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Antibacterial Study on Lauha Bhasma with Special Reference to Gokshura Kwatha Bhavana and Puta

Dr. Urmila Waxar

Associate Professor, Department of Rasa Shastra and Bhaishajya Kalpana, Pt. S. S. Govt Auto. Ayurveda College, Burhanpur (M. P.), India
Email: drurmilawaxar[at]gmail.com

Abstract: ***Rasa-aushadhies are dynamic medicines in our science. Herbomineral preparations are time tested drugs used for various acute and chronic diseases from time immemorial. They are needed to be study on modern parameters. Metals and minerals are known to have antimicrobial properties in modern science. Many herbs also shown antibiotic properties. Bhasmas are also needed to be studied to explore antibiotic properties. In present study three different of Lauha bhasma samples were subjected for antibacterial activity against 04 pathogenic bacteria to assess the individual as well as comparative antibacterial effect with special reference to effect of gokshura (Tribulus terrestris) kwatha bhavna and puta on lauha bhasma. Moderate sensitivity was observed in some samples on specific concentrations suggestive of bacteriostatic nature at some level.***

Keywords: Lauha, Bhasma, Antibacterial, Gokshura (Tribulus terrestris)

1. Introduction

Bhasmas are very potent broad spectrum therapeutic agents in Ayurveda. The original component of particular metal as well as the pharmaceutical procedure applied including the herbs for bhasma nirmana responsible for their dynamic therapeutic potential. Lauha bhasma finds significant place in treating various disorders. It is indicated for mutrakrichhra treatment in chakradutta. The disease is compared with UTI (urinary tractinfection) where antibiotic treatment plays significant role. In today's scenario there is a need to explain pharmacological nature of drugs on modern parameter also. So keeping all these points in consideration study was planned to evaluate antibacterial effect. Under present study three different samples of Lauha bhasma were investigated for their antibacterial activities against some selected pathogenic bacteria.

1. LB – A-Lauha bhasma plane (Amritikrita)
2. LB – B-Lauha bhasma processed (Triturated) with Gokshura kwatha (21 times Bhavna)
3. LB – C-Lauha bhasma incinerated with Gokshura kwatha Bhavna (21 times Puta after Gokshura kwatha Bhavna) then Amritikaran.

The screening was done to find out antibacterial properties of different concentration of aqueous extract of drug sample.

2. Material & Method

The material & method adopted in the study are as follows.

Bacterial strains

Two strains of gram-negative bacterial i. e. Escherichia coli and Pseudomonas aeruginosa and two strains of Gram-positive bacterial Staphylococcus aureus and Bacillus subtilis were used as the test organisms; the cultures of bacterial were maintained in their appropriate agar slant at 4^0C and used as stock cultures in the study. Bacterial strains as test organisms were obtained from the center of excellence in Biotechnology of Advanced Research and Instrument action Facility of Madhya Pradesh Council of Science and Technology, Bhopal.

Preparation of water soluble extract

Water soluble extract of drug samples were used in the study. To prepare the water soluble extract of drugs, protocol mentioned in API was followed.

Microbiological techniques adopted

Kirby-Baurer method (Disc Diffusion method) was followed to test the antibacterial activity. The paper disc having the same diameter impregnated with different concentration of extract is placed on agar plates. The charged disc diffuses the drug in agar. If an organism placed in agar is sensitive to the drug it will not grow around the disc and makes a clear area, this area of no growth around the disc is known as "Zone of inhibition".

Inoculation

Nutrient broth was used to activate, grow and to dilute the bacterial suspensions. All the culture media were prepared and treated according to the guidelines (Hi media Laboratories ltd. Mumbai. India). To start bacterial culture a number of cells (the inoculums) are to be transferred (inoculated) in to sterilized broth media. The bacterial strains were grown to the exponential phase in nutrient broth at 37^0C for 18 hrs and adjusted to a final density of 10^8 cfu/ml by diluting fresh cultures and comparing them with Mc Farland density.

Swabbing

Bacteria grow very well in fluid media i. e. nutrient broth. Hence they are used as enriched media before plating on solid media. Solid media is essential for isolation of organism in pure form, so for isolation of microorganisms in pure form without contamination, swabbing was done on solid plates. The petri dishes containing nutrient agar

media were labelled according to the organism which was to be plated.

A sterile swab is dipped in suitable dilution of a broth culture of the test organisms and spreaded over the surface of a solid medium in plates, by streaking the swab over the entire sterile agar surface. Care should be taken that entire media should be plated.

Preparation of different concentrations of the extract

50mg of accurately weighed extract (previously prepared aqueous extract) of each sample was dissolved in 25ml of distilled water. The solution was stirred for one hour using a magnetic stirrer. It was then filtered to get rid of the particles, this stock solution was further diluted to prepare different concentrations (i. e.25%, 50%, 75%) stock solution was 100% concentration.

Preparation of Discs

Discs of same diameter (0.5 mm) were prepared by using standard whatman filter paper no.1. These discs were placed in a Mac-cartney's bottle and autoclaved at 15 lbs pressure for 20 mnts.

Evaluation of Antibacterial study

This was carried out on solid media by disc diffusion method. The sterile discs were charged with 10ul of required concentration of drug with the help of micropipette. The disc was dried at room temperature to remove excess moisture. The charged disc was then applied on solid media in previously marked zones of different concentration on the plates. A force was used in this process. It was sterilized by red hot each time. The disc placed should not be close than 24mm. The petridishes were then incubated at 37^0C within 15 minutes after disc were applied.

