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Research Article

PHARMACEUTICO ANALYTICAL STUDY AND COMPARATIVE ANTIMICROBIAL EFFECT OF *HARTALA BHASMA* AND *HARTALAGODANTI BHASMA*

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ABSTRACT

Hartala (Orpiment) is a deep orange-yellow colored mineral with formula As_2S_3 and *Godanti* (Gypsum) is a very soft sulfate mineral with the chemical formula $CaSO_4$ $2H_2O$. *Hartala bhasma* and *Hartalagodanti bhasma* is being in practice widely in *Ayurvedic* formulation in spite of its toxic nature. This study includes, *Sodhan* (purification) of *Haratala* and *Godanti*, *Marana* of *Hartala*, *Godanti* and *Hartalagodanti*, Chemical Analysis of *Hartala bhasma* and *Hartalagodanti bhasma* and Comparative antimicrobial study of *Hartala bhasma* and *Hartalagodanti bhasma*. Antimicrobial activity of *Hartala bhasma* and *Hartalagodanti bhasma* was conducted against gram positive, gram negative bacteria to evaluate its efficacy as broad spectrum antibiotic. So an attempt had been made to put *Hartala bhasma* and *Hartalagodanti bhasma* as an antimicrobial agent. *Hartala bhasma* and *Hartalagodanti bhasma* has an effective antimicrobial activity against Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus in both Diffusion methods (Kirby- Bauer disc diffusion method and Stokes disc diffusion method) and Dilution methods (Broth dilution method & Agar dilution method).

KEY WORDS: *Hartala bhasma, Hartalagodanti bhasma*, Orpiment, Gypsum, Antibacterial agent.

INTRODUCTION

Ayurveda the science of life is the oldest health science which becomes more and more popular as alternative and complementary medicine around the globe over recent years [1]. *Hartala* comes under *Uparasa* [2] and *Godanti* first come under 20 century in rasa classics [3]. *Hartala* is highly potent mineral which is included in the list of poisonous drug by Drug & Cosmetic Act 1940 under Schedule E because of its highly toxic nature in crude form [4]. In the rasa classics *Hartala bhasma* [5] and *Hartalagodanti bhasma* [6] has its own significant role to eliminate the *Doshas* [7-8] like *Vata-rakta* (Gout), *Kushthas* (skin disorders), *Visharp*, *Shvas* (bronchial asthma), *Kaphaj roga*, *Kasha* (cough), Malaria, Pneumonia, Typhoid fever etc. An Antimicrobial is an agent that kills microorganism or inhibits their growth and Antibiotics are useful for the treatment of infectious disease in situations where the normal host defence cannot destroy pathogens. Antimicrobial agents differ not only in there action and activity but also in their distribution, metabolization and excretion from the body. When immediate antimicrobial therapy is essential there is no time to culture and identify the disease causing agent. So drug (*Hartala bhasm* and *Hartalagodanti bhasm*) specificity was checked with methods by Kirby- Bauer disc diffusion method, Stokes disc diffusion method, Broth dilution method and Agar dilution method.

AIM AND OBJECTIVE

1. *Sodhan* of *Haratala* and *Godanti*
2. *Marana* of *Hartala*, *Godanti* and *Hartalagodanti*
3. Chemical Analysis of *Hartala bhasma* and *Hartalagodanti bhasma*
4. Comparative antimicrobial study of *Hartala bhasma* and *Hartalagodanti bhasma*

Antimicrobial sensitivity tests were takes place by two types

- **Diffusion methods**

  A. Kirby- Bauer disc diffusion method
  B. Stokes disc diffusion method

- **Dilution methods**
  A. Broth dilution method
  B. Agar dilution method

MATERIAL AND METHODS

Step 1: *Sodhana* of Crude *Hartala* and *Godanti*

Materials

- Crude *Haratala* (1.30kg), *Churnudaka*[9] (10 kg) and *Dola yantra*[10] for *Haratala bhasma.*
- Crude *Haratala* (200gm), *Hulhula svarasa (Tilaparni-Gynandropsis gynandra* Brique) (5 liter) and *Dolayantra* for *Hartalagodanti bhasma.*
- *Godanti* (2.00kg), *Nimbu svarasa* (5 liter) and *Dola yantra* for *Hartalagodanti bhasma.*

