



## PHARMACEUTICO-ANALYTICAL AND ANTIMICROBIAL STUDY OF JARIT VANGA BHASMA PREPARED WITH SPECIAL REFERENCE TO RASATARANGINI

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### ABSTRACT

Background: Vanga Bhasma is said to possess antimicrobial activity (Jantughna Prabhava). Hence it was decided to evaluate the antimicrobial activity of Jarit Vanga Bhasma (JVB) prepared with special reference to Rasatarangini 18/29-33. Objectives: JVB was synthesized, analysed and its antimicrobial effects were studied in *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Escherichia coli* and *Candida albicans*. Materials and Methods: The JVB was prepared and analyzed for the quality parameters mentioned in the Ayurvedic texts as well as the modern parameters like XRD, SEM and EDX to find out the nature of the Vanga Bhasma samples. The anti-microbial study was done to find out the anti-microbial efficacy of the JVB samples. Results and Conclusions: The adopted method for preparation of JVB was able to produce a Bhasma compatible to organoleptic parameters mentioned in the ancient texts. The obtained JVB was grayish white with the formation of the small sized particles as small as a nanoparticle which was confirmed by SEM study. XRD study confirms that Tin oxide is the major compound found in all the JVB samples. JVB showed antimicrobial activity in inhibiting the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Escherichia coli* and *Candida albicans* with a concentration of 100mg/ml. The mean zone of inhibition was 12.33mm, 12.33mm, 14.66mm, 10.33mm and 16mm respectively. This outcome further supports the Krumighna and Jantughna properties (Anti-microbial activity) of Vanga Bhasma. Hence, JVB is said to possess Anti-Microbial activity.

**Keywords:** Analytical, Anti-microbial, Jaran, Maran, Shodhan, Vanga

### INTRODUCTION

Rasaushadhis are the potent herbomineral preparations better known for their quicker action, lesser dose quantity, prolonged shelf life and a better palatability<sup>1</sup>. Today's modern era demands such Bhasma preparations due to their Rasayana and Yogavahi effects along with a wide range of therapeutic indications. But preparation of Bhasma is a skillful task which requires a longer time for its preparation. Moreover, different texts of Rasashastra have mentioned a number of Bhasma preparations with a wide range of indications and off course with a variety of pharmaceutical processing and ingredients. Vanga, a Putiloha<sup>2</sup> is a widely prescribed drug with a broad-spectrum therapeutic indication. This article is aimed at evaluating the antimicrobial activity of JVB prepared with special reference to Rasatarangini 18/29-33<sup>3</sup>.

### MATERIALS AND METHODS

This study was done under the sections- Pharmaceutical Study, Analytical Study and Antimicrobial Study.

#### Pharmaceutical Study of JVB

The different materials used for the preparation of Vanga Bhasma; raw Vanga (Tin), Tila<sup>4</sup> Taila (Sesame oil), Takra<sup>5</sup> (Butter milk), Gomutra<sup>6</sup> (Cow's urine) and powder of Ashwattha Twak<sup>7</sup> (*Ficus religiosa*) were procured from local retailers. The Kulattha

Kwath<sup>8</sup> (Decoction of *Dolichos biflorus* Linn.) and Churnodaka<sup>9</sup> (Lime water) were prepared in the departmental laboratory. All medicinal plants used in the study were authenticated at Department of Botany.

The preparation of Vanga Bhasma consists of steps such as Shodhan (Samanya and Vishesha) and Jaran.

#### Shodhan of Vanga

Ashuddha Vanga was subjected to Samanya<sup>10</sup> and Vishesha Shodhan.<sup>11</sup>

The Samanya Shodhan of Vanga was done by quenching the molten Vanga subsequently into Tila Taila, Takra, Gomutra, Kanji<sup>12</sup> and Kulattha Kwath; 7 times each. Then this Samanya Shodhit Vanga was subjected to Vishesha Shodhan. The Samanya Shodhit Vanga was melted and further quenched into Churnodak (lime water) for 7 times. Each quenching was done in a fresh liquid. These purification methods not only remove the impurities from Vanga but also enhance the therapeutic properties of it.

#### Preparation of JVB

JVB (Batch A1, B1, C1) were processed with Ashwattha Twak along with the Shuddha Vanga.

