SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDY OF VANGA BHASMA PREPARED WITH SPECIAL REFERENCE TO RASATARANGINI

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ABSTRACT

Vanga Bhasma which has been prepared with Parada and Haratala or even without Parada and Haratala is widely used for a broad spectrum of diseases. It is also said to possess Jantughna Prabhava (Antimicrobial activity). Hence it was decided to synthesize, analyse and study the antimicrobial activity of Vanga Bhasma prepared with special reference to Rasatarangini 18/25-28. The present study deals with the preparation of Vanga Bhasma with reference to Rasatarangini 18/25-28. The synthesized Bhasma samples were characterized by various analytical techniques. The antimicrobial effects of these Bhasma samples were studied. The samples were characterized with the contemporary parameters like XRD, SEM and EDX to find out the nature of the Vanga Bhasma samples. These samples were further tested against certain Gram +ve, Gram-ve and fungal organisms, so as to find out the anti-microbial efficacy of the Vanga Bhasma samples. The adopted method for preparation of Vanga Bhasma (Ref. Rasatarangini 18/25-28) 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 nano-particle was confirmed by SEM study. Average 14 Putas are required to prepare Vanga Bhasma and XRD study confirms that Tin oxide is the major compound. 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 Krumintha and Jantughna properties (anti-microbial activity) of Vanga Bhasma.

KEYWORDS: Analytical, Anti-microbial, Maran, Shodhan, Vanga bhasma, Rasatarangini.

INTRODUCTION

Bhasma, a unique dosage form, mentioned in the ancient Ayurveda texts is an incinerated metal or mineral prepared after certain rounds of processing like Shodhana, Marana, Amritikarana etc. The Bhasmas are known to offer miraculous results in clinical practice[1]. Quality of a drug depends upon its raw material selection, processing and applications with standard guidelines. It is essential to apply certain standards for manufacture of drugs so that the genuineness of the drug is not compromised. There have been concerns regarding the safety and efficacy of Ayurvedic drugs mainly the Bhasma with special reference to the heavy metal toxicity[2]. Keeping this fact in mind, the Vanga Bhasma, was prepared for the present study and analyzed for quality control checks, on the parameters described in the Ayurvedic texts as well as modern technology like SEM, EDX and XRD. These Bhasmas should exhibit efficacy against the disease inducing microbes. Hence, it was tested against certain Gram +ve, Gram-ve and fungal organisms, so as to find out the anti-microbial efficacy of the Vanga Bhasma samples.

MATERIALS AND METHODS

The different materials used for the preparation of Vanga Bhasma; raw Vanga (Tin)[3], Parad[4], Haratala[5], Tila[6] Taila (Sesame oil), Takra[7] (Butter milk), Gomutra[8] (Cow’s urine), Rasonal[9], powder of Ashwatha[10] Twak (Ficus religiosa), Rajika[11], Saindhava[12], Tandula[13], Vamshapatra[14], Shunthi[15], Hingul[16], Harida[17], Masha[18] and Jeeraka[19] were procured from local retailers. The Kanji[20] (Sour gruel), Kulattha Kwath[21] (Decoction of Dolichos biflorus Linn.), Churnodaka[22] (Lime water) and Arkapatra Swaras[23] (Expressed juice of leaves of Calotropis procera) 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[24] (Samanya and Vishesha), Bhavana[25] and Maran[26].
Shodhan of Vanga

Ashuddha Vanga was subjected to Samanya and Vishesha Shodhan.

The Samanya Shodhan of Vanga was done by quenching the molten Vanga subsequently into Tila Taila, Takra, Gomutra, Kanji and Kulattha Kwath 7 times each. Then this Samanya Shodhit Vanga was subjected to Vishesha Shodhan. Here Samnya Shodhit Vanga was melted and further quenched into lime water for 7 times. Each quenching was done in a fresh media.