Method

Crude *Haratala* and Godanti was crushed into small pieces and made a three *Pottali* (*Haratala*- 1.30kg, *Haratala*- 200gm and *Godanti*- 2kg) of cotton cloth. This *Pottali* hung in the *Churnudaka, Hulhula svarasa (Tilaparni-Gynandropsis gynandra* Brique) and *Nimbu svarasa* respectively filled pot with help of wooden stick and subjected to *Mridu agani* (mild heat) for 3 hours[11-13]. After self cooling, *Haratala* and *Godanti* was removed and washed with hot water. After drying it was weighted.

Step 2: *Marana* of *Hartala* and *Godanti*

Materials

- *Suddha Haratala* (500gm), bark of *Ashvatha (Ficus Religiosa* L.*)* (6.5kg) for decoction, bark of *Ashvatha* (60kg) for ash.
- *Suddha Godanti* (1.920gm), *Ghritkumari* (*Aloe Vera* L.) - Q.S.

Method

1. *Suddha Haratala* was crushed into fine powder and ground well with decoction of bark of *Ficus Religiosa* for 21 days *Bhavana* is given, then contents are made into thin and flat *Chakrikas* and dried under the sunrays. These *Chakrikas* are kept in a *Sharavasampt* (earthen vessel) and *Sandhibandhan* is done. Now bark of *Ficus Religiosa* was fired and form ash which was used in Bhasmayantra. After this take an earthen pot and filled half with ash of *Ficus Religiosa* and placed *Saravasampt* and then again filled ash of *Ficus Religiosa* in the earthen pot and *Sandhibandhan* is done (*Bhasm yantra*). There after three *Gajput* was given with *Uplas*. After *Svangshita bhasm yantra* was put out *from Gajput* and collect the *Hartala bhasm*.
2. *Suddha Godanti* powder was taken and made round *Chakrikas* by giving *Bhavana* with *rasa* of *Ghritkumari* and dried. Then keep in *Saravasamputa* and three *Gajput* was given [14]. After *Swangshita*, *Saravasamput* was put out *from Gajput* and collect the *Godanti bhasm*.

Step 3: Formation of *Hartalagodanti bhasm*

Materials

- *Suddha Haratala* (200gm), *Godanti bhasm* (1.00kg) and *Ghritkumari* (Q.S.)

Method

Suddha Hartala and *Godanti bhasm* was taken and made round *Chakrikas* by giving *Bhavana* with *rasa* of *Ghritkumari* and dried. Then keep in *Saravasamputa* and three *Gajput* was given. After *Swangshita*, *Saravasamput* was put out *from Gajput* and collect the *Hartalagodanti bhasm*.

Step 4: Antimicrobial study of *Hartala bhasma* and *Hartalagodanti bhasma*

Table 1: Maintenance of culture

S.N.	Bacteria	Type of Bacterial media used
1.	Streptococcus pneumonia	Blood Agar
2.	Klebsiella pneumonia	Mac Conkey Agar, Muller Hinton Agar
3.	Pseudomonas aeruginosa	Mac Conkey Agar, Muller Hinton Agar
4.	Staphylococcus aureus	Blood agar, Nutrient Agar, Muller Hinton Agar

Strains of Bacterial species were selected by their various antibiograms prevalent in local population. Evaluation of antibacterial activity done by using Glucose Broth liquid medium, Muller Hinton Agar and blood agar solid medium by Bacterial Broth dilution method maintained vide information. Solution of both medicinal preparation (*Harital bhasma* and *Hartalgodanti bhasma*) was prepared in Glucose Broth containing concentration of 250mg/ml, 2000mg/ml, 150mg/ml. 1×10^6 / ml concentration of bacteria equivalent to 0.5

McFarland $BaCl_2$ solution prepared and 250mg/ml, 200mg/ml, 150mg/ml of *Hartal bhasma* and *Hartalgodanti bhasma* is mixed in same Glucose broth and incubated for 24 hr. at 37^0C. Plating of this Broth did according its standard procedure, after 24 hours incubation colonies count done as per standard procedure [15, 16].