**Jarana of Shuddha Vanga<sup>13</sup>**

The specified amount of Vishesh Shodhita Vanga was taken in the Lauha Kadahi. The Shuddha Vanga was heated, till it completely melted. A measured quantity of Ashwattha Tvak Churna (ATC) ranging from 5g to 7g was sprinkled over the molten Vanga and a forceful rubbing was done with the ladle. The ATC was added at a regular interval followed by a rubbing with a pressure. This was done, until the Vanga got converted into a

powder form and none of the metal particles remained in a visibly metallic form. The powdered Vanga was collected in the centre of the iron pan; covered with the Sharava and a maximum amount of heat was offered. Intermittently the Sharava was slightly lifted to ensure that the powdered Vanga has turned to red hot. Once all the powdered Vanga changed to red hot, the heating was stopped and left for self-cooling. Next day, Jarit Vanga was carefully collected and was subjected to weighing.

This process was done in three batches viz. A1, B1 and C1.

In nutshell, it can be summarized that during the preparation of JVB-

Average temperature of Lauha Kadhai during procedure:	640 °C
Average temperature of Gas stove:	650°C
Average temperature of Jarit Vanga:	600°C
Average time taken for Jaran procedure:	3.5 h
Average time taken for attaining a red-hot stage:	1.30 h
Average increase in weight of Jarit Vanga -	31.60g (10.21%)

**RESULTS****Table 1: Observation regarding Jaran of Vanga**

No. of Batch	Wt. of Shuddha Vanga (g)	Wt. of Ashwattha Panchanga (g) 1/4 <sup>th</sup> part	Duration	Wt. of Jarit Vanga (g)	Wt Increase in %
Batch A1	277.81	69.45	3 h 55 min	303.04	8.32
Batch B1	276.10	68.77	4 h 00 min	309.63	10.82
Batch C1	277.14	69.28	4 h 15 min	313.2	11.51

**Table 2: Observation regarding pH of water of Jarit Vanga and wt. of Vanga after Kshalana**

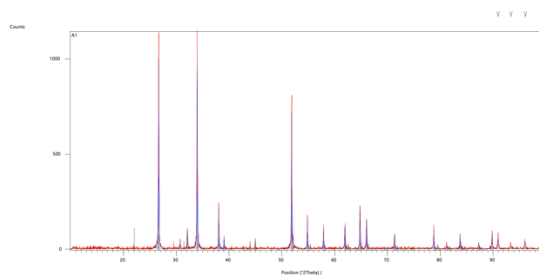
No. of Batch	1 <sup>st</sup> Wash	2 <sup>nd</sup> Wash	3 <sup>rd</sup> Wash	Wt. of Vanga after Kshalana (g)	Wt. decrease in %
Batch A1	12	9.2	7.62	287.96	4.97
Batch B1	11	8.56	7.45	291.77	6.12
Batch C1	13	9.40	7.20	295.18	6.126

**Analytical Study****Table 3: Organoleptic characters<sup>14</sup> of JVB**

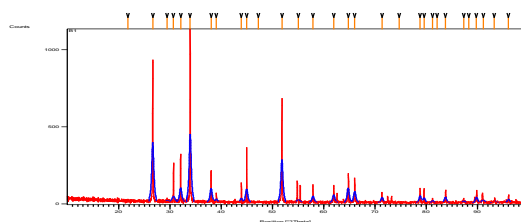
Parameter	JVB
Shabda	Anupasthita
Sparsha	Slightly rough
Rupa	Greyish
Susnigdhatva	Alpa snigdha
Nischandratva	No metallic luster
Rekhapurnatva	Upasthita
Varitaratva	Anupasthita
Unama	Anupasthita
Rasa	Tasteless
Gandha	Not specific

**Table 4: Analytical Study<sup>15</sup>**

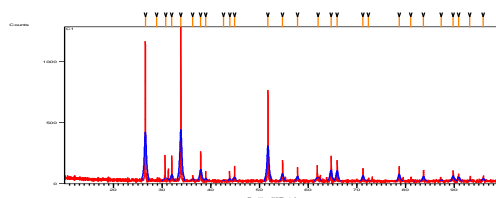
Sr. No.	Sample of JVB	Ash Content	Acid Insoluble Matter	Water Soluble Extractives	Alcohol Soluble Extractives	pH
1	A1	95.38%	93.15%	0.48%	0.86%	8.14
2	B1	93.38%	90.61%	0.35%	0.80%	7.99
3	C1	96.17%	92.22%	0.22%	0.84%	7.98



