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Research Article

ANTI-MICROBIAL EFFICACY OF VANGA BHASMA ON MUTRAKRICHHA W.S.R TO LOWER URINARY TRACT INFECTION

Tanuj Bhatia¹, Chinky Goyal^{2*}, Amandeep Kaur³, Amrit Malik⁴, Sudhir Malik⁵ and Jadhav Viraj Vilas⁶

^{1,2}Dept. of Rasa Shastra & Bhaishjya Kalpana, Saraswati Ayurved Hospital and Medical College, Gharuan, Mohali
 ³Department of Samhita & Siddhanta, Saraswati Ayurved Hospital and Medical College, Gharuan, Mohali,
 ⁴Dept. of Agad Tantra, Shri Dhanwantry Ayurvedic College & Hospital, Chandigarh
 ⁵Dept. of Bala Roga, Shri Krishna Govt. Ayurvedic College, Kurukshetra, Haryana
 ⁶Saraswati Ayurved Hospital and Medical College, Gharuan, Mohali

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ABSTRACT

Vanga (Shukra Loha) is a unique metal mentioned in Ayurvedic lexicon with indication in Mutrakriccha after converting it to Vanga Bhasma. Mutrakriccha is a condition of urogenital system with dysuria as a prime symptom which includes urinary tract infection. As manifestation of *Mutrakriccha* and lower urinary tract infection are similar, an attempt has been made in the present study to evaluate the anti-microbial efficacy of vanga bhasma on three pathogens viz. Streptococcus aureus, Candida albicans and E.coli. by in vitro method. Two samples of Vanga Bhasma have been prepared as per the reference of Rasa Tarangini for the present study. Disc diffusion method was adopted for the present study and three readings were noted at 24hrs, 48hrs and 72 hrs after initial inoculation as well as secondary inoculation. Results indicated that both the samples have shown anti-microbial activity against E.coli with inoculums of 1.4 x 10^2 cells/ml but no anti-microbial activity was shown against the other two pathogens viz. Streptococcus aureus and Candida albicans. So, it can be concluded that both the samples of Vanga Bhasma Sample 1 and Sample 2 are having antibacterial activity against Esherichia coli. as no growth was found in all the cultures after 60 hours of inoculation and Vanga Bhasma can be successfully used to treat urinary tract infection caused due to E.coli. infection.

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INTRODUCTION

The term *Mutrakrichha* comprises of two words *Mutra* and *kriccha*, the disease in which urine is passed with difficulty is called *Mutrakriccha*. (1) Considering this, it can be stated that *mutrakriccha* is a condition of urogenital system with dysuria as a prime symptom which include UTI. Urinary tract infection is defined as multiplication of organisms in the urinary tract. When the infection is restricted to lower urinary tract i.e. urethra, bladder, and prostate then it is called as Lower urinary tract infection (LUTI). (2) Urinary tract infections are second in frequency after upper respiratory tract infection. (3) Incidence and degree of morbidity and mortality form infections are greater with those in the urinary tract than with those of the upper respiratory tract. Bacteria are by far the most common invading organisms but fungi, yeasts and viruses also produce urinary tract infection.

Vanga bhasma is a unique preparation mentioned in Rasaśāstra. Vanga is having alchemical as well as therapeutic importance. It has a synonym_Śhukraloha', which indicates

its efficacy in correcting the disorders related to lower urinary tract. The other ingredients of this formulation are *Nirgundi*, *Haridra* and *Hingula*.

Mutrakrichha is a disorder which comes under disarrays of Mutravaha Srotas, description of this disease is mentioned in almost all classical texts which reflect its prevalence in ancient period. It is a disease involving basti marma. As basti is one among the tri-marma (main three vital organs), it has great therapeutic importance. Acharya mentioned and elaborately explained the mutrakriccha and its type in comprehensive manner.

Urinary tract infection refers to both microbial colonization of the urine and tissue invasion of any structure of the urinary tract. Bacteria are most commonly responsible, although yeast, fungi and viruses may produce urinary infection. When it affects the lower urinary tract it is known as bladder infection (cystitis) and when it affects the urinary tract it is known as kidney infection (pyelonephritis). Symptoms from a lower urinary tract include pain with urination, frequent urination, and feeling the need to urinate despite having an empty

*Corresponding author: Chinky Goyal

bladder (4). Infections of the urethra and bladder are often considered superficial or (mucosal) infections, pyelonephritis and renal suppuration signify tissue invasion. The 3 basic form of UTI are pyelonephritis, cystitis and asymptomatic bacteriuria. Focal pyelonephritis and renal abscess are less common. (5) From a microbiological perspective, urinary tract infection exists when pathogenic microorganisms are detected in the urine, urethra, and kidney. Symptoms include Burning sensation, urgency and frequency of micturition accompanied by significant bacteriuria have been termed as acute urethral syndrome.