1. Kirby- Bauer disc diffusion method

Within 15 minutes after adjusting the turbidity of the inoculums suspension to that of standard, dip a sterile nontoxic cotton swab into the inoculum and rotate the swab several times with firm pressure on the inside wall of the tube to remove excess fluid. Inoculate the dried surface of Mueller - Hinton agar plate that has been brought to room temperature by streaking the swab three times over the entire agar surface. Replace the lid of the dish. Allow 3-5 minutes but no longer than 15 minutes for surface of the agar to dry before applying the antibiotic discs. Place the appropriate antimicrobial-impregnated discs on the surface of the agar, using either sterile forceps or multidisc dispenser. After placement, press the disc on the surface of the medium to provide uniform contact. Do not move the disc once it has contacted the agar, because some of the drug diffuses almost immediately. The discs must be evenly distributed on the agar so that they are not closer than 24mm centre to centre. On a plate of 100mm diameter, seven discs may be placed one in the centre and six in the periphery. As soon as the antibiotic impregnated disc comes in contact with the moist agar surface, it absorbs moisture from the agar and the antibiotic diffuses into the surrounding medium.

The plates are then incubated at 35^0C for 16-18 hours. Visible growth of the bacteria occurs on the surface of the agar where the concentration of the antibiotic has fallen below its inhibitory level for the test strain. Bacterial growth occurs in the form of a circle with middle of the disc forming the centre of the circle. By using caliper or a transparent plastic ruler, the zones of complete growth inhibition around each of the discs are carefully measured. The diameter of the disc is included in this measurement. The zone size that is observed in a disc diffusion test has no meaning by itself. The interpretation of zone size into susceptible (infection interpretation of zone dosage), moderately susceptible (infection that may responds to therapy with higher dosage) or resistant (not treatable with this agent) is based on the interpretation chart. Reference strains of S. aureus, E. coli, P. aeruginosa, should be tested each time a new batch of discs, agar is used.

2. Stokes disc diffusion method

The plate is divided into three parts. The test organism is inoculated on central one third and control on upper and lower thirds of the plate. However, in modified Stokes disc diffusion method, the test organism is inoculated in the upper and lower thirds and control on central one third. An uninoculated gap 2-3 mm wide should separate the test and control areas on which antibiotic discs are applied. The plates are then incubated at 35-37^{o}C for 16-18 hours. For reading and reporting of results, discard any plate on which growth is not semi-confluent and repeat the test. Measure the zone size. i.e. the distance in mm from edge of the disc to the zone edge, if that is obvious, if it is not, measure to the point of 80% or more inhibition of growth. However, if the test zones of inhibition at all, it is not necessary to perform any measurement. Each zone size is interpreted as follows:

A. Sensitive: The zone- size is equal to, larger than or not more than 3 mm smaller than the control.

B. Intermediated: The zone size of the test strain is at least 2mm, but also at least 3mm smaller than that of the control strain.