**XRD<sup>16</sup> Study of JVB: - Batch A1-**



**XRD Study of JVB: - Batch B1-**



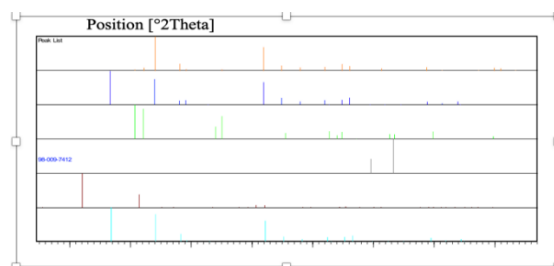
**XRD Study of JVB: - Batch C1-**

#### Identified Patterns List:<sup>17</sup>

**Table 5: Identified pattern list of JVB (Batch A1, B1 & C1)**

Visible	Ref. Code	Score	Compound Name	Displacement [°2Th.]	Scale Factor	Chemical Formula
*	98-009-7412	28	Rutile (OH)-containing)	0.000	0.320	H1.72 O2 Sn0.57
*	98-015-7134	14	Tin-Beta, Rapidly Solidified	0.000	0.044	Sn0.875
*	98-018-0454	No Matching Lines	Magnesium-Hp, Sh	0.000	0.000	Mg1
*	98-016-3534	0	Calcium (7.98) – Vi	0.000	0.095	Ca7.9776
*	98-018-1276	5	Tin Dioxide - Rutile-type, Np Stable	0.000	0.242	O2 Sn1

#### Plot of Identified Phases-



## XRD pattern of Identified Phases

### Batch A1-

- Totally 22 peaks were identified in Vanga Bhasma (Batch A1) at different angles (2 Theta) from 26.7452 to 96.1289.
- 5 strong peaks were chosen as strong with their relative intensity and compared to standard X-ray powder diffraction file.
- 4<sup>th</sup>, 1<sup>st</sup>, 8<sup>th</sup>, 5<sup>th</sup> & 12<sup>th</sup> peak with relative intensity of 100%, 97.26%, 69.28 %, 20.54% & 19.31% were considered as significant at 34.0314<sup>0</sup>, 26.7452<sup>0</sup>, 51.9248<sup>0</sup>, 38.1115<sup>0</sup>, & 64.8787<sup>0</sup> having 2.63, 3.33, 1.75, 2.35 & 1.43 d space value respectively.

### Batch B1-

- Totally 30 peaks were identified in Vanga Bhasma (Batch B1) at different angles (2 Theta) from 21.7998 to 96.0022.
- 5 strong peaks were chosen as strong with their relative intensity and compared to standard X-ray powder diffraction file.
- 6<sup>th</sup>, 2<sup>nd</sup>, 12<sup>th</sup>, 16<sup>th</sup> & 5<sup>th</sup> peak with relative intensity of 100%, 86.41%, 62.64%, 20.86% & 20.55% were considered as significant at 33.9261<sup>0</sup>, 26.6276<sup>0</sup>, 51.8129<sup>0</sup>, 64.7371<sup>0</sup>, & 32.0626<sup>0</sup> having 2.64, 3.34, 1.76, 1.43 & 2.78 d space value respectively.

### Batch C1-

- Total 27 peaks were identified in Vanga Bhasma (Batch B1) at different angles (2 Theta) from 26.5385 to 95.9087.
- 5 strong peaks were chosen as strong with their relative intensity and compared to standard X-ray powder diffraction file.
- 5<sup>th</sup>, 1<sup>st</sup>, 12<sup>th</sup>, & 7<sup>th</sup> peak with relative intensity of 100%, 94.61%, 69.10%, 22.98% were considered as significant at 33.8193<sup>0</sup>, 26.5385<sup>0</sup>, 51.7117, 37.9049<sup>0</sup> having 2.64, 3.35, 1.76, 2.37 d space value resp.

