

**PHARMACEUTICAL AND IN VITRO ANTIMICROBIAL STUDY OF VINDHYAVASI
YOG: AN AYURVEDIC HERBOMINERAL FORMULATION****Dr. Vinod Dalvi*, Dr. Mrunali Patil**

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ABSTRACT

Vindhyavasi yog is a herbo-mineral preparation mentioned in Bhaishajya Ratnavali. The present study complied under section as pharmaceutical study, and Antimicrobial study. Main ingredient of this yoga is Loha Bhasma which is prepared by Ayurvedic procedure. Shodhan was done in Til Tail, Takra, Gomutra, Kanji and Kulttha kwath. Vishesh shodhan done in Triphala kwath. During shodhan in Triphala kwath Loha become more brittle. Total 20 putas was required for preparation of Loha Bhasma. Highest temperature attend during process of puta was 1000.5 °C. Ayurvedic bhasma parikshan was done which fulfil the criteria. Modern Analytical test like XRD, SES-EDS shows nano crystalline catalyst material which has an average particle size of 80 nm and mainly contain O and Fe element. According to Bhaishajya Ratnavali all ingredient are taken and mixed together and triturated till become homogenous mixture. Dark coffee brown colored preparation of Vindhyavasi yoga was formed. Analytical study of Vindhyavasi yog was done. For understanding action of Vindhyavasi yog in kanthagat vyadhi, antimicrobial study was done against *Streptococcus pyogenes*, *Haemophilus influenza*, and *klebsiella pneumoniae* which shows significant on these bacteria as compare to standard *Streptomycin*. It was done by Well diffusion method by observing their zone of inhibition.

KEYWORDS: Vindhyavasi yog, Loha Bhasma, Bhasma parikshan, Nano, Antimicrobial.**INTRODUCTION**

In Vedic and Samhita period mainly herbal drugs was used in different forms but from the period of Nagarjuna 8th century compounds of herbo-mineral drugs are used profusely. Rasashastra is specialized branch of Ayurveda in which mineral and metal based medicinal preparation are described. To increase the efficacy, fast action, shelf life and easy administration different Kaplan's are developed.

In Kharaliya kalpanas ingredients are triturated in khalva yantra. It is unique & most widely used due to easy preparation method, more potency and good results. *Bhavana* and *Mardan* sanskar are the main principle behind preparation of kharaliya kalpa. These are useful for curing many diseases and healthy life. In Bhaishajya Ratnavali one of the best formulation mentioned in

Rajaykshma roghadhikar^[1] which contain Vyosha^[5,9], Shatavari,^[17] Triphala^[8,9,23], Bala, Atibala^[16] and Loha bhasma.^[18,19,20,22] It is mainly indicated for Vakshashata, Kanthagat vyadhi, Rajayakshma, Bahustamba, Ardit vyadhi.

Loha bhasma is nano iron particles based medicine. Iron is crucial element for human body, its main function is oxygen transport. Its deficiency leads to various health infirmities. Iron containing drugs are widely used in modern medicine in anaemia as hematinics. In Ayurveda it is used with different drugs with different conditions. It is indicated in Pandu (anaemia), Kamala, Liver diseases, Shotha, Shool, and Kshaya etc.^[30] This Loha bhasma is used in Vindhyavasi yog^[1,2] as key ingredients.

Now a days we are suffering from different respiratory tract infection. Upper respiratory tract infection is common illness in both children & adults. It will caused by viruses, bacteria or allergens. *Streptococcus pyogenes*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis* are commonly related with respiratory tract infection.^[32, 33] Respiratory tract infections which can range from mild colds to life-threatening conditions. These are one of the top reasons of people visit doctors worldwide. These infections not only harm people's quality of life but also create significant financial challenges for individuals and health care systems.