C. Resistant: The zone size of the test strain is smaller than 2mm.

3. Bacterial Broth Dilution Methods

Serial dilution of the drug in Mueller-Hinton broth is taken in tubes and a standardized suspension of the test bacterium inoculated. The inoculum is prepared as in case of disk diffusion methods by comparing with 0.5 McFarland opacity standards. An organism of known sensitivity should also be titrated. Incubate at 35-37^{0}C for 17-18 hours and read the results. Incubate at 30^{0}C for determination of minimum inhibitory concentration (MIC) of methicillin. MIC is the lowest concentration of microbial agent at which there is no visible growth. For determination of minimum bactericidal concentration (MBC), subculture from each tube showing no growth over a quarter of a nutrient medium free from antimicrobial agent. Incubate and examine them for growth. The tube containing lowest concentration of the antimicrobial agent that fails to yield growth, on subculture, is the MBC of the antimicrobial agent for test strain. MIC inhibits the bacterial growth

while MBC kills the bacterium. Determining the MIC and MBC using these conventional methods gives precise information regarding an organism's susceptibility.

4. Agar dilution methods

For this method, concentrations of antimicrobial agents are corporate into agar plates, one plate for each concentration to be tested. The organisms to be tested are diluted to a slightly greater turbidity than that of McFarland 0.5 standard. This initial suspension is then further diluted, and an aliquot of each organism is placed into one well of a replicating inoculators device. This device has metal prongs that are calibrated to pick up a small amount of the bacterial suspension (usually 0.001 ml) and deliver it to the agar surface. At least 25 different strains plus controls can be placed in the wells of the inoculators for delivery to each plate in a single manual movement. In this manner, approximately 1 x 10^4 colony forming units (CFU) are delivered in a discrete drop to the surface of agar plates containing different concentrations of antimicrobial agents. After overnight incubation the organisms will grow on those plates that do not contain enough antimicrobial agents to inhibit them. The lowest concentration of agent that allows no more than one or two CFU or only a slight haze to grow is the MIC.

RESULTS

After preparation of *Hartala Bhasma* and *Hartalagodanti Bhasma* the samples were taken for chemical analysis, Sample (A) - *Hartala Bhasma* and Sample (B) - *Hartalagodanti Bhasma*. Samples were carried out at Oasis Test House Limited, Jaipur. (No.-M677/2008 and M678/2008).

Table 2: Chemical Analysis of *Hartala Bhasma*

Appearance	White Yellowish
Odour	Odourless
UV Analysis	0.025% w/v solution in water at 200nm to 380 nm of UV range (a) 229nm = 0.907 (b) 283nm = 0.052
PH of 5% w/v suspension	11.24
Ash value w/w %	99.10
Acid insoluble ash w/w %	2.06
Loss of drying (at 110^{0}C) w/w %	0.555
Arsenic as As w/w %	10.28
Sulphur as S w/w %	20.914

Table 3: Chemical Analysis of *Hartalagodanti Bhasma*

Appearance	White
Odour	Odourless
UV Analysis	No absorption at 200nm to 380 nm of UV range
PH of 5% w/v suspension	10.46
Ash value w/w %	99.802
Acid insoluble ash w/w %	5.275
Loss of drying (at 110^{0}C) w/w %	2.00
Arsenic as As w/w %	1.625
Sulpher as S w/w %	8.574
Calcium as Ca w/w %	31.970

Comparative antimicrobial effect of *hartala bhasma* and *hartalagodanti bhasma* was done under supervision of Dr. Arvind Singh Centre Head and Chief Pathologist of Wellspring Amolak Pathology Lab, Udaipur (Raj.). Antimicrobial sensitivity tests four strains prevalent in population (selected by antibiograms) of all four bacteria's were used, plated and observed by standard methods.

