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Analytical and antimicrobial study of Vanga Bhasma with special reference to Ayurved Prakash

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ABSTRACT

Background: Bhasma, the Ayurvedic organometallic preparation, is an incinerated metal or mineral prepared after several rounds of processing through Puta. Vanga Bhasma is also a novel preparation routinely prescribed for Prameha and genito-urinary disorders. Objectives: The present study deals with the preparation of Vanga Bhasma according to the procedures mentioned in Ayurved Prakash. The synthesized Bhasma samples were characterized by various analytical techniques. The Antimicrobial effects of these samples were studied against certain Gram +ve, Gram -ve and fungal organisms. Materials and Methods: The different steps involved in the synthesis of Vanga Bhasma include Shodhan, Jaran, Bhavana and Maran. Bhasma was incinerated by the traditional method of heating i.e., Puta. The obtained samples were analyzed for the quality control checks, on the parameters described in Ayurvedic texts as well as modern techniques such as SEM, EDX and XRD 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 Vanga Bhasma samples. Results and Conclusions: This study reveals that the synthesized Bhasma was converted into its nontoxic oxide form and had a highly reduced particle size observed from SEM images. Average 13 Puta are required to prepare Vanga Bhasma which is grayish white coloured. 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.

Key words: Analytical, Anti-microbial, Jaran, Maran, Shodhan, Vanga.

INTRODUCTION

Today's modern era has tremendous expectations from Ayurved for fulfilling its health requirements. This is possible through Rasaushadhis which have quick action, lesser dose, tastelessness, prolonged shelf life and better palatability.^[1] Due to these innate

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qualities, Rasaushadhis have conquered won the confidence of the patients and the society at large. Among the Rasaushadhies, Bhasmas are the mostly prescibed medicines. Vanga Bhasma^[2] is one of it, mainly indicated in Genito-urinary tract diseases and Diabetes mellitus. There is a necessary to fix some standards for manufacture of Bhasmas so as to ensure a guality product and for that characterization essential. The like is verv classical texts Rasaratnasamuchhaya, Rasatarangini etc. have mentioned specific indications of Vanga Bhasma regarding its antimicrobial activity (Jantughna Prabhava). This article is meant to evaluate the antimicrobial activity of Vanga Bhasma prepared w.s.r. to Ayurved Prakash (Ref. 3/170).^[3]

MATERIALS AND METHODS

The different materials used for the preparation of Vanga Bhasma; raw Vanga^[4] (Tin), Parad^[5], Haratal^[6],

Tila Taila^[7] (Sesame oil), *Takra*^[8] (Butter milk), Gomutra^[9] (Cow's urine), Rasona^[10], powder of Twak^[11] (Ficus religiosa), Rajika^[12], Ashwattha Saindhava^[13], Tandula^[14], Vamshapatra^[15], Shunthi^[16], Hingu^[17], Haridra^[18], Masha^[19] and Jeeraka^[20] were procured from local retailers. The *Kanji*^[21] (Sour gruel), Kulattha^[22] Kwath (Decoction of Dolichos biflorus Linn.), Churnodaka^[23] (Lime water) and Arkapatra^[24] Swaras (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^[25] (Samanya and Vishesha), Jaran^[26], Bhavana^[27] and Maran.^[28]

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^[29] Vanga was subjected to Vishesha Shodhan.^[30] Here Samanya Shodhit Vanga was melted and further quenched into lime water for 7 times. Each quenching was done in a fresh liquid media.

Table 1: Observation regarding Weight of Vangabefore 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 Vangabefore 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

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Materials & Methods for preparation of Vanga Bhasma

Jarana of Shuddha Vanga

The specified amount of Vishesha Shodhita Vanaa was taken in the Lauha Kadahi. The Shuddha Vanga was heated, till it completely melted. A measured quantity of Ashwattha Tvak Churna ranging from 5g to 7g was sprinkled over the molten Vanga and a forceful rubbing was done with the ladle. The Ashwattha Tvak Churna 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, Jarita Vanga was carefully collected and was subjected to weighing.