MATERIALS AND METHODS

Materials include

a) Major raw drugs

Sunthi Churna (Rizome), Marich Churna (Seeds), Pipali Churn (Seeds), Shatavari Churn (Root), Haritaki Churn (Fruit), Bibhitak Churn (Fruit), Amalki Churn(Fruit), Bala Churn (Root), Atibala Churn (Root), Loha (Iron turning).

b) Associated drugs

- Drugs for Lola shodhan:-Til tail, Takra, Gomutra, Kanji, Kulattha.
- For Loha Vishesh shodhan:-Triphala Bharad.
- For Loha Marana:-Dadim patra.
- For Kanji Nirman:-Tandul.

c) Equipments

Gas stove, Khalva yantra, Sharav, Mulatani mati, Cloth, Lighter, Holder, Weighting Machine, Cow dunk (upal), Air tight container, Iron vessel, Sieve, Stainless steel bowl, Heating torch with Burner, Mortar pestle, Pyrometer.

Method Include

1) Collection of raw materials

- Raw Loha (Iron Turning) was purchased from Dynamic engg. and authenticated in System services (Material testing division).
- Raw herbal churnas was procured from Certified Pharmaceutical Company and authenticated in their GMP Certified lab.
- Til tail, Gomutra, Kulattha beej purchased from local market.
- Kanji was prepared in Rasashastra department.

2) Loha Bhasma Nirman- It includes a) Loha Samanya Shodhana

b) Loha Vishesh Shodhana

c) Loha Maran

a) Loha Samanya Shodhana^[18]

तैले तद्रे गवां मूत्रे ह्यारनाले कुलत्थजे । क्रमान्निषेचयेत्तप्तं द्रावे द्रावे तु सप्तधा ॥

स्वर्णादिलोहपत्राणां शुद्धिरेषा प्रशस्यते ॥

(रस.रत्न.समु.5/13)

- Raw Loha (Iron turning) was heated in Iron vessel with the help of special heating torch which burner till become red hot.
- After becoming red hot it was dipped in the Til Taila.
- The same Procedure was repeated for 7 times.
- Fresh samples of Til Tail was used for every time.
- Every piece of Loha was collected and weight was taken.
- Same procedure was done in Takra, Gomutra, Aranala(kanji) and, Kulattha kwatha for 7 times.



Fig.1: Loha Samanya Shodhana in Til Tail, Takra, Gomutra, Aranala (kanji) and, Kulattha kwatha.

b) Loha Vishesh Shodhana^[19]

तीक्ष्णलोहोन्मितं सूक्ष्म सूक्ष्म रजो दविकासंस्थितं वह्नि सन्तपितम् ।
सप्तधा स्तापितं त्रैफलेनाम्भसा लोहमेव द्रुतं याति शुद्धि पराम् ॥
रसतरङ्गिणी 20/15

- One part authenticated Triphala bharad churn was taken in steel vessel and 16 parts of tap water was added and boiled up to its remains ¼th (quarter). After cooling it was filtered by cloth.

- Shodhit Loha churna was heated in Iron vessel with the help of special heating torch which burner till become red hot.
- After becoming red hot it was dipped in the Triphala kwath.
- The same Procedure was repeated for 7 times.
- Fresh samples of Triphala kwath was used for every time.



Fig. 2: Loha vishesh shodhan in Triphala kwath.

c) Loha Maran-^[24]

It was done according to procedure mentioned in Yogratnakar.

दाडिमीपत्रजरसैलोहवूर्णं च भावितम् ।आतपे सप्तधा तेन पुनर्गजपुटद्वयम् ॥११॥

इत्थं कृतं च तद्द्रव्यं शुद्धं वारितारं भवेत्प्रायोजयेत्सर्वरोगेषु सत्यं गुरुत्वो यथा॥१२॥

सो.र. पूर्वार्ध

- Dadimpatra swaras was prepared by fresh Dadimapatra.
- One Part of Shuddha Tikshna loha churn was taken in clean khalva yantra. And sufficient quantity of dadimpatra swaras was added then put in sunlight.
- When it became dry again fresh dadimpatra swaras was added and put in sunlight. Same procedure was repeated for 7 times. Then enclosed in sharav sampute and subjected to gajput.^[18]
- Procedure was repeated until it becomes fine powder. Total 20 gajput was required.
- Then bhasmapariksha (Varna, Rekhapurnatva, Varitartav, Apunarbhav, Unam, Nischandrika, Sukshmatwa and Niswadu etc.) was done.
- After fulfillment of these bhasmapariksha loha Bhasma was used in preparation of Vindhyavasi yog.