### Table 1: Observation regarding Weight of Vanga before and after Samanya Shodhan

| Weight (g)       | Batch 1 | Batch 2 | Batch 3 |
|------------------|---------|---------|---------|
| Initial          | 500     | 500     | 500     |
| Final (dried weight) | 437     | 433.15  | 434.31  |
| % change in weight | 12.6    | 13.37   | 13.13   |

### Table 2: Observation regarding Weight of Vanga before and after Vishesha Shodhan

| Weight (g)       | Batch 1 | Batch 2 | Batch 3 |
|------------------|---------|---------|---------|
| Initial          | 432     | 428.15  | 429.31  |
| Final (dried weight) | 416.72  | 414.16  | 415.72  |
| Weight loss(g)   | 15.28   | 13.99   | 13.59   |
| % change in weight | 3.53    | 3.26    | 3.16    |

Preparation of Vanga Bhasma- (Batch A3, B3, C3)- Shuddha Vanga + Ashwattha Twak Churna + Shuddha Parada + Shuddha Haratala

### Table 3: Batch wise observation regarding Maran ingredients of Vanga

| Batch No. | Shuddha Vanga (g) | Shuddha Parad (1/4th of Vanga) (g) | Total quantity of amalgam Formation (g) | Wt. decrease in % | Shuddha Hartal (g) | Total wt.=Shuddha Vanga + Shuddha Parada + Shuddha Haratala (g) |
|-----------|-------------------|-----------------------------------|---------------------------------------|------------------|-------------------|---------------------------------------------------------------|
| Batch A3  | 138.90            | 34.72                             | 157.39                                | 9.34             | 69.45             | 226.84                                                        |
| Batch B3  | 138.05            | 34.51                             | 156.11                                | 9.53             | 69.02             | 225.13                                                        |
| Batch C3  | 138.57            | 34.64                             | 157.75                                | 8.92             | 69.28             | 227.03                                                        |

### Table 4: Observation Regarding Vanga Bhasma- Batch A3

| Puta   | Shuddha Vanga + Shuddha Parada + Shuddha Haratala | Bhavana Drava-Arkapatra Swaras (ml) | Wt. of Chakrikas Before Puta (Dry Chakrikas-g) | Wt. of Chakrikas After Puta (g) | Cow Dung Cakes (No.) | Cow Dung Cakes (Wt.) Kg | Max. Temp. (°C) | Time reqd. to attain the Max. Temp. (minutes) | Colour of Chakrikas after Puta | Hardness/Softness of Chakrikas | Hardness | % Wt. loss |
|--------|--------------------------------------------------|------------------------------------|-----------------------------------------------|-----------------------------|----------------------|-----------------------|---------------|-----------------------------------------------|---------------------------------|-----------------------------|---------|-----------|
| 1<sup>st</sup> | 226.84                                          | 90                                 | 240.4                                         | 224.28                      | 10                   | 5                    | 773           | 18                                            | Yellowish                        | Hard                        | 2.56    | 1.12      |
| 2<sup>nd</sup> | 224.28                                          | 90                                 | 239.48                                        | 221.8                       | 10                   | 5                    | 758           | 20                                            | Light yellowish                 | Hard                        | 2.48    | 1.10      |
| 3<sup>rd</sup> | 221.8                                           | 90                                 | 237.2                                         | 219.17                      | 10                   | 5                    | 690           | 18                                            | Dark Greyish                     | Hard                        | 2.63    | 1.18      |
| 4<sup>th</sup> | 219.17                                          | 80                                 | 232.65                                        | 217.95                      | 10                   | 5                    | 705           | 15                                            | Dark Greyish                     | Hard                        | 2.79    | 1.27      |
| 5<sup>th</sup> | 217.95                                          | 80                                 | 231.4                                         | 214.33                      | 10                   | 5                    | 680           | 12                                            | Dark Greyish                     | Hard                        | 3.62    | 1.66      |
| 6<sup>th</sup> | 214.33                                          | 80                                 | 229.18                                        | 210.34                      | 9                    | 4.5                  | 664           | 10                                            | Dark Greyish                     | Soft                        | 3.99    | 1.86      |
| 7<sup>th</sup> | 210.34                                          | 80                                 | 226.87                                        | 207.79                      | 9                    | 4.5                  | 622           | 12                                            | Dark Greyish                     | Soft                        | 2.55    | 1.21      |
| 8<sup>th</sup> | 207.79                                          | 80                                 | 224.02                                        | 204.46                      | 8                    | 4.0                  | 596           | 11                                            | Dark Greyish                     | Soft                        | 3.33    | 1.60      |
| 9<sup>th</sup> | 204.46                                          | 80                                 | 219.02                                        | 201.34                      | 8                    | 4.0                  | 542           | 9                                             | Dark Greyish                     | Soft                        | 3.12    | 1.47      |
| 10<sup>th</sup> | 201.34                                         | 70                                 | 216.76                                        | 198.6                       | 6                    | 3.0                  | 498           | 12                                            | Dark Greyish                     | Soft                        | 2.74    | 1.36      |
| 11<sup>th</sup> | 198.6                                           | 70                                 | 214.06                                        | 195.39                      | 6                    | 3.0                  | 475           | 10                                            | Dark Greyish                     | Soft                        | 3.21    | 1.55      |
| 12<sup>th</sup> | 195.39                                         | 60                                 | 209.62                                        | 193.57                      | 4                    | 2.0                  | 320           | 5                                             | Dark Greyish                     | Soft                        | 3.7     | 1.89      |
| 13<sup>th</sup> | 193.57                                         | 60                                 | 208.13                                        | 196.85                      | 4                    | 2.0                  | 312           | 8                                             | Dark Greyish                     | Soft                        | 3.28    | 1.69      |
### Table 5: Observation regarding Vanga Bhasma- Batch B3

