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SCREENING ANTIBACTERIAL ACTIVITY OF SOME BHASMA (METAL-BASED HERBAL MEDICINES) AGAINST ENTERIC PATHOGENS

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Abstract

Bhasma has been used along with appropriate herbs for treatment of critical ailments as medicinal preparation in Ayurveda. Very little or no published information is available on the antibacterial activity of Bhasmas. Considering the use of Ayurvedic metal based herbal preparations to treat various disorders, we studied the antibacterial potential of some Ayurvedic preparations such as Mandura bhasma, Tamra bhasma, Lauha bhasma and Kashis bhasma against enteric bacterial pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Salmonella typhimurium* and *Proteus vulgaris* using a disc diffusion method. The results revealed that Tamra bhasma possesses strong antibacterial activity while Lauha bhasma and Mandura bhasma showed significant antibacterial activity. Study suggest that the use these bhasma may useful to control the enteric bacterial infection.

Keywords: Antibacterial activity, Bhasma, Enteric bacterial pathogens

Introduction

In India, many ayurvedic herbo-mineral preparations (Bhasmas) traditionally are used for the treatment of chronic ailments. Metals have been used in disease treatment since time immemorial. Gold in medicine was mentioned by Roman physician Pliny and Greek philosopher Dioscrides. Hippocrates, the father of modern medicine, explained the beneficial healing and anti-disease properties of silver. In olden days, people used silver bottles for storing water, wine and milk and to prevent spoiling (Nadkarni, 2005). In Siddha medicines, apart from gold and silver, mercury, sulphur, mica, arsenic, zinc and several other minerals, gems, shells, horns are treated with indigenous herbs and are given as bhasma. In ayurveda, several metallic herbal preparations with organic macromolecules termed "Bhasmas", are employed in the treatment of a variety of disorders (Patel, 1986). Bhasma preparations involve the conversion of the metal into its mixed oxides, during which, the zero valent metal state is converted to a higher oxidation state. The significance of this "Bhasmikarana" is that the toxic nature of the resulting metal oxide is completely destroyed while introducing the medicinal properties into it (Wadekar *et al*, 2005). A bhasma means a fine ash obtained through incineration (Subbarayappa, 1997). Silver, gold, zinc, copper and other metals which are well known to have anti-microbial effect in modern medicines are used as wonderful life saving drugs against infectious diseases for thousands of years without any adverse effects. Role of these herbo-mineral preparations for curing skin diseases such as psoriasis, eczema, alopecia,

diabetic ulcer, warts, vitiligo and leprosy are well studied (Joseph, 2008). Most of the medicines are mixture of compounds and because of its synergistic action; toxicity is being diminished, thereby increasing bioavailability through the cells of the body. Various commercially available medicines such as Lauha bhasma, Mandura bhasma, Tamra bhasma etc these medicines are particularly used for treating infectious diseases. So far, little is known about the antibacterial properties of these medicines and the scientific basis of its application in treating infectious bacterial diseases. Hence the attempt was made to determine the antibacterial properties of some available (Bhasma) metal based herbal preparations.

Materials and Methods

Metal-Based Herbal Medicines (Bhasma) such as Mandura bhasma, Tamra bhasma, Lauha bhasma and Kashis bhasma were procured from local market. These commercially available formulations are used for treating various diseases in traditional clinical practice in India and are usually prepared from purified metal, triturated with decoction of herbal juices. They are generally prescribed in the dose of 100-200 mg day⁻¹ and recommended to be taken with a suitable adjuvant

Bacterial cultures: The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The bacterial cultures were rejuvenated in Mueller-Hinton broth (Hi-media laboratories, Mumbai, India) at 37°C for 18h and then stocked at 4°C in Mueller-Hinton Agar. The inoculum size of the bacterial culture was

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standardized according to the National committee for Clinical Laboratory Standards (NCCLS, 2002) guideline. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37°C for 3h until

the culture attained a turbidity of 0.5 McFarland units. The final inoculum size was standardized to 10⁵ CFU/mL with the help of SPC and Nephelo-turbidometer.