A. *HARTAL BHASMA*

Table 4: Streptococcus Pneumonia

250mg/ml	200mg/ml	150mg/ml	cons. mg/ml
0	1	4	Growth observed in strains
100%	75%	0%	Inhibition

Table 5: Klebsiella Pneumoniae

250mg/ml	200mg/ml	150mg/ml	cons. mg/ml
1	3	4	Growth observed in strains
75%	25%	0%	Inhibition

Table 6: Pseudomonas Aeruginosa

250mg/ml	200mg/ml	150mg/ml	cons.mg/ml
2	4	4	Growth observed in strains
50%	0%	0%	Inhibition

Table 7: Staphylococcus Aureus

250mg/ml	200mg/ml	150mg/ml	cons.mg/ml
0	2	4	Growth observed in strains
100%	50%	0%	Inhibition

B. *HARTALGODANTI BHASMA*

Table 8: Streptococcus Pneumoniae

250mg/ml	200mg/ml	150mg/ml	cons. mg/ml
0	4	4	Growth observed in strains
100%	0%	0%	Inhibition

Table 9: Klebsiella Pneumoniae

250mg/ml	200mg/ml	150mg/ml	cons.mg/ml
2	4	4	Growth observed in strains
50%	0%	0%	Inhibition

Table 10: Pseudomonas Aeruginosa

250mg/ml	200mg/ml	150mg/ml	cons. mg/ml
3	4	4	Growth observed in strains
25%	0%	0%	Inhibition

Table 11: Staphylococcus Aureus

250mg/ml	200mg/ml	150mg/ml	cons. mg/ml
2	4	4	Growth observed in strains
50%	0%	0%	Inhibition

Reading method

0.005 ml delivery calibrated loop was used to inoculate the media plates were incubated further 24 hour and colonies were counted and multiplied by 200 to get number of CFU/ml of broth. As mentioned in theoretical section up to 1000 CFU/ml were considered as inhibitory effect of drug.

A. *HARTAL BHASMA*

Table 12: Streptococcus Pneumoniae

Strains	250mg/ml	200mg/ml	150mg/ml
I	<1000/ml	>1000/ml	Confluent growth
II	<1000/ml	No Growth	Confluent growth
III	N o Growth	<1000/ml	Confluent growth
IV	<1000/ml	<1000/ml	Confluent growth

Table 13: Klebsiella Ppneumoniae

Strains	250mg/ml	200mg/ml	150mg/ml
I	<1000/ml	<1000/ml	Confluent growth
II	>1000/ml	Confluent growth	Confluent growth
III	<1000/ml	Confluent growth	Confluent growth
IV	No growth	Confluent growth	Confluent growth

Table 14: Pseudomonas Aeruginosa

Strains	250mg/ml	200mg/ml	150mg/ml
I	<1000/ml	Confluent growth	Confluent growth
II	>1000/ml	Confluent growth	Confluent growth
III	>1000/ml	Confluent growth	Confluent growth
IV	<1000/ml	Confluent growth	Confluent growth

Table 15: Staphylococcus Aureus

Strains	250mg/ml	200mg/ml	150mg/ml
I	<1000/ml	<1000/ml	Confluent growth
II	<1000/ml	No Growth	Confluent growth
III	No Growth	>1000/ml	Confluent growth
IV	No Growth	Confluent growth	Confluent growth

B. *HARTALGODANTI BHASMA*

Table 16: Streptococcus Pneumonia

Strains	250mg/ml	200mg/ml	150mg/ml
I	<1000/ml	Confluent growth	Confluent growth
II	<1000/ml	Confluent growth	Confluent growth
III	<1000/ml	Confluent growth	Confluent growth
IV	<1000/ml	Confluent growth	Confluent growth

Table 17: Klebsiella Pneumonia

Strains	250mg/ml	200mg/ml	150mg/ml
I	<1000/ml	Confluent growth	Confluent growth
II	<1000/ml	Confluent growth	Confluent growth
III	>1000/ml	Confluent growth	Confluent growth
IV	>1000/ml	Confluent growth	Confluent growth

Table 18: Pseudomonas Aeruginosa

Strains	250mg/ml	200mg/ml	150mg/ml
I	<1000/ml	Confluent growth	Confluent growth
II	>1000/ml	Confluent growth	Confluent growth
III	>1000/ml	Confluent growth	Confluent growth
IV	>1000/ml	Confluent growth	Confluent growth