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

Table 3: Observation regarding Jarana of Vanga

Table 4: Observation regarding pH of water of JaritaVanga and wt. of Vanga after Kshalana

No. of Batch	1 st Wash	2 nd Wash	3 rd Wash	Wt. of <i>Vanga</i> after <i>Kshalana</i> (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

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C1 0.12 0.12 0.120 0.120	Batch C1	13	9.40	7.20	295.18	6.126
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Table 5: Observation regarding Vanga Bhasma- Batch A2

Puta	Total wt.= <i>Jarita Vanga</i> + Shuddha Haratala (g)	Bhavana Drava-Arkapatra Swaras	Wt. of <i>Chakrikas</i> Before <i>Puta</i> (Dry <i>Chakrikas-</i> g)	Wt. of <i>Chakrikas</i> After P <i>uta</i> (g)	Cow Dung Cakes (No.)	Cow Dung Cakes (Wt.) Kg	Max. Temp. (°C)	Time reqd. to attain the Max. Temp. (minutes)	Colour of <i>Chakrikas</i> after <i>Puta</i>	Hardness/ Softness of Chakrikas	Wt. loss after <i>Puta</i> (g)	% Wt. Loss
1 st	287.96	100	298.26	284.76	10	5	652	18	Yellowish	Soft	13.4	4.66
2 nd	284.76	100	297.96	280.73	10	5	684	20	Light yellowish	Hard	14.7	5.36
3 rd	280.73	100	295.69	276.77	10	5	752	16	Greyish	Hard	10.8	4.17
4 th	276.77	100	292.61	272.91	10	5	724	15	Greyish	Hard	9.23	3.70
5 th	272.91	100	288.54	269.39	10	5	732	14	Greyish	Soft	9.26	3.86
6 th	269.39	90	284.84	265.94	9	4.5	655	12	Greyish	Soft	7.82	3.39
7 th	265.94	90	281.20	262.68	9	4.5	668	10	Greyish	Soft	6.16	2.76
8 th	262.68	90	277.54	259.56	9	4.5	562	12	Greyish	Soft	5.59	2.58
9th	259.56	90	276.45	256.70	8	4.0	588	10	Greyish	Soft	2.34	1.10
10 th	256.70	90	272.26	253.94	8	4.0	594	9	Greyish	Soft	2.48	1.18
11 th	253.94	80	267.94	251.26	8	4.0	493	8	Greyish	Soft	1.77	0.83
12 th	251.26	80	266.22	248.74	6	3.0	486	7	Greyish White	Soft	1.93	0.92
13 th	248.74	70	263.97	246.39	5	2.5	418	12	Greyish White	Soft	1.94	0.93
14 th	246.39	70	261.65	244.37	4	2.0	310	10	Greyish White+	Soft	1.81	0.88

 Table 6: Observation regarding Vanga Bhasma- Batch B2

Puta	Total wt. <i>=Jarita Vanga</i> + Shuddha Haratala (g)	Bhavana Drava- Arkapatra Swaras (ml)	Wt. of <i>Chakrikas</i> Before <i>Puta</i> (Dry <i>Chakrikas-</i> g)	Wt. of <i>Chakrikas</i> After <i>Puta</i> (g)	Cow Dung Cakes (No.)	Cow Dung Cakes (Wt.) Kg	Мах. Тетр. (⁰ С)	Time reqd. to attain the Max. Temp. (minutes)	Colour of <i>Chakrikas</i> after <i>Puta</i>	Hardness/ Softness of Chakrikas	Wt. loss after <i>Puta</i> (g)	% Wt. Loss
1 st	291.77	100	307	287.54	10	5	754	18	Yellowish	Soft	4.23	1.44
2 nd	287.54	100	301.77	283.01	10	5	686	16	Light	Hard	4.53	1.57