Table: 1. Antibacterial Potential of bhasma against various enteric bacterial pathogens at 5mg/disc in degree of decreasing order (Zone of inhibition of growth in mm)

Herbal Preparation	Solvent extract	<i>S. typhimurium</i> (MTCC 98)	<i>P. vulgaris</i> (MTCC426)	<i>E. aerogenes</i> (MTCC 111)	<i>S. typhi</i> (MTCC 733)	<i>E. coli</i> (MTCC1739)	<i>S. paratyphi-B</i> (MTCC 733)	<i>K. pneumoniae</i> (MTCC 109)	<i>S. aureus</i> (MTCC96)	<i>S. epidermidis</i> (MTCC435)	<i>P. aeruginosa</i> (MTCC424)	Antibacterial Sensitivity Index (ASI)	ASI of Product
Tamra bhasma	Aqueous	13	12	12	14	13	12	12	14	15	15	3.5	16.3
	Ethanol	14	15	15	17	15	20	14	17	17	20	11.4	
	Acetone	16	19	17	20	17	22	15	22	23	24	14.8	
Lauha bhasma	Aqueous	14	13	-	20	-	16	13	13	17	15	13.2	15.1
	Ethanol	16	15	12	20	-	18	16	16	18	17	16.4	
	Acetone	20	17	14	24	12	20	18	20	20	19	19.5	
Kashis bhasma	Aqueous	-	13	12	16	-	13	12	13	13	14	12.1	13.1
	Ethanol	-	15	14	15	12	15	14	16	15	17	14.8	
	Acetone	12	16	16	17	13	18	18	17	17	20	18.4	
Mandura bhasma	Aqueous	-	12	-	-	-	-	11	12	-	-	10.6	9.9
	Ethanol	11	15	12	13	12	-	12	14	13	12	13.3	
	Acetone	13	17	12	15	15	12	14	19	16	15	16.4	
Negative control	Aqueous	-	-	-	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-	-	-	-
	Acetone	-	-	-	-	-	-	-	-	-	-	-	-
Positive control	Ampicillin 10 mcg/disc	19	16	14	18	11	18	30	24	25	16	-	-

Preparation of disc for antibacterial activities:

The aqueous, methanol and acetone solution of bhasma (powder preparation) were prepared in their respective solvents and the sterile blotting paper disc (10 mm) were soaked in the diluted solution in such concentration that the amount of solution absorbed by each disc was 5 mg of each extract. The prepared disc were dried in controlled temperature to remove excess of solvent and used in study.

Antibacterial Activity using disc diffusion method:

The modified paper disc diffusion method (NCCLS, 2002) was employed to determine the antibacterial activity of both aqueous and organic solution of ayurvedic preparations. Turbidity of inoculums was matched with McFarland turbidity

standard. Inoculums were spread over the Nutrient agar plate using a sterile cotton swab in order to get a uniform microbial growth. Then the prepared antibacterial disc were placed over the lawn and pressed slightly along with positive and negative controls. Ampicillin 10mcg/disc (Hi-Media, Mumbai) were used as positive control while disc soaked in sterile distilled water and organic solvents and dried were placed on lawns as negative control. The plates were incubated for 18h at 37°C. The antibacterial activity was evaluated for 5mg/disc and diameter of inhibition zones were measured. Experiment was carried out in triplicate and the averages diameter of zone of inhibition was recorded. Antimicrobial Sensitivity Indices (ASI) was calculated by following formula:

Antimicrobial Sensitivity Index for Herbal preparation

$$\frac{\text{Total zone of growth inhibition}}{\text{No. of Antimicrobial agents tested} \times \text{No. of bacterial Pathogens}}$$

Antimicrobial Sensitivity Index for a Bacteria

$$\frac{\text{Total score of antimicrobial sensitivity of bacteria}}{\text{No. of antimicrobial agent}}$$

Results and Discussion

Metallic herbal preparations offer advantages over plant drugs by virtue of their stability over a period, lower dosage, easy storability, sustained availability

and contain minerals and metals as integral part of the formulations (Kumar *et al*, 2006). They are being used with an intention to give therapeutic efficacy to the designated illness. The metals and minerals are mixed

with herbs because they are considered non-living and by treating them with herbs they are converted to a living state thereby becoming bio-compatible. The same metal processed with different herbs acts on different organs in the human body.

