the analysis of all the parameters, Sample 1 and Sample 2 were considered better for usage and internal administration compared to Sample 3 as Sample 3 contains *Ashuddha Dantimoola Kwatha Bhavana* which might cause excessive effect of drug and toxicity.
- Hence Antifungal study was conducted on Sample 1 and 2 to test for its effect on fungal species causing Mucormycosis
- The results of Sample 1 might be due to the balanced effect of ingredients and *Bhavana Dravya.*
- ***Tankana* and *Ardraka Swarasa*** are also known for their antidotal activity on *Vatsanabha* and *Jayapala*, which helps in controlling their action.
- Drugs also have *Krimighna* action thereby helpful in mitigating the *Mucor* and *Rhizopus* species.
- ***Dantimoola kwatha*** due to its synergetic effect of *Krimihara* property might have upgraded the action of *Tripurabhairava Rasa* over Mucormycosis species.
- The results of Sample 2 may be attributed to the additional effect of ***Ardraka Swarasa.*** However the comparative decrease in the antifungal effect of Sample 2 on *Mucor* species might be due to the overpowering effect of *Ardraka Swarasa* over the other ingredients as *Ardraka Swarasa* is an antidote for *Vatsanabha, Hingula* and *Jayapala,* which is not present in Sample 1.

Images

Image 01-05: Ingredients of Tripurabhairava Rasa- Shuddha Vatsanabha, Shuddha Tankana, Shuddha Gandhaka, Shuddha Hingula and Shuddha Jayapala.

Image 06-09: Tripurabhairava rasa nirmana.

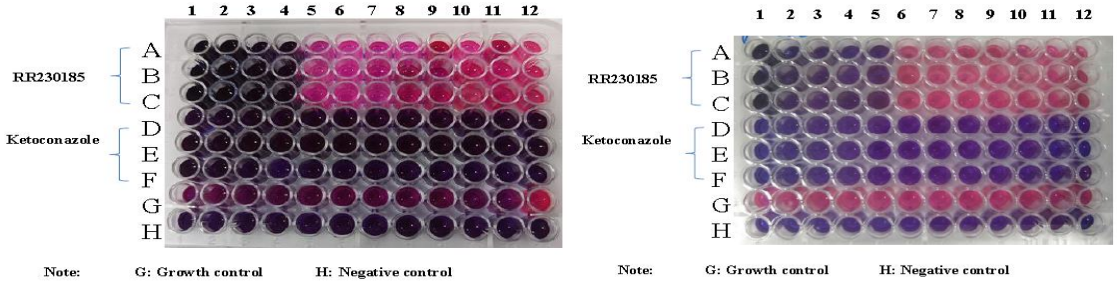

Image 10-11: MIC of Sample 1 against *Rhizopus oryzae* and *Mucor racemosus.*

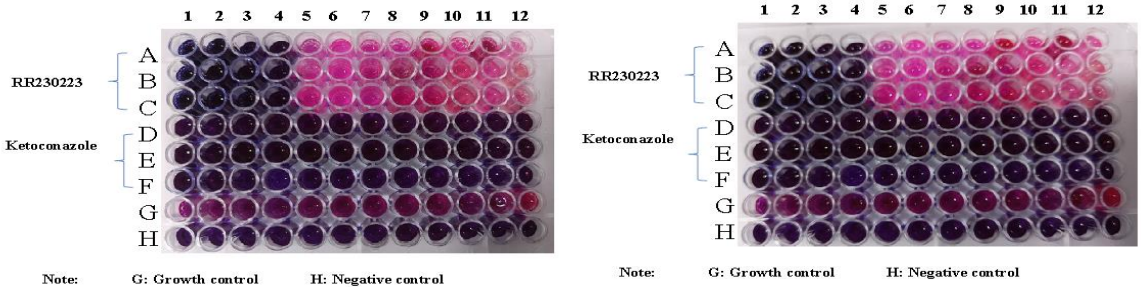

Image 12-13: MIC of Sample 2 against *Rhizopus oryzae* and *Mucor racemosus.*

CONCLUSION

- *Kharaleeya Rasayana* is one among the *Chaturvidha Rasayana* mentioned in classics among which *Tripurabhairava Rasa* is one of the formulation mentioned in the context of *Jwara Adhikara*, wherein the ingredients possess *Jwaraghna- Krimighna* action.
- Hence the study was taken up to evaluate the pharmaceutico-analytical parameters of *Tripurabhairava Rasa* along with Antifungal effect on the species causing Mucormycosis, a complication of Covid 19 pandemic.
- In this regard the present study enlightens the scope of Ayurvedic formulations specifically *Rasadravyas* in tackling such grave diseases. It also addresses the concern of society regarding the use of *Visha- Upavisha* and such poisonous drugs in treatment of health ailments.

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