Bacterial infections are the most common cause of UTI, with E.coli being the most frequent pathogen, causing 75-90% of acute infections in patients without catheters (6). Other gram negative bacilli, especially Proteus and Klebsiella and occasionally Enterobacter, account for a smaller proportion of uncomplicated infections. Gram positive cocci play a lesser role in urinary tract infections, nonetheless Staphylococcus saprophyticus, Enterococci, Staphylococcus aureus are associated with acute urinary tract infection in young females and in-patient with renal stone or previous instrumentation. (7)

MATERIAL AND METHODS

As manifestation of *mutrakriccha* and lower urinary tract infection are similar, an attempt has been made to understand the concept of Lower urinary tract infection and effect of *Vanga bhasma* by in vitro study. Two samples of *Vanga Bhasma* viz. Sample I and Sample II has been prepared as per the reference of *Rasa Tarangini* (8) and antimicrobial efficacy of both the samples were studied against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*.

Study Site: The study was conducted at Interstellar Testing Centre Lab, Panchkula, Haryana.

Microorganisms: Escherichia coli, Staphylococcus aureus and Candida albicans

Equipments: Test tubes, oven, water bath apparatus, dropper, microscope, nutrient agar, sterilesaline etc.

Microbiological analysis of Vanga Bhasma (Sample I and Sample II)- For the antimicrobial study of Vanga Bhasma, standard cultures of these micro-organisms were taken. As per following steps the study was conducted.

Antimicrobial Activity Response Against

Staphylococcus aureus ATCC 6538

- 1. Escherichia coli ATCC 8739
- 2. Candida albicans 20°C/48 hrs ATCC 10231

Inoculum preparation

- Sub- culturing of these organisms with nutrient agar was done and then thesewere incubated at 30° to 37° for 24hrs.
- Staphylococcus aureus ATCC 6538
- Escherichia coli ATCC 8739
- Candida albicans 20°C/48 hrs ATCC 10231

Young culture suspension was prepared with nutrient broth and again incubated.

Staphylococcus aureus ATCC 6538 Escherichia coli ATCC 8739 Candida albicans 20°C/48 hrs ATCC 10231 **Preparation of cell suspension:** Preparation of cell suspension of about, 100orgs/m1 with the help of Neplometer Standard chart was carried out. A cell suspension of 24 hrs old culture of specified micro-organisms was prepared with sterile saline. The cell density was matched to the density of suspension prepared as in Tube 3 of above chart. Then the cell density was adjusted using sterile saline. As given in the chart, the cell suspension thus formed will had approximate cell density of 1.4 x 10A9 cells /ml.

Above cell suspension was used to prepare a cell suspension of 1.4 x 10A2 cells /ml approx as given below:-

1.4 x 19A9 cells/ml ----->1.4 x 10A2 cells/ml. Theoretically the last dilution should contain approximate 1.4 x 10A2 cells/ml. This dilution was confirmed by plate count method.

Sample Preparation: Nutrient Broth was prepared and sterilized at 121°C in mini autoclave for 15 min. Sample was heated in water bath to 42°C to melt it. 1 ml of the melted sample i.e. Vanga Bhasma was added to the nutrient broth at 40°C aseptically; and then it was shaken vigorously; after that it was cooled to 30°C and then 1 ml of cell suspension having approximate count 1.4 x10A2 cells/ml was added as mentioned in the following table and this tubes are incubated and readings were taken after 24hrs, 48 hrs and 72hrs and readings were taken.

After 60 hrs of initial inoculation sub culturing from all the above suspensions is carried out with 0.1 ml of above suspension into freshly prepared nutrient broth without culture in pre-sterilised nutrient broath media tube containing 10 ml of broth and reading were taken after 24hrs, 48hrs and 72hrs.

RESULTS

Table No.1 Showing initial inoculation of *Vanga Bhasma* (Sample I)

Cultures	Observations			
	24 hrs	48 hrs	72 hrs	
Staphylococcus	Negative AtNegative		At	
aureus ATCC 653	8 30/37° C	37° C		
Escherichiacoli	Negative AtNegative		At	
ATCC 8739	30/37° C	37° C		
Candida albicans Negative AtNegative			At Negative At 20°/25 °C	
ATCC 10231	20°/25° C	20°/25° C	Negative At 20°/25°C	
REMARK	No growth fo	ound in all the ab	oove cultures after 60 hrs.	

Table No.2: Showing secondary inoculation of *Vanga Bhasma* (Sample I)

Media		Observation	
	24 hrs	48 hrs	72 hrs
Nutrient Broth	Negative	Negative	
	At 30° to 37°C	At 37° C	
Nutrient Broth	Negative	Negative	
	At 30° to 37° C	At 37° C	
Nutrient Broth	Negative	Negative	Nagativa At 200/250 C
	At 20 to 25° C	At 20°/25° C	Negative At 20°/25° C
REMARK	No growth fou	nd for all above	cultures after 60 hrs.