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									yellowish			
3rd	283.01	100	296.27	278.15	10	5	693	20	Greyish	Hard	4.86	1.71
4 th	278.15	100	292.68	273.73	10	5	700	18	Greyish	Hard	4.42	1.58
5 th	273.73	100	288.21	269.53	10	5	652	14	Greyish	Hard	4.20	1.53
6 th	269.53	90	283.65	265.38	9	4.5	660	15	Greyish	Soft	4.15	1.53
7 th	265.38	90	278.83	261.30	9	4.5	690	12	Greyish	Soft	4.08	1.53
8 th	261.30	90	276.16	257.35	9	4.5	565	10	Greyish	Soft	3.95	1.51
9 th	257.35	90	272.31	253.52	8	4.0	584	10	Greyish	Soft	3.83	1.48
10 th	253.52	80	267.75	249.77	8	4.0	572	9	Greyish	Soft	3.75	1.47
11 th	249.77	80	264.05	245.97	8	4.0	482	8	Greyish	Soft	3.80	1.52
12 th	245.97	80	261.50	242.18	6	3.0	443	10	Greyish White	Soft	3.79	1.54
13 th	242.18	70	255.44	238.65	5	2.5	418	12	Greyish White	Soft	3.53	1.45
14 th	238.65	70	253.88	235.30	4	2.0	350	12	Greyish white+	Soft	3.35	1.40
15 th	235.30	60	249.53	232.34	4	2.0	308	9	Greyish White+	Soft	2.96	1.25

 Table 7: Observation regarding Vanga Bhasma - Batch C2

Puta	Total wt.= <i>Jarita Vanga</i> + Shuddha Haratala (g)	Bhavana Drava-Arkapatra Swaras (ml)	Wt. of <i>Chakrikas</i> Before <i>Puta</i> (Dry <i>Chakrikas-</i> g)	Wt. of <i>Chakrikas</i> After <i>Puta</i> (g)	Cow Dung Cakes (No.)	Cow Dung Cakes (Wt.) Kg	Max. Temp. (ºC)	Time reqd. to attain the Max. Temp. (minutes)	Colour of <i>Chakrikas</i> after <i>Puta</i>	Hardness/ Softness of <i>Chakrikas</i>	Wt. loss after <i>Puta</i> (g)	% Wt. Loss
1 st	295.18	100	305.98	290.10	10	5	752	20	Yellowish	Hard	5.08	1.72
2 nd	290.10	100	305.33	284.97	10	5	685	20	Light yellowish	Hard	5.1 3	1.7 6
3 rd	284.97	100	300.23	279.94	10	5	652	16	Greyish	Hard	5.03	1.76
4 th	279.94	100	294.80	274.96	10	5	696	15	Greyish	Hard	4.98	1.77
5 th	274.96	100	289.82	270.08	9	4.5	620	12	Greyish	Soft	4.88	1.78
6th	270.08	90	285.94	265.32	9	4.5	598	10	Greyish	Soft	4.76	1.76
7th	265.32	90	281.28	260.70	9	4.5	580	12	Greyish	Soft	4.62	1.74
8th	260.7	80	275.26	256.44	8	4.0	530	10	Greyish	Soft	4.26	1.63
9th	256.44	80	270.67	252.32	8	4.0	448	9	Greyish	Soft	4.12	1.60
10th	252.32	80	267.55	248.34	6	3.0	425	8	Greyish	Soft	3.98	1.57

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									White			
11th	248.34	70	262.90	244.48	6	3.0	470	7	Greyish White	Soft	3.96	1.55
12th	244.48	70	259.71	240.92	4	2.0	328	5	Greyish White+	Soft	3.56	1.45
13th	240.92	60	255.50	237.44	4	2.0	330	4	Greyish White+	Soft	3.48	1.46

Table 8: Organoleptic Characters^[31]

Parameter	Vanga Bhasma No. 2
Shabda	Anupasthita
Sparsha	Soft, no coarse Particles
Rupa	Greyish white
Susnigdhatva	Alpa snigdha
Nischandratva	No metallic luster
Rekhapurnatva	Upasthita
Varitaratva	Upasthita
Unama	Upasthita
Rasa	Tasteless
Gandha	Not specific

Table 9: Analysis of Jarit Bhasma^[32]