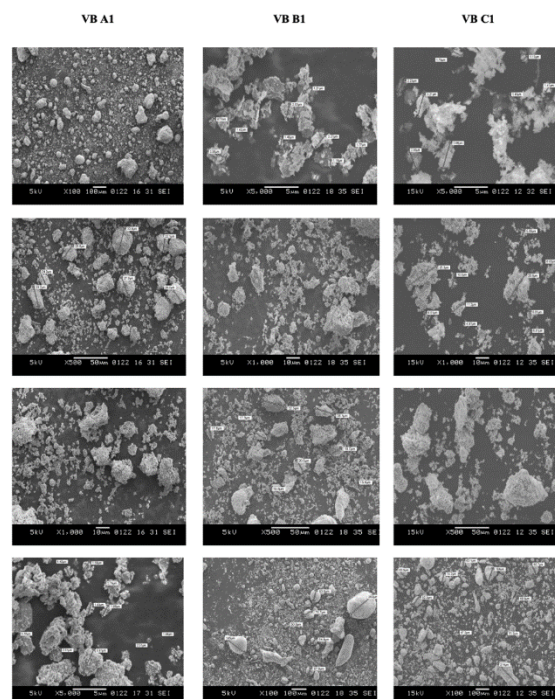
### Batch A1, B1 & C1: -

- Vanga Bhasma (Batch A1, B1 & C1) peaks are compared with standard 2 theta values with ref. No.98-009-7412 confirmed the presence of Tin Oxide (SnO<sub>2</sub>) with hydroxide in tetragonal rutile structure.
- Also, peaks compared with standard 2 theta values with ref.No.98-015-7134 confirmed presence of Tin (Sn) in Tetragonal form. Beta in Tin – Beta is different form of crystal structure in tetragonal Tin.
- Also peaks which compared with standard 2 theta values with ref. No.98-018-0454 confirmed the presence of Magnesium (Mg) in its hexagonal closed pack structure.
- Also peaks which compared with standard 2 theta values with ref. No. 98-016-3534 confirmed the presence of Calcium with anorthic (also called triclinic). In general Calcium exists in Face-centered-cubic. But because of different conditions during preparation such as different pressure and temperature may affect crystal structure.
- Also peaks which compared with standard 2 theta values with ref. No.98-018-1276 confirmed the presence of Tin Oxide

(SnO<sub>2</sub>) in its tetragonal rutile structure.

## Scanning Electron Microscopy<sup>18</sup> of JVB-

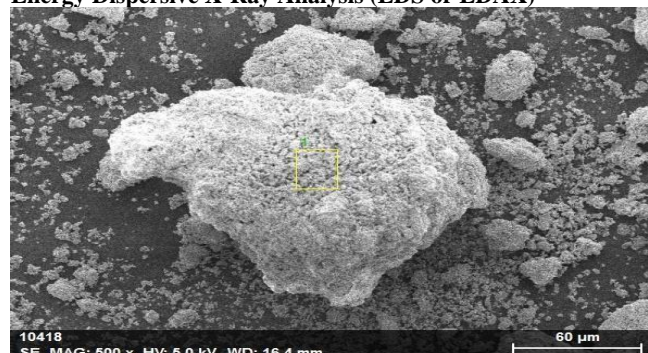
### SEM Batch A1, B1, C1-



Sample A1 has a particle size ranging from 1.42µm-3.81µm; some of these particles have the particle size of 22.1 µm-33.8 µm. Sample B1 has a particle size of 977nm; with few particles ranging from 1.16 µm-3.06 µm. Sample C1 has a particle size ranging from 1.13 µm-3.66 µm.

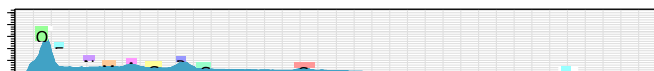
It may be noted that Sample A1, B1 and C1 are the Jarit Vanga preparations of Shuddha Vanga + Ashwattha Twak Churna (without classical Puta). Even then the considerably smaller particle size was obtained.

## Energy Dispersive X-Ray Analysis (EDS or EDAX)<sup>19</sup>



### EDS-BATCH A1

HV:5.0Kv 10418Date:3/3/2020 12:45:39 PM Image size:1000 x 750 Mag:500x



EDS Batch B1

Date:3/3/2020 12:47:49 PM HV:5.0kV Puls th.:9.15kps

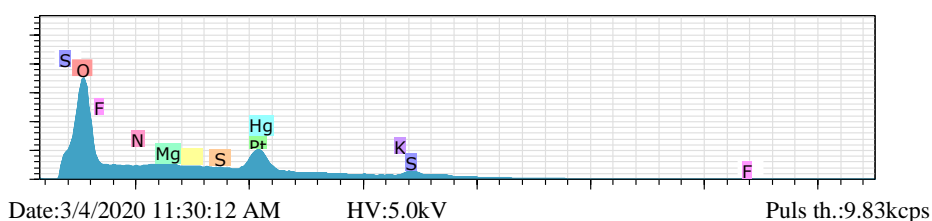
El AN Seriesun. C norm.C Atom. C Error (1 Sigma)  
[wt. %] [wt. %] [at. %] [wt. %]

Sn 50 M-series	99.69	99.69	98.74	16.17
O 8 K-series	0.13	0.13	0.98	0.04
Pt 78 M-series	0.11	0.11	0.07	0.03
Fe 26 L-series	0.04	0.04	0.08	0.03
Al 13 K-series	0.02	0.02	0.07	0.03
Si 14 K-series	0.01	0.01	0.02	0.03
Mg 12 K-series	0.00	0.00	0.02	0.03
S 16 K-series	0.00	0.00	0.01	0.03
Na 11 K-series	0.00	0.00	0.00	0.00