| Puta | Shuddha Vanga + Shuddha Parada + Shuddha Haratala (g) | Bhavana Drava-Arkapatra Swaras (ml) | Wt. of Chakrikas Before Puta (Dry Chakrikas-g) | Wt. of Chakrikas After Puta (g) | Cow Dung Cakes (No.) | Cow Dung Cakes (Wt.) Kg | Max. Temp. (°C) | Time reqd. to attain the Max. Temp. (minutes) | Colour of Chakrikas after Puta | Hardness/Softness of Chakrikas | Wt. loss after Puta (g) | % Wt. loss |
|------|------------------------------------------------------|--------------------------------------|-----------------------------------------------|-------------------------------|----------------------|-------------------------|----------------|-----------------------------------------------|-------------------------------|---------------------------|-------------------------|------------|
| 1st  | 225.13                                              | 90                                   | 240.53                                        | 227.48                        | 10                   | 5                      | 693            | 19                             | Yellowish                        | Hard                      | 2.35                    | 1.00       |
| 2nd  | 227.48                                              | 90                                   | 246.04                                        | 230.01                        | 10                   | 5                      | 762            | 20                             | Light yellowish                  | Hard                      | 2.53                    | 1.09       |
| 3rd  | 230.01                                              | 90                                   | 245.76                                        | 227.24                        | 10                   | 5                      | 693            | 16                             | Dark Greyish                     | Hard                      | 2.77                    | 1.20       |
| 4th  | 224.24                                              | 80                                   | 242.5                                         | 224.55                        | 10                   | 5                      | 625            | 14                             | Dark Greyish                     | Hard                      | 2.69                    | 1.19       |
| 5th  | 224.55                                              | 80                                   | 243.44                                        | 221.96                        | 10                   | 5                      | 686            | 12                             | Dark Greyish                     | Hard                      | 2.59                    | 1.15       |
| 6th  | 221.96                                              | 80                                   | 240.67                                        | 218.85                        | 9                    | 4.5                    | 652            | 10                             | Dark Greyish                     | Soft                      | 3.11                    | 1.40       |
| 7th  | 218.85                                              | 80                                   | 235.71                                        | 216.04                        | 9                    | 4.5                    | 635            | 12                             | Dark Greyish                     | Soft                      | 2.81                    | 1.28       |
| 8th  | 216.04                                              | 80                                   | 232.6                                         | 213.46                        | 8                    | 4.0                    | 585            | 10                             | Dark Greyish                     | Soft                      | 2.58                    | 1.19       |
| 9th  | 213.46                                              | 70                                   | 231.32                                        | 210.73                        | 8                    | 4.0                    | 492            | 10                             | Dark Greyish                     | Soft                      | 2.73                    | 1.28       |
| 10th | 210.73                                              | 70                                   | 226.18                                        | 207.4                         | 8                    | 4.0                    | 586            | 8                              | Dark Greyish                     | Soft                      | 3.33                    | 1.58       |
| 11th | 207.4                                               | 70                                   | 224.18                                        | 205.26                        | 6                    | 3.0                    | 492            | 8                              | Dark Greyish                     | Soft                      | 2.14                    | 1.03       |
| 12th | 205.26                                              | 60                                   | 222.74                                        | 201.89                        | 6                    | 3.0                    | 384            | 5                              | Dark Greyish                     | Soft                      | 3.37                    | 1.64       |
| 13th | 201.89                                              | 60                                   | 216.79                                        | 199.73                        | 4                    | 2.0                    | 356            | 4                              | Dark Greyish                     | Soft                      | 2.16                    | 1.07       |
| 14th | 199.73                                              | 60                                   | 214.58                                        | 197.73                        | 4                    | 2.0                    | 342            | 4                              | Dark Greyish                     | Soft                      | 2                       | 1.00       |