S N	Name of Sampl e of Jarit Vanga Bhasm a	Ash Conte nt	Acid Insolubl e Matter	Water Soluble Extractiv es	Alcohol Soluble Extractiv es	рН
1.	A1	95.38 %	93.15%	0.48%	0.86%	8.1 4
2.	B1	93.38 %	90.61%	0.35%	0.80%	7.9 9
3.	C1	96.17 %	92.22%	0.22%	0.84%	7.9 8

Table 10: Analysis of Vanga Bhasma

S N	Name of Sampl e of Vanga Bhasm a	Ash Conte nt	Acid Insolubl e Matter	Water Soluble Extractiv es	Alcohol Soluble Extractiv es	рН
1.	A2	94.87 %	91.15%	0.53%	0.79%	8.8 1
2.	В2	95.17 %	93.30%	0.43%	0.91%	8.2 0
3.	C2	95.57 %	90.84%	0.37%	0.76%	8.8 4

X-Ray Diffraction study of Vanga Bhasma^[33]





Identified Patterns List^[34]

Table 11: Showing identified pattern list of VangaBhasma (Batch A2, B2 & C2)

Visib le	Ref. Cod e	Scor e	Compoun d Name	Displacem ent [°2Th.]	Scal e Fact or	Chemic al Formul a
*	98- 008 - 457 6	84	Tin(IV) Oxide	0.000	0.87 0	O2 Sn1
*	98- 001 - 533 8	1	Ottemann ite	0.000	1.23 8	S3 Sn2

Plot of Identified Phases



XRD pattern of Identified Phases

Results

Batch A2

 a) Totally 17 peaks were identified in *Vanga Bhasma* (Batch A2) at different angles (2 Theta) from 26.5857 to 96.0343.

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- b) 2strong peaks were chosen as strong with their relative intensity and compared to standard X-ray powder diffraction file.
- c) 1st, 2nd, 5th peak with relative intensity of 100%, 84.11%, 50.65%, were considered as significant at 26.58570, 33.88000, & 51.77660 having 3.35, 2.64&1.76 d space value respectively.

Batch B2

- a) Totally 24 peaks were identified in *Vanga Bhasma* (Batch B1) at different angles (2 Theta) from 23.9863 to 98.7995.
- b) 2 strong peaks were chosen as strong with their relative intensity and compared to standard X-ray powder diffraction file.
- c) 3rd, 5th, & 9th peak with relative intensity of 100%, 78.55% & 32.01 were considered as significant at 26.58180, 33.87580 & 51.75270, having 3.35, 2.64 & 1.76 d space value respectively.

Batch C2

- a) Totally 22 peaks were identified in *Vanga Bhasma* (Batch B1) at different angles (2 Theta) from 26.5385 to 95.9087.
- b) 2 strong peaks were chosen as strong with their relative intensity and compared to standard X-ray powder diffraction file.
- c) 2nd, 3rd, 7th peak with relative intensity of 100%, 81.17%, 57.94% were considered as significant at, 26.58400, 33.87660, 51.77610 having 3.35, 2.64, 1.76d space value respectively.

Batch A2, B2 & C2

- a) Vanga Bhasma (Batch A2, B2 & C2) peaks are compared with standard 2 theta values with ref.
 No. 98-008-4576 confirmed the presence of Tin Oxide (SnO2) with hydroxide in tetragonal structure.
- b) Also peaks compared with standard 2 theta values with ref.No.98-001-5338 confirmed presence of Tin Sulphide (Ottemannite-Sn₂S₃) with orthorhombic structure.

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Scanning Electron Microscopy study^[35]

SEM Batch A2, B2, C2



Sample A2 has a particle size ranging from 1.29 μ m-5.24 μ m while sample B2 with a particle size of 625nm-738nm with few particles ranging from 1.05 μ m-2.78 μ m. Similarly particle size of the sample C2 ranges from 1.34 μ m-4.24 μ m.

Here samples A2, B2 and C2 preparations are made from *Shuddha Vanga + Shuddha Haratala*. This batch also attained a nano particle size.