Total: 100.00 100.00 100.00



10426 Date:3/4/2020 11:29:46 AM Image size:1000 x 750  
Mag:500x HV:5.0kV



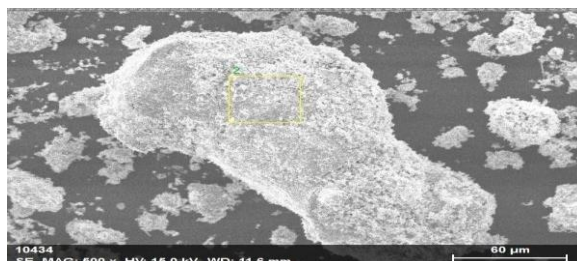
Date:3/4/2020 11:30:12 AM HV:5.0kV Puls th.:9.83kps

El AN Seriesun. C norm.C Atom. C Error (1 Sigma)  
[wt. %] [wt. %] [at. %] [wt. %]

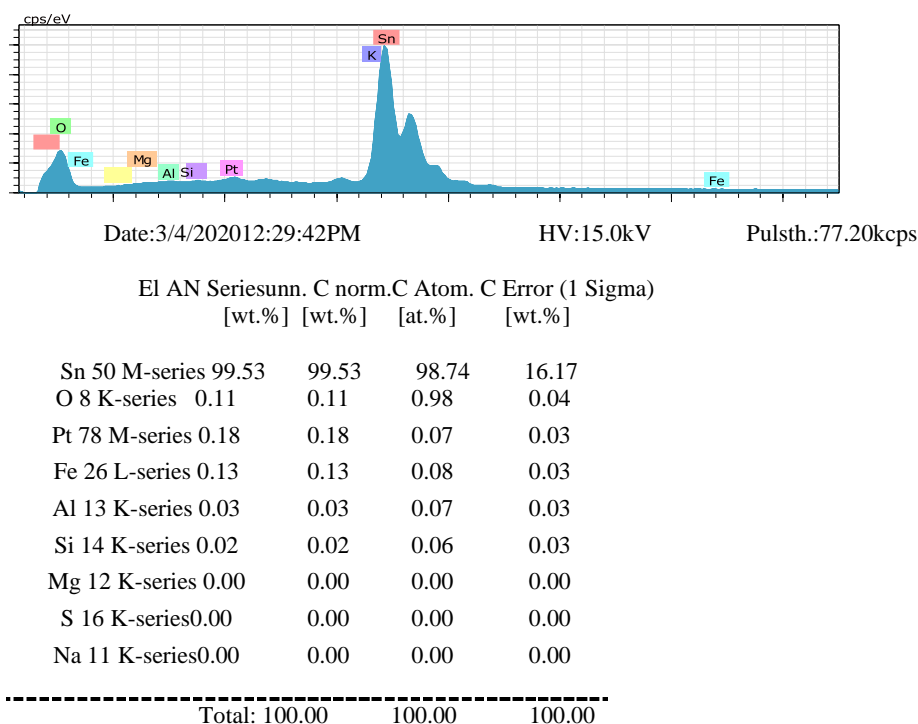
Sn 50 M-series	99.32	99.32	98.74	16.17
O 8 K-series	0.18	0.18	0.98	0.04
Pt 78 M-series	0.17	0.17	0.07	0.03
Fe 26 L-series	0.29	0.29	0.08	0.03
Al 13 K-series	0.02	0.02	0.07	0.03
Si 14 K-series	0.02	0.02	0.06	0.03
Mg 12 K-series	0.00	0.00	0.00	0.00
S 16 K-series	0.00	0.00	0.00	0.00
Na 11 K-series	0.00	0.00	0.00	0.00

Total: 100.00 100.00 100.00

EDS Batch C1



10434 Date:3/4/2020 12:29:06 PM Image size:1000 x 750 Mag:500x HV:15.0kV



#### Antimicrobial Activity<sup>20</sup>

##### Preparation of test Solutions/Stock solution:

The Suspensions of Vanga Bhasma samples A1, B1 & C1 were prepared with the help of following method:

Vanga Bhasma sample: 100mg

Tween 80: 1 g Distilled water: 10 ml

So, the final concentration of the test solution obtained was- 10 mg/ml.