### Table 6: Observation regarding Vanga Bhasma- Batch C3

| Puta | Shuddha Vanga + Shuddha Parada + Shuddha Haratala(g) | Bhavana Drava-Arkapatra Swaras (ml) | Wt. of Chakrikas Before Puta (Dry Chakrikas-g) | Wt. of Chakrikas After Puta (g) | Cow Dung Cakes (No.) | Cow Dung Cakes (Wt.) Kg | Max. Temp. (°C) | Time reqd. to attain the Max. Temp. (minutes) | Colour of Chakrikas after Puta | Hardness/Softness of Chakrikas | Wt. loss after Puta (g) | % Wt. loss |
|------|------------------------------------------------------|--------------------------------------|-----------------------------------------------|-------------------------------|----------------------|-------------------------|----------------|-----------------------------------------------|-------------------------------|---------------------------|-------------------------|------------|
| 1st  | 227.03                                              | 90                                   | 241.29                                        | 224.45                        | 10                   | 5                      | 705            | 19                             | Yellowish                        | Hard                      | 2.58                    | 1.09       |
| 2nd  | 224.45                                              | 90                                   | 239.68                                        | 221.79                        | 10                   | 5                      | 735            | 20                             | Light yellowish                  | Hard                      | 2.66                    | 1.13       |
| 3rd  | 221.79                                              | 90                                   | 238.24                                        | 219.28                        | 10                   | 5                      | 685            | 18                             | Dark Greyish                     | Hard                      | 2.51                    | 1.08       |
| 4th  | 219.28                                              | 80                                   | 235.14                                        | 216.84                        | 10                   | 5                      | 624            | 15                             | Dark Greyish                     | Hard                      | 2.44                    | 1.06       |
| 5th  | 216.84                                              | 80                                   | 237.18                                        | 214.45                        | 9                    | 4.5                    | 658            | 12                             | Dark Greyish                     | Hard                      | 2.39                    | 1.05       |
| 6th  | 214.45                                              | 80                                   | 229.65                                        | 211.78                        | 9                    | 4.5                    | 660            | 10                             | Dark Greyish                     | Soft                      | 2.67                    | 1.19       |
| 7th  | 211.78                                              | 80                                   | 226.66                                        | 208.66                        | 9                    | 4.5                    | 632            | 9                              | Dark Greyish                     | Soft                      | 3.12                    | 1.4        |
| 8th  | 208.66                                              | 80                                   | 222.52                                        | 206.19                        | 8                    | 4.0                    | 583            | 10                             | Dark Greyish                     | Soft                      | 2.47                    | 1.13       |
| 9th  | 206.19                                              | 70                                   | 219.81                                        | 203.48                        | 8                    | 4.0                    | 550            | 10                             | Dark Greyish                     | Soft                      | 2.71                    | 1.25       |
| 10th | 203.48                                              | 70                                   | 216.37                                        | 200.41                        | 8                    | 4.0                    | 480            | 8                              | Dark Greyish                     | Soft                      | 3.07                    | 1.44       |
| 11th | 200.41                                              | 70                                   | 215.10                                        | 198.72                        | 6                    | 3.0                    | 456            | 8                              | Dark Greyish                     | Soft                      | 1.69                    | 0.8        |
Table 7: Organoleptic Characters\textsuperscript{[27]}

| Parameter          | Vanga Bhasma No. 3 |
|--------------------|--------------------|
| Shabda             | Anupasthita        |
| Sparsha            | Soft, nocorase Particles |
| Rupa               | Dark grayish       |
| Susnidhatva        | Alpa snigda        |
| Nischandratva      | No metallic luster |
| Rekhapurnatva      | Upasthita          |
| Varitaratva        | Upasthita          |
| Unama              | Upasthita          |
| Rasa               | Tasteless          |
| Gandha             | Not specific       |