Energy Dispersive X Ray^[36]





EDS Batch B2



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(1 Sigma)	[wt.%][wt.%	6][at.%]	[wt.%]
Sn 50 M-series	99.34	499.34	96.18	24.60
O 8K-series	0.47	0.47	3.38	0.09
Pt 78 M-series	0.09	0.09	0.05	0.03
Si 14 K-series	0.06	0.06	0.25	0.03
Fe 26 L-series	0.02	0.02	0.05	0.03
Mg 12 K-series	0.01	0.01	0.05	0.03
Al 13 K-series	0.01	0.01	0.04	0.03
Na 11 K-series	0.00	0.00	0.00	0.00
K 19 L-series	0.00	0.00	0.00	0.00

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Sn 50 M-series	99.22	99.22	96.18	24.60
O 8 K-series	0.65	0.65	3.38	0.09
Pt 78 M-series	0.07	0.07	0.09	0.03
Si 14 K-series	0.02	0.02	0.26	0.03
Fe 26 L-series	0.04	0.04	0.09	0.03
Mg 12 K-series	0.00	0.00	0.00	0.00
Al 13 K-series	0.00	0.00	0.00	0.00
Na 11 K-series	0.00	0.00	0.00	0.00
K 19 L-series	0.00	0.00	0.00	0.00

Antimicrobial Activity^[37]

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: 1 g Distilled water: 10 ml

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

2) Standards used in study

Positive Control: Cepfodoxime 10 mcg (Himedia Labs, Mumbai, India) was used as standard or positive control for bacteria while Flucanozol 25 mcg (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^[38]
- b) Bacillus subtilis^[39,40]

- c) Klebsiella pneumonia^[41]
- d) E.coli^[42]
- e) Candida albicans^[43]

Determination of Minimum inhibitory concentration Microdilution assay^[44]

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).^[45] 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.

Table 12: Zone of Inhibition of Vanga Bhasmaagainst organisms.

S N	Name of Organism	Zone of Inhibition in mm									
		Sample A2			Sample B2			Sample C2			
		1 0 g / m I	50m g/ml	100 mg /ml	10 mg /ml	50 mg /ml	100 mg /ml	10 mg /ml	50 mg /ml	100 mg /ml	
1	Staphylo coccus aureus	1 0	12	15	11	12	14	10	13	15	
2	Bacillus subtilis	8	10	12	8	09	12	8	10	14	
3	Klebsiell a pneumo nia	1 0	14	16	9	12	15	9	14	17	
4	E.coli	8	10	13	8	11	13	8	12	15	

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5	Candida albicans	1 1	13	16	10	12	15	10	13	15
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Table 13: Zone of Inhibition of control drug againstorganisms

SN	Name of Organism	Distilled Water +	Cepfodoxime	Fluconazole	
		Tween80	10mcg	25mcg	
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

Zol 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 14.66 mm, as a mean zone of inhibition is more effective.

Zol of VB against Bacillus Subtilis

In a ZoI of VB against Bacillus Subtilis, the sample with strength of 100 mg/ml, having 12.66 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 15.00 mm, as a mean zone of inhibition is more effective.

Zol of VB against E. Coli

The sample with strength of 100 mg/ml, having 13.66 mm, as a mean zone of inhibition is more effective. This is followed by sample strength of 50 mg/ml.

Zol of VB against Candida Albicans

The sample with strength of 100 mg/ml, having 15.33 mm, 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.

As compared to the Cefpodoxime^[46] (Cephalosporin) and Fluconazole^[47], 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 VB (Ref. *Ayurved Prakash* 3/170) as 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.

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 preparation showed antifungal activity also. This outcome further supports the *Krumighna* and *Jantughna* properties (Anti-microbial activity) of *Vanga Bhasma* (Ref. Ayurved Prakash 3/170).

CONCLUSION

The adopted methods for preparation of *Vanga Bhasma*, (Ref. *Ayurved Prakash* 3/170) 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. XRD study confirms that Tin oxide is the major compound found in all the *Vanga Bhasma* samples. VB (Ref. *Ayurved Prakash* 3/170) 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

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supports the *Krumighna* and *Jantughna* properties (Anti-microbial activity) of *Vanga Bhasma*.

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