##### Standards used in study

**Positive Control:** Cefpodoxime 10 mcg (Himedia Labs, Mumbai, India) was used as standard or positive control for bacteria while Fluconazole 25 mcg (Himedia Labs, Mumbai, India) was used as standard or positive control for fungi in this study.

**Negative Control:** Distilled water + Tween 80

##### Microorganisms:

- a. *Staphylococcus aureus*<sup>21</sup>
- b. *Bacillus subtilis*<sup>22,23</sup>
- c. *Klebsiella pneumonia*<sup>24</sup>
- d. *E. coli*<sup>25</sup>
- e. *Candida albicans*<sup>26</sup>

##### Determination of Minimum inhibitory concentration Micro dilution assay<sup>27</sup>

The minimum inhibitory concentration was defined as the lowest concentration of the compound to inhibit the growth of microorganisms (Kumar, G.S. et al., 2007)<sup>28</sup>. The minimum inhibitory concentration values were determined by broth dilution assay of micro dilution assay. Varying concentrations of the solutions of Bhasma (10mg/ml, 50mg/ml, 100mg/ml) were prepared. 0.1ml of standardized test organism of Controls was equally set up by using solvents and test organisms without extract. The tube with least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration.

#### Antimicrobial Activity

**Table No. 6: Zone of Inhibition of Vanga Bhasma against organisms**

Sr. No.	Name of Organism	Zone of Inhibition in mm								
		Sample A1			Sample B1			Sample C1		
		10 Mg/ml	50 Mg/ml	100 Mg/ml	10 Mg/ml	50 Mg/ml	100 Mg/ml	10 Mg/ml	50 Mg/ml	100 Mg/ml
1	<i>Staphylococcus aureus</i>	8	10	13	9	10	12	9	11	12



2	<i>Bacillus subtilis</i>	-	8	10	-	7	13	-	9	14
3	<i>Klebsiella pneumonia</i>	10	12	15	09	10	14	09	10	15
4	<i>E. coli</i>	-	7	10	06	07	10	-	7	11
5	<i>Candida albicans</i>	10	12	15	10	14	16	09	12	17

Table No.7 : Zone of Inhibition of control drug against organisms

Sr. No	Name of Organism	Distilled Water + Tween80	+Cefpodoxime	Fluconazole
			10mcg	25mcg
1	<i>Staphylococcus aureus</i>	0	24	-
2	<i>Bacillus subtilis</i>	0	25	-
3	<i>Klebsiella pneumonia</i>	0	30	-
4	<i>E.coli</i>	0	17	-
5	<i>Candida albicans</i>	0	-	28

**Statistical Study****ZoI of VB against *Staphylococcus Aureus* -**

In a ZoI of VB against *Staphylococcus Aureus*, at 95% Confidence Interval (CI), there is significant difference in the means of three different sample strengths. The sample with strength of 100 mg/ml, having 12.33 mm, as a mean zone of inhibition is more effective.

**ZoI of VB against *Bacillus Subtilis* -**

In a ZoI of VB against *Bacillus Subtilis*, the sample with strength of 100 mg/ml, having 12.33 mm, as a mean zone of inhibition is more effective.

**ZoI of VB against *Klebsiella Pneumoniae***

The sample with strength of 100 mg/ml, having 14.66 mm, as a mean zone of inhibition is more effective.

**ZoI of VB against *E. Coli***

The sample with strength of 100 mg/ml, having 10.33 mm, as a mean zone of inhibition is more effective. This is followed by sample strength of 50 mg/ml.

**ZoI of VB against *Candida Albicans*-**

The sample with strength of 100 mg/ml, having 16 mm, as a mean zone of inhibition is more effective.

As compared to the Cefpodoxime (Cephalosporin) and Fluconazole, the VB preparations were found having less antimicrobial activity against all the pathogens. However, it is worth noting that this VB preparation showed antifungal activity also.

**DISCUSSION**

The finding of the antimicrobial studies confirmed the action of JVB as an antimicrobial drug and is useful in inhibiting the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Escherichia coli* and *Candida albicans* with a concentration of 100mg/ml.

As compared to the Cefpodoxime<sup>29</sup> (Cephalosporin) and Fluconazole<sup>30</sup>, the VB preparations were found having less antimicrobial activity against all the pathogens. However, it is worth noting that this preparation showed antifungal activity also.

This outcome further supports the Krumighna and Jantughna properties (Anti-microbial activity) of Jarita Vanga Bhasma.