Table 8: Analysis Details\textsuperscript{[28]}

| Sr. No. | Name of Sample of Vanga Bhasma | Ash Content | Acid Insoluble Matter | Water Soluble Extractives | Alcohol Soluble Extractives | pH |
|---------|--------------------------------|-------------|-----------------------|----------------------------|------------------------------|----|
| 1       | A3                             | 98.76%      | 94.43%                | 0.33%                      | 0.642%                       | 7.90 |
| 2       | B3                             | 98.18%      | 94.19%                | 0.58%                      | 0.73%                        | 8.75 |
| 3       | C3                             | 99.36%      | 93.79%                | 0.27%                      | 0.72%                        | 8.75 |

Analytical Study

X-Ray Diffraction Study\textsuperscript{[29]}

Batch A3

Batch B3
Batch C3

![XRD pattern of Vanga Bhasma (Batch C3)](image)

Identified Patterns List[^30^]

| Visible | Ref. Code   | Score | Compound Name                  | Displacement [°2Th.] | Scale Factor | Chemical Formula |
|---------|-------------|-------|--------------------------------|-----------------------|--------------|------------------|
| *       | 98-009-0609 | 87    | Cassiterite                    | 0.000                 | 0.960        | O2 Sn0.912       |
| *       | 98-009-7513 | 4     | Tin(IV) Sulfide                | 0.000                 | 0.439        | S3 Sn2           |
| *       | 98-063-9165 | 3     | Mercury Sulfide (1/1)          | 0.000                 | 0.516        | Hg1 S1           |

Plot of Identified Phases

RESULTS

Batch A3
1. Totally 16 peaks were identified in *Vanga Bhasma* (Batch A3) at different angles (2 Theta) from 26.5681° to 90.8571°.
2. 3 strong peaks were chosen as strong with their relative intensity and compared to standard X-ray powder diffraction file.
3. 1st, 3rd, 6th peak with relative intensity of 100%, 78.24%, 49.52%, were considered as significant at 26.5681°, 33.8633°, & 51.7593° having 3.35, 2.80 & 1.76 d space value respectively.

Batch B3
1. Totally 25 peaks were identified in *Vanga Bhasma* (Batch B1) at different angles (2 Theta) from 14.9732° to 95.9566°.
2. 3 strong peaks were chosen as strong with their relative intensity and compared to standard X-ray powder diffraction file.
3. 2nd, 5th, & 11th peak with relative intensity of 100%, 80.17% & 55.93% were considered as significant at 26.5469°, 33.8378° & 51.7206°, having 3.35, 2.64 & 1.76 d space value respectively.

Batch C3
1. Totally 21 peaks were identified in *Vanga Bhasma* (Batch B1) at different angles (2 Theta) from 26.5725° to 95.9920°.
2. 3 strong peaks were chosen as strong with their relative intensity and compared to standard X-ray powder diffraction file.
3. 2nd, 4th, 7th peak with relative intensity of 100%, 76.79%, 55.26% were considered as significant at, 26.5840°, 33.8766°, 51.7761° having 3.35, 2.64, 1.76 d space value respectively.

**Batch A3, B3 & C3**

1. *Vanga Bhasma* (Batch A3, B3 & C3) peaks are compared with standard 3 theta values with ref. No.98-009-0609 confirmed the presence of Tin Oxide (Cassiterite SnO2) with hydroxide in tetragonal structure.

2. Also peaks compared with standard 2 theta values with ref.No.98-009-7513 confirmed presence of Tin Sulphide (SnS3) with orthorhombic structure.

3. Also peaks compared with standard 2 theta values with ref.No.98-063-9165 confirmed presence of Mercury Sulphide (HgS) with hexagonal structure.

**Scanning Electron Microscopy**[31]

**SEM Batch A3, B3, C3**
Sample A3 has a particle size of 1.22 µm - 2.79 µm. Sample B3 has a particle size of 744 nm - 953 nm while sample C3 has a particle size ranging from 9.37 µm - 38.5 µm.