In the present study all the required apparatus and material were sterilized in autoclave and placed in a laminar air flow cabinet under pathogen free condition.

Measurement of Inhibition zone

After overnight incubation the degree of sensitivity is determined by measuring the diameters of the zones of complete inhibition, including the diameter of the disc. The zone of inhibition of bacterial growth around the disc was measured in mm. with the help of scale. The readings were taken at a different planes and mean was calculated for final reading. This result was based upon the scale of inhibition developed by Arora D. S. et. al. (1997). Interpretation was made as given under.

Table 1: Showing relation between zone of inhibition and drug sensitivity

SNo.	Zone of inhibition (in m. m.)	Drug sensitivity
1	Below 6	Insensitive
2	6<9	Less sensitive
3	9<12	Moderate sensitive
4	>12	Highly sensitive

The antibacterial study was done by quality control laboratory, Bhopal (M. P).

Observation and Results

Zone of inhibition observed in different LB-samples (Four concentration on the test organisms selected is presented below.

Table 2: Showing sensitivity of Escherichia coli

Drug Conc.	Zone of Inhibition (mm)		
	L. B.-A	LB-B	LB-C
25% (0.5mg/ml)	6	8	6
50% (01.00mg/ml)	8	9	6
75% (1.5mg/ml)	9	9	8
100% (2 mg/ml)	12	12	9

Table 3: Showing sensitivity of Staphylococcus aureus

Drug Con.	Zone of Inhibition (mm)		
	L. B.-A	LB-B	LB-C
25% (0.5mg/ml)	6	7	7
50% (01.00mg/ml)	6	8	8
75% (1.5mg/ml)	8	8	8
100% (2 mg/ml)	10	9	11

Table 4: Showing sensitivity of Bacillus subtilis

Drug Conc.	Zone of Inhibition (mm)		
	L. B.-A	LB-B	LB-C
25% (0.5mg/ml)	7	6	8
50% (01.00mg/ml)	8	10	8
75% (1.5mg/ml)	5	10	8
100% (2 mg/ml)	9	10	8

Table 5: Showing sensitivity of Pseudomonas aeruginosa

Drug Conc.	Zone of Inhibition (mm)		
	L. B.-A	LB-B	LB-C
25% (0.5mg/ml)	8	8	7
50% (01.00mg/ml)	8	9	9
75% (1.5mg/ml)	9	9	9
100% (2 mg/ml)	11	10	10

Analysis of above reading shows that

1. E-coli was moderately sensitive (9mm-12mm inhibition) to 75% & 100% concentration of LB-A & LB-B & 50% concentration of LB-B (9mm only).
2. Staphylococcus aureus was moderately sensitive in 100% concentration of all three samples of drug. While less sensitive in other three concentrations.
3. Bacillus Subtilis was moderately sensitive (9mm-12mm inhibition) in 50%, 75% & 100% concentration of LB-B & 100% concentration of LB-A.
4. Pseudomonas aeruginosa was moderately sensitive (9mm to 11mm) in 75%, 100% concentration of all the three samples of drug, while 50% concentration of LB-B & LB-C.

3. Discussion on Antibacterial Study

In the research work "Lauha bhasma was selected to evaluate its efficacy on mutrakrichhra roga. The study was planned in special reference to study the role of bhavna dravya and maraka dravya on the therapeutic properties of Lauha bhasma (mutrakrichhrahara prabhava) hence

gokshura (Tribulus terrestris) the well-established classical drug is selected for the purpose of bhavna and puta. As the conceptual study reflects the interaction of gokshura kwatha with Lauha bhasma will impregnate mutrakrichhra hara properties as well as potentiates the action of Lauha bhasma on the disease.

Mutrakrichhra roga which is compared with UTI in modern science. So antibacterial evaluation was planned.

The results of antibacterial study shows moderate zone of inhibition in all the drugs samples at 100% concentration except for E. coli and P. aeruginosa, where moderate sensitivity is seen even in lesser concentration of drug (Table No.2 to 5) other concentration were found sensitive but in lesser zone. Modern studies support this fact.

Results are encouraging for antibacterial effect but it was clinically more significant, which can be better explained through ayurvedic concept. The drugs samples have some variation in their constitutions, according to that their pharmacological properties were calculated for better explanation of the entire drug sample.

- LB-B bears tridoshahara properties along with mutrakrichhra hara vastisodhana and ashmari nashaka prabhava.
- LB-C bears predominantly pitta kapha hara property with vatahara property in less extent also bear vastisodhana, ashmarinashaka and mutrakrichhra hara prabhava in less percentage.

All the samples have rasayana effect with more % in LB-B which will work as immunity stimulant.

Hence the Louha bhasma will subside the symptom with its pitta shamaka property and supports the immune factor with rasayana effect and gokshura will support this action more broadly with its tirdoshahara, mutracrichhra-hara, ashmarinashak, vastisodhana properties. Modern pharmacological studies support antibacterial potential as bacteriostatic nature in the entire sample but more pronounced in LB-B.

Abbreviations

☑%-Percentage
☑A. P. I-Ayurvedic Pharmacopoeia of India
☑B. P. N.-Bhava Prakash Nighantu
☑L. B.-Lauha bhasma
☑M. N.-Madanpal Nighantu

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