**CONCLUSION**

The adopted methods for preparation of Vanga Bhasma was able to produce a Bhasma compatible to organoleptic parameters mentioned in the ancient texts. Formation of the small sized particles as small as a nanoparticle was confirmed by SEM study. The colour variation could be due to heat offered during the processes as well as the quality of the raw material. XRD study confirms that Tin oxide is the major compound found in all the Vanga Bhasma samples. Jarita Vanga Bhasma showed antimicrobial activity in inhibiting the growth of *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Escherichia coli* and *Candida albicans* with a concentration of 100mg/ml. This outcome further supports the Krumighna and Jantughna properties (Anti-microbial activity) of Vanga Bhasma.

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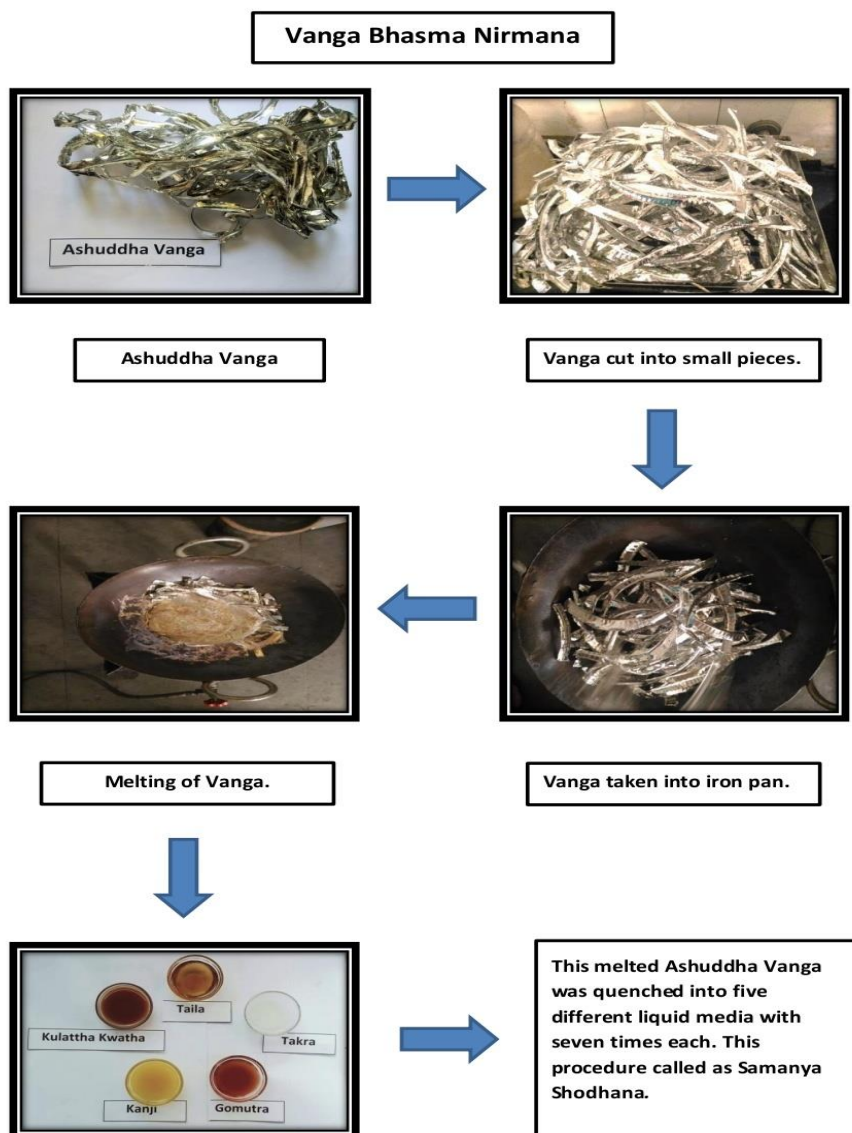
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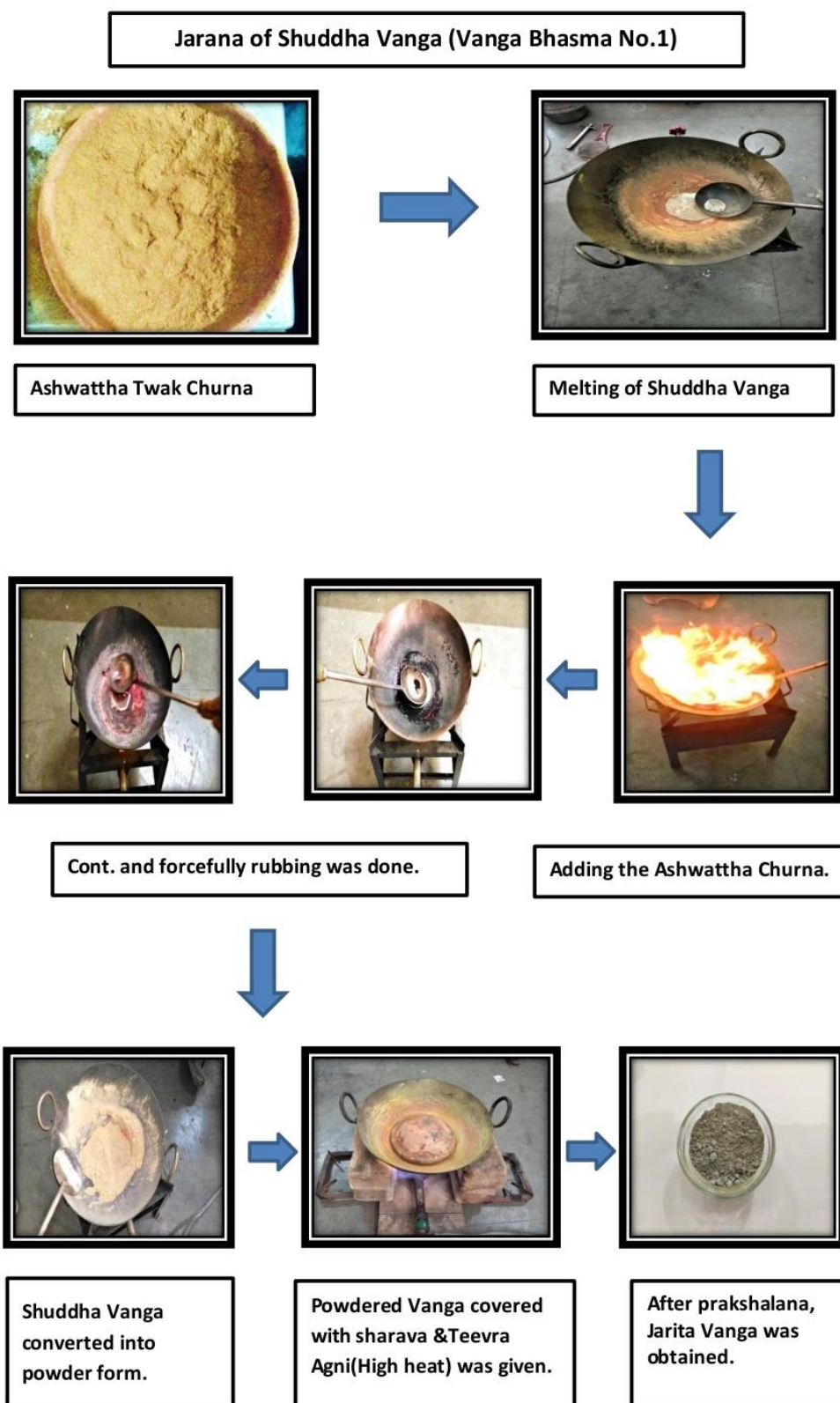
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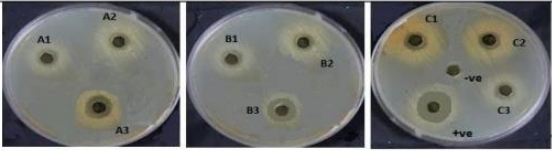

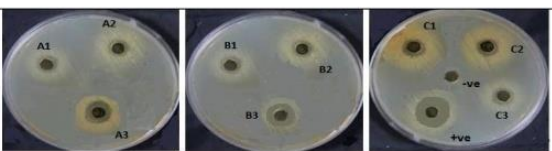
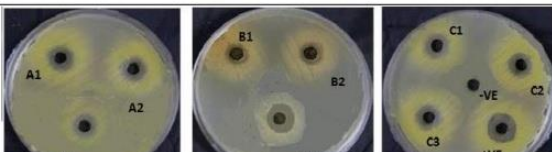
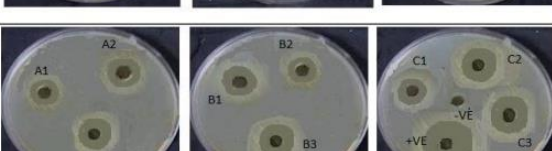
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### Anti- microbial Study

Sr. no.	Micro-organism	Zone of inhibition of Vanga bhasma
1	Staphylococcus aureus	
2	E. coli	
3	Bacillus subtilis	
4	Klebsiella pneumonia	
5	Candida albicans	

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