Table no.3 Showing initial inoculation of *Vanga Bhasma* (Sample II)

Cultures	Observations			
	24 hrs	48 hrs	72 hrs	
Staphylococcus	Negative	Negative		
aureus ATCC 653	8 30/37° C	37° C		
Escherichia	Negative	Negative		
ATCC 8739	30/37° C	37° C		
Candida				
albicans ATCC 10231	Negative 20°/25° C	Negative 20°/25° C	Negative At 20°/25 °C	
REMARK	No growth found in all the above cultures after 60 hrs.			

Table no.4 Showing secondary inoculation of *Vanga Bhasma* (Sample II)

Media		Observation	
24 hrs	48 hrs	72 hrs	
Negative	Negative		
At 30° to 37°C	At 37° C		
Negative At 30° Negative			
to 37° C	37° C		
Negative	Negative	Magativa At 200/250 C	
20°/25° C	20°/25° C	Negative At 20°/25° C	
No growth four	nd for all above	e cultures after 60 hrs.	
	24 hrs Negative At 30° to 37°C Negative At 30 to 37° C Negative 20°/25° C	24 hrs A8 hrs Negative Negative At 30° to 37°C At 37° C Negative At 30° Negative to 37° C 37° C Negative Negative	

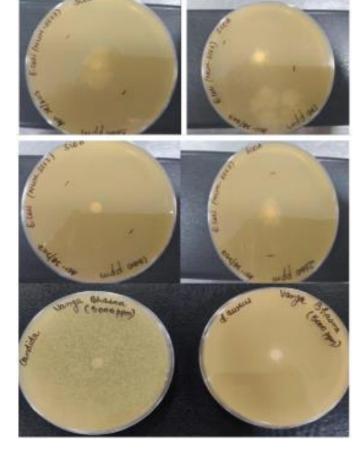


Figure No.1

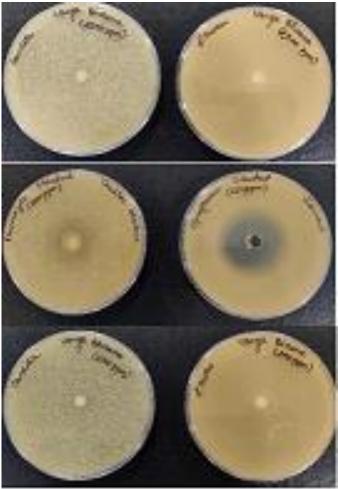


Figure no- 2

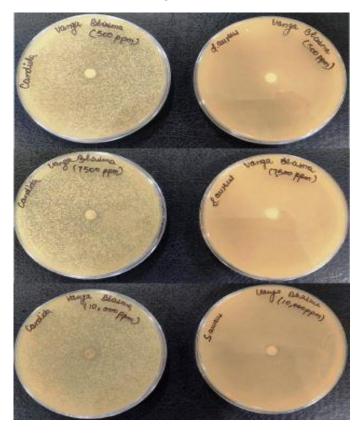


Figure no.- 3

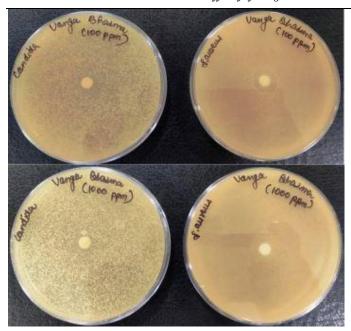


Figure no.- 4

DISCUSSION

To evaluate the Anti-microbial activity of two different samples of *Vanga Bhasma*, this study was performed. Since, no documented study is found mentioned in *Ayurveda* regarding antimicrobial activity in the modern medicine were followed. *Vanga Bhasma* is a unique Ayurvedic preparation having indication in lower urinary tract disorders. Therefore for this study, three common pathogenic microorganisms responsible for lower urinary tract infection .i.e. *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* were taken. In Vitro studies were undertaken to assess the antimicrobial activity at Interstellar Testing Centre Panchkula Haryana. For the antimicrobial study of *Vanga Bhasma* (sample I and II) standard culture of these microorganisms were taken.

- Staphylococcus aureus, ATCC 6538
- Escherichia coli, ATCC 8739
- Candida albicans 20°C/48 hrs, ATCC10231

These samples are mostly used in LUTI disease. Study performed specially against these three most common bacteria which is more responsible for skin disease.

- 1. *Staphylococcus aureus* these are gram positive cocci, non sporing bacteria responsible for infection.
- 2. *Escherichia coli* gram negative Bacteria, non sporing cause of grequent opportunistic infections in mucosal area etc.
- 3. *Candida albicans*-yeast species. Infections of the LUTRI commonly involve the moist area may involve ureters, urethra etc.

It is noted that the preparation under study i.e. *Vanga Bhasma* (*Sample* I and II) are having antibacterial activity against *Escherichia coli* with inoculums of 1.4 x 10² cells/ml under the above said condition.

CONCLUSION

For this study standard cultures and subcultures were taken of these microorganisms. Sample prepared for 1ml of *Vanga Bhasma* against the 1ml of cell suspension. It is noted that the preparation under study (*Vanga Bhasma* Sample 1 and Sample 2) are having antibacterial activity against *Esherichia coli* as no growth was found in all the cultures after 60 hours.

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