Energy Dispersive X-Ray Analysis [32]
EDS Batch A3
| Element | Series | wt.% | at.% |
|---------|--------|------|------|
| Sn      | 50 L-series | 50.95 | 73.34 | 32.30 | 1.52 |
| O       | 8 K-series  | 13.47 | 19.39 | 63.34 | 1.61 |
| Pt      | 78 L-series | 1.87  | 2.69  | 0.72  | 0.08 |
| Hg      | 80 L-series | 1.64  | 2.25  | 0.61  | 0.07 |
| K       | 19 K-series | 0.80  | 1.15  | 1.54  | 0.05 |
| Fe      | 26 K-series | 0.38  | 0.55  | 0.52  | 0.04 |
| Si      | 14 K-series | 0.35  | 0.51  | 0.94  | 0.04 |
| S       | 16 K-series | 0.01  | 0.12  | 0.03  | 0.03 |
| Al      | 13 K-series | 0.00  | 0.00  | 0.00  | 0.00 |
| Mg      | 12 K-series | 0.00  | 0.00  | 0.00  | 0.00 |
| Na      | 11 K-series | 0.00  | 0.00  | 0.00  | 0.00 |

Total: 69.46 100.00 100.00

EDS Batch B3

![EDS Image]

Available online at: [http://ijapr.in](http://ijapr.in)
El AN Series unn. C norm. C Atom. Error(1Sigma) wt.\%[wt.\%][at.\%] [wt.\%]

| Element | L-series | 50 | 61.10 | 70.60 | 27.72 | 1.85 |
|---------|----------|----|-------|-------|-------|------|
| Sn      | 8 K-series | 17.89 | 20.67 | 60.23 | 2.18 |
| K       | 19 K-series | 2.67 | 3.09 | 3.35 | 0.11 |
| N       | 7 K-series | 1.64 | 0.75 | 6.31 | 0.32 |
| Pt      | 78 M-series | 1.18 | 1.37 | 0.33 | 0.07 |
| Fe      | 26 K-series | 1.06 | 1.22 | 1.02 | 0.06 |
| Hg      | 80 M-series | 0.72 | 1.85 | 0.19 | 0.05 |
| Si      | 14 K-series | 0.27 | 0.31 | 0.52 | 0.04 |
| S       | 16 K-series | 0.16 | 0.14 | 0.33 | 0.03 |
| Al      | 13 K-series | 0.00 | 0.00 | 0.00 | 0.00 |
| Mg      | 12 K-series | 0.00 | 0.00 | 0.00 | 0.00 |

Total: 86.69 100.00100.00

EDS Batch C3

Date:3/4/2020 12:54:14 PM Image size:1000 x 750
Mag:1000x HV:25.0kV
EDS studies confirm the presence of 'Sn' as a major ingredient. 'O' was found as a second major ingredient in all the samples. However presence of 'O' was remarkably higher in VB. Since Parada was an ingredient of VB, its presence was confirmed with all the samples. Fe, Pt, K, S and Si were found in all the Bhasma samples;

**Antimicrobial Activity**[33]

1. **Preparation of test Solutions/Stock solution:**
   The Suspensions of Vanga Bhasma samples A, B & C were prepared with the help of following method:
   Vanga Bhasma sample: 100mg
   Tween 80: 1g Distilled water: 10ml
   So, the final concentration of the test solution obtained was- 10 mg/ml.

2. **Standards used in study**
   Positive Control: Cepfodoxime 10mcg (Himedia Labs, Mumbai, India) was used as standard or positive control for bacteria while Flucanozol 25mcg (Himedia Labs, Mumbai, India) was used as standard or positive control for fungi in this study.
   Negative Control: Distilled water + Tween 80

3. **Microorganisms:**
   a. Staphylococcus aureus[34]
   b. Bacillus subtilis[35,36]
   c. Klebsiella pneumonia[37]
   d. E.coli[38]
   e. Candida albicans[39]

**Determination of Minimum inhibitory concentration Microdilution assay**[40]

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)[41]. 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 setup 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.

### Table 10: Zone of Inhibition of Vanga Bhasma against organisms

| Sr.No | Name of Organism      | Zone of Inhibition in mm | 
|-------|-----------------------|--------------------------|
|       |                       | Sample A3                | Sample B3                | Sample C3                |
|       |                       | 10Mg/ml                  | 50Mg/ml                  | 100Mg/ml                 | 10Mg/ml                  | 50Mg/ml                  | 100Mg/ml                 |
| 1     | Staphylococcus aureus | 11                       | 15                       | 18                       | 10                       | 14                       | 17                       | 10                       | 12                       | 17                       |
| 2     | Bacillus subtilis     | 9                        | 13                       | 15                       | 10                       | 13                       | 18                       | 9                        | 11                       | 14                       |
| 3     | Klebsiella pneumonia  | 11                       | 14                       | 18                       | 12                       | 14                       | 19                       | 10                       | 13                       | 18                       |
| 4     | E.coli                | 10                       | 12                       | 17                       | 12                       | 14                       | 17                       | 10                       | 13                       | 16                       |
| 5     | Candida albicans      | 12                       | 15                       | 18                       | 13                       | 15                       | 19                       | 12                       | 14                       | 19                       |