Fig.3: Dadim patra swaras bhavit loha churna for maran.



Fig.4: Different steps of Loha maran.

3) Preparation of Vindhyavasi yog^[1, 2]

Vindhyavasi yog was prepared according to Bhaishajya

Ratanavali.

व्योषं शतावरीं त्रीणि फलानि द्वे बले तथा । सर्वामयहरो योगः सोऽयं
लौहजोऽन्वितः ॥८१॥

एष वक्षःक्षतं हन्ति कण्ठजांश्च गदांस्तथा । राजयक्ष्माणमत्युग्रं

बाहुस्तम्भमथादितम् ॥८२॥

भेषज्य रत्नावली (राजयक्ष्मा)

Table No. 1: Preparation of Vindhyavasi yog.

Sr.no	Name of drug	Quantity
1.	Sunthi Churna(Rizome)	10 gm
2.	Marich Churna(Seeds)	10 gm
3.	Pipali Churn (Seeds)	10 gm
4.	Shatavari Churn(Root)	10 gm
5.	Haritaki Churn(Fruit)	10 gm
6.	Bibhitak Churn(Fruit)	10 gm
7.	Amalki Churn(Fruit)	10 gm
8.	Bala Churn(Root)	10 gm
9.	Atibala Churn(Root)	10 gm
10.	Lohabhasma (Incinerated Iron)	90 gm

1. Measured quantity of all ingredients are taken in khalwa yanta and triturated until become homogenous mixture.
2. Then prepared Vindhyavasi yog was packed in airtight container and stored in dry place.
3. Organoleptic tests and analytical tests such as loss on drying, total ash value, Acid insoluble ash, Alcohol soluble extractive, water soluble extractive and particle size was done.



Fig.5 Preparation of Vindhyavasi yog.

4) Procedure of Antimicrobial Activity

The Antimicrobial activity of a drug was done by Agar Well Diffusion method against three bacteria namely.

1. *Streptococcus Pyogenes*.
2. *Haemophilus Influenza*
3. *Klebsiella Pnumoniae*

Procedure

1. The inoculum of the microorganism was prepared from the bacterial cultures. 15ml of nutrient agar (Hi media) medium was poured in clean sterilized Petri plates and allowed to cool and solidify.
2. 100 µl of broth of bacterial strain was pipette out and spread over the medium evenly with a spreading rod till it dried properly.
3. Wells of 6mm in diameter were bored using a sterile cork borer. Solutions of the compounds (100µl/ml)

were prepared in DMSO and 100µl of prepared test solutions (1mg/ml) and standard was added to the wells. The petri plates incubated at 37°C for 24 h.

4. Streptomycin (1mg/ml) was prepared as a positive

control and DMSO was taken as negative control.

5. Antibacterial activity was evaluated by measuring the diameters of the zone of inhibitions (ZI) all the determination were performed in triplicates.

OBSERVATIONS AND RESULTS

Table No. 2: Quantity of Loha after each shodhan.

Sr.no	Shodhan dravya	Ave.time required to become red hot loha	Qty.of liquid media for each nirvap in ml	Wt. Of loha before nirvap in gm.	Wt. Of loha after nirvap in gm.
1.	Til Tail	12 min	500	200	214.20
2.	Takra	12 min	500	214.20	217.25
3.	Gomutra	11 min	500	217.25	222.94
4.	Kanji	10 min	500	222.94	218.24
5.	Kulattha kwath	11 min	500	218.24	207.04
6.	Triphala kwath	10 min	500	207.04	202.34

Table No. 3: Result of Shodhana of Loha.

Sr. No	Details	Quantity
1.	Quantity of raw Loha(Turnings)	200 gm
2.	Total quantity of each media for shodhan	3.5 lit.
3.	Quantity obtained after samanya shodhan	207.04 gm.
4.	Quantity obtained after vishesh shodhan	202.34 gm.
5.	Total loss during samanya shodhan	No loss
6.	Total loss during Vishesh shodhan	No loss

Table No. 4: Observations of Loha Marana w.r.t.Temp. And Bhasma pariksha.