According to the antibacterial activity of bhasma (Table 1) it was observed that Mandura bhasma was found strong antibacterial against *S. aureus* and *P. vulgaris* moderate antibacterial against *K. pneumoniae*, *S. epidermidis*, *S. typhi*, *E. coli*, *P. aeruginosa*, *S. typhimurium*, *E. aerogenes*, and where as it was mild antibacterial against *S. paratyphi-B* (fig.1). Mandura bhasma, an ayurvedic preparation of iron (prepared using complex ayurvedic processes like Shodhana, Marana etc.) has long been used in the treatment of liver diseases (Sharma, 1977). Tamra Bhasma is derived from metallic copper that is recommended for different ailments of liver and spleen, abdominal pains, colitis, heart problems, anemia, tumors, loss of appetite, eye troubles and tuberculosis. Copper is an integral part of several enzymes and influences the immune system. Pharmacological investigations have reported the use of Tamra bhasma for treating gastric ulcers and secretion, the management of lipid peroxidation in the liver of albino rats and as an antioxidant (Tripathi and Singh, 1996; Pattanaik, 2003). The present study shows that the Tamra bhasma strong antibacterial against *P. aeruginosa*, *S. epidermidis*, *S. aureus*, *S. typhi*, *S. paratyphi-B*, *P. vulgaris*, *E. coli*, *E. aerogenes* and *S. typhimurium*, while moderate antibacterial against *K. pneumoniae* (fig.2). Lauha bhasma is an iron-based ayurvedic preparation. Iron nourishes blood, enhances vigor and its astringency prevents blood from becoming too hot or too fluid. It is an essential element of hemoglobin, playing an important role in oxygen transport (O'dell and Sunde, 1997). Lauha bhasma is used in anemia similar to iron tablets/capsules/syrup prescribed as iron supplementation in allopath. These Bhasmas are prepared from purified iron filings/ferric oxide or magnetic iron incinerated with decoction of Triphala, Ghritkumari ras, vinegar and sesame oil (Kumar *et al*, 2006). Lauha bhasma was found strong antibacterial against *S. typhi*, *S. epidermidis*, *S. paratyphi-B*, *P. aeruginosa*, *S. typhimurium*, *S. aureus*, *K. Pneumoniae*, *P. vulgaris*, and while moderate antibacterial against *E. aerogenes* and mild antibacterial against *E. coli* (fig.3). Kashis bhasma is useful in anemia adnominal pain and general weakness. It was found strong antibacterial against *P. aeruginosa*, *S. typhi*, *S. paratyphi-B*, *S. aureus*, *S. epidermidis*, *P. vulgaris*, *K. pneumoniae*, *E. aerogenes* and moderate antibacterial against *S. typhimurium* and *E. coli* (fig.4). In the present investigation among the ayurvedic preparation Tamra bhasma and Lauha bhasma were potent antibacterial followed by Kashis bhasma and Mandura bhasma against enteric bacterial pathogens.

Fig.1: Antibacterial activities of Mandura bhasma against various bacterial pathogens

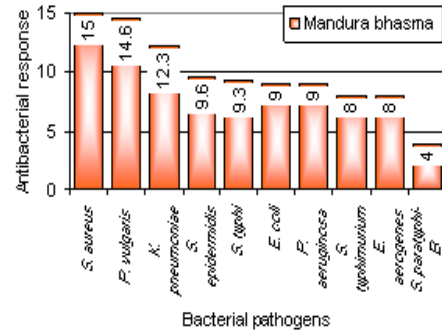


Fig.2: Antibacterial activities of Tamra bhasma against various bacterial pathogens

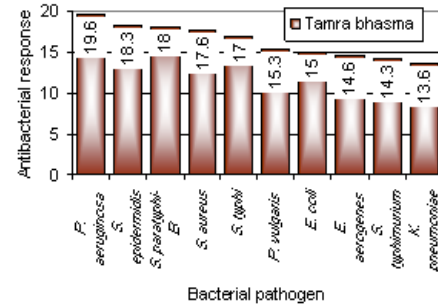


Fig.3: Antibacterial activities of Lauha bhasma against various bacterial pathogens

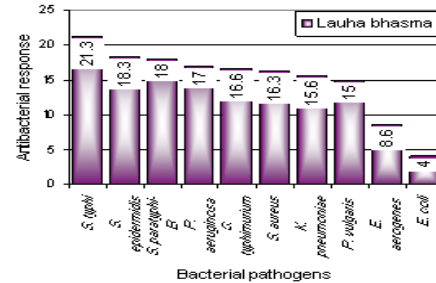
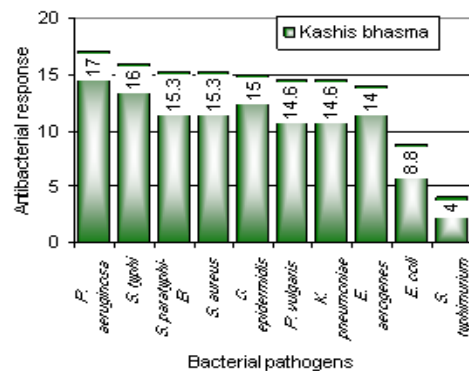


Fig.4: Antibacterial activities of Kashis bhasma against various bacterial pathogens



Conclusion

From above study it was observed that the Bhasma such as Mandura bhasma, Tamra bhasma, Lauha bhasma and Kashis bhasma was found significant antibacterial activity. It was concluded that these ayurvedic preparation can used to control or prevent the enteric bacterial infection. Modern techniques are necessary to standardize and bring out high quality herbal products owing to their complex nature.

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