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Table 11: Zone of Inhibition of control drug against organisms

| Sr. No | Name of Organism       | Distilled Water + Tween80 | Cepfodoxime 10 mcg | Fluconazole 25 mcg |
|--------|------------------------|---------------------------|---------------------|--------------------|
| 1      | Staphylococcus aureus  | 0                         | 24                  | -                  |
| 2      | Bacillus subtilis      | 0                         | 25                  | -                  |
| 3      | Klebsiella pneumonia   | 0                         | 30                  | -                  |
| 4      | E.coli                 | 0                         | 17                  | -                  |
| 5      | Candida albicans       | 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 100mg/ml, having 17.33mm, 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 100mg/ml, having 15.66mm, as a mean zone of inhibition is more effective.

**ZoI of VB against Klebsiella Pneumoniae**

The sample with strength of 100mg/ml, having 18.33mm, as a mean zone of inhibition is more effective.

**ZoI of VB against E. Coli**

The sample with strength of 100mg/ml, having 16.66mm, as a mean zone of inhibition is more effective.

**ZoI of VB against Candida Albicans**

The sample with strength of 100mg/ml, having 18.66mm, as a mean zone of inhibition is more effective.

The findings indicate that Vanga Bhasma prepared with the stated reference possesses Antimicrobial property against Candida Albicans, Klebsiella Pneumoniae, Staphylococcus Aureus, E.Coli and Bacillus Subtilis in their decreasing order with a concentration of 100mg/ml.

As compared to the Cefpodoxime and Fluconazole, 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 Vanga Bhasma (Ref. Rasatarangini 18/25-28).

**CONCLUSION**

The adopted methods for preparation of Vanga Bhasma, (Ref. Rasatarangini 18/25-28) 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 nano-particle 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.

1. XRD study confirms that Tin oxide is the major compound found in all the Vanga Bhasma samples.
2. VB (Ref. Rasatarangini 18/25-28) is an antimicrobial drug and is useful in inhibiting the growth of Candida Albicans, Klebsiella Pneumoniae, Staphylococcus Aureus, E.Coli and Bacillus Subtilis in their decreasing order 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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Cite this article as:
Raman Belge, Rameshwar Pandey, Prakash Itankar. Synthesis, Characterization and Antimicrobial Study of Vanga Bhasma Prepared with Special Reference to Rasatarangini. International Journal of Ayurveda and Pharma Research. 2021;9(4):1-16.

Source of support: Nil, Conflict of interest: None Declared

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Vanga Bhasma Nirmana

Ashuddha Vanga

Vanga cut into small pieces.

Melting of Vanga.

Vanga taken into iron pan.

This melted Ashuddha Vanga was quenched into five different liquid media with seven times each. This procedure called as Samanya Shodhana.

Preparation of Amalgam.

1 Part of Shuddha Vanga

1/4th Part of Shuddha Paraada.

Amalgam formation was done after triturating.
Marana of Vanga (VangaBhasma No.3)

1 Part Amalgam (Shuddha Vanga & Parada).

Shuddha Hartala (1/2\textsuperscript{nd} Part of Shuddha Vanga)

Arkapatra Swarasama

After puta

This process was repeated, until to obtain appropriate Bhasma.

Arkapatra Swarasama was added & trituration was done.

Puta was given.

Sharava samputa was done.

After equal trituration, chakrika formation was done.
### Anti-microbial Study

| Sr. no. | Micro-organism                | Zone of inhibition of Vanga bhasma |
|---------|-------------------------------|-----------------------------------|
| 1       | Staphylococcus aureus         | ![Image of inhibition zones](image1) |
| 2       | E. coli                       | ![Image of inhibition zones](image2) |
| 3       | Bacillus subtilis              | ![Image of inhibition zones](image3) |
| 4       | Klebsiella pneumonia           | ![Image of inhibition zones](image4) |
| 5       | Candida albicans               | ![Image of inhibition zones](image5) |