No.of puta	Max.temp attained	Time required to attain Max. temp	Weight of Loha After Puta (gm)	Bhasma pariksha			
				Colour	Touch	Rekha purnatwa	Varitaratwa
1st	860°C	2hrs. 10 min	220 gm	Black	Rough	Absent	Absent
5th	830.2°C	1 hrs. 45 min	230.56gm	Black	Rough	Absent	Absent
7th	725°C	1 hrs. 15 min	230.30 gm	Black	Rough	Absent	Absent
8th	844.4°C	1 hrs. 30 min	228.40 gm	Black	Rough	Absent	Absent
9 th	853.6°C	1hrs. 10 min	228.26 gm	Black	Rough	Absent	Absent
10th	1000.5°C	1hrs. 58 min	222.30 gm	Rough	Rough	Absent	Absent
12th	952°C	1hrs. 18 min	220 gm	Blackish red	Slight rough	50%	Absent
16th	930°C	1 hrs. 42 min	181.32 gm	Blackish red	Smooth	Present	Absent
18th	879°C	1hrs. 50 min	170.40 gm	Blackish red	Smooth	+	60%
20th	905°C	2 hrs.	152.58 gm	Blackish red	Smooth, Fine	+	90%

a) Finish drug analysis

In organoleptic characters Vindhyavasi yog shows Dark

coffee brown in colour, soft in touch, Slightly Pungent in teats and Characteristic Smell.

Table No. 5 Analysis of Vindhyavasi Yog.

Sr. No	Test	Obtained Values
1.	Loss on drying	5.67%
2.	Total Ash	54.67%
3.	Acid Insoluble Ash	45.29%
4.	Alcohol soluble extractive	8.37%
5.	Water soluble extractive	29.53%
6.	Particle size	Passes through 80#

Table No. 6: Antimicrobial activity of Vindhyavasi yog.

Sr.No.	Samples	Zone in Diameter		
		<i>Streptococcus Pyogenes</i>	<i>Haemophilus influenza</i>	<i>Klebsiella Pnumoniae</i>
1.	Control	00	00	00
2.	(Standard) streptomycin	26	30	28
3.	Vindhyavasi yog	13	15	14



Fig. 6: Antimicrobial activity of Vindhyavasi yog.

DISCUSSION

In ayurvedic literature many herbo-mineral yoga are mentioned. Vindhyavasi yog contain 9 herbal drug and 1 mineral drug. It contain combination of ushna and sheet virya dravya. In this yoga all ingredients are Madhur vipaki except Maricha (Katu vipak). It is mainly indicated for Vakshashata, Kanthagat vyadhi, Rajayakshma, Bahustamba, Ardit vyadhi.

Shodhan was done in Til tail, Takra, Gomutra, Ariana (kanji) and Kulattha kwatha all dravyas are acidic in nature except Gomutra which is slightly alkaline in nature. For preparation of Loha bhsma, 20 putas was required which passes all bhasma parikshas. Analytical test of Vindhyavasi yog was done.

Main aim of this yog to study how it works on kanthagat vyadhi. Antibacterial activity was done which shows satisfactory result (50%).

CONCLUSION

In physiochemical parameter, the analytical test results are Loss on drying 5.67 %, Total ash 54.67%, Acid insoluble ash 45.29%. Alcohol Soluble extractive 8.37% and particle size passes through 80 mesh. All these findings are within the normal limits. The antibacterial activity of Vindhyavasi yog was evaluated by measuring the zone of inhibition against *Streptococcus pyogenes*, *Haemophilus influenza*, and *klebsiella pnumoniae* bacteria stain in vitro study by well diffusion method. Vindhyavasi yog exhibited good antibacterial activity against these 3 bacteria as compared to standard Streptomycin.

From this above work we conclude that we can use Vindhyavasi yog in different condition of respiratory tract infection and gives quality of life to peoples. And also offers our treatment cost effective and more accurate. We need to prepare novel doses forms, animal study, and clinical trials on Vindhyavasi yog to make our treatment scientifically more validated. So further study is needed.

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