Table 19: Staphylococcus Aureus

Strains	250mg/ml	200mg/ml	150mg/ml
I	<1000/ml	Confluent growth	Confluent growth
II	<1000/ml	Confluent growth	Confluent growth
III	>1000/ml	Confluent growth	Confluent growth
IV	>1000/ml	Confluent growth	Confluent growth

CONCLUSION

1. In *Shodan* process *Hartala* (for *Hartala bhasm*) was taken 1.30kg - obtained 1.27kg, *Hartala* (for *Hartalagodanti bhasm*) 200gm - obtained 200gm and *Godanti* 2kg - obtained 1.92kg. This little loss of weight of *Hartala* and *Godanti* due to purification.
2. In *Marana* process *Suddha hartala* was taken 500gm – obtained 121gm, *Suddha godanti* 1.92kg – obtained 1.43kg, *Hartalagodanti* 1.2kg – obtained 1.01kg.
3. In chemical analysis, w/w % of Arsenic was 10.28 & Sulphur 20.914 for *Hartala bhasm* and Arsenic 1.625, Sulphur 8.57 and calcium 31.97 for *Hartala bhasm* was measured.
4. For antimicrobial effect of *Hartala bhasma* and *Hartalagodanti bhasma* first prepare three concentrations (250mg/ml, 200mg/ml &150mg/ml) on gram +ve (Streptococcus pneumonia & Staphylococcus aureus) and Gram –ve (Klebsiella pneumoniae & Pseudomonas aeruginosa).
5. In 250mg/ml conc. of *Hartala bhasm* 100% Inhibition result come out on Streptococcus pneumonia & Staphylococcus aureus but 75% on Klebsiella pneumonia and 50% on Pseudomonas aeruginosa.
6. In 200mg/ml conc. of *Hartala bhasm* 75% Inhibition result come out on Streptococcus pneumonia, 50% on Staphylococcus aureus, 250% on Klebsiella pneumonia but no any antimicrobial effect seen on Pseudomonas aeruginosa. So for *Hartala bhasma* Antibacterial property on Gram +ve > Gram –ve
7. In 150mg/ml conc. of *Hartala bhasm* no any antimicrobial effect seen on gram +ve and gram –ve bacteria.
8. In 250mg/ml conc. of *Hartalagodanti bhasm* 100% Inhibition result come out on Streptococcus pneumonia, 50% on Staphylococcus aureus & Klebsiella pneumonia and 25% on Pseudomonas aeruginosa.
9. In conc. of 200mg/ml and150mg/ml of *Hartalagodanti bhasm* has no any antimicrobial effect seen on gram +ve and gram –ve bacteria.
10. So 250mg/ml conc. of *Hartala bhasma* and *Hartalagodanti bhasma* was more effective on gram +ve bacteria and in both Bhasms, *Hartala bhasma* was more effective than *Hartalagodanti bhasm*.

Present antimicrobial study of *Hartala bhasm* and *Hartalagodanti bhasm* has done out of the body (In vitro) but in future this can be helpful in the medication of infected patient (In vivo) of these bacteria and also in other research work.

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Photographs

Method of *Sodhan*

Asuddha Hartala (i)

Hartala Sodhan (i)

Suddha Hartala (i)

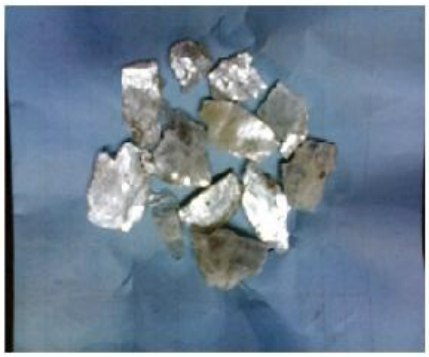
Asuddha Hartala(ii)

Hartala Sodhan(ii)

Suddha Hartala(ii)

Asuddha Godanti

Godanti Sodhan



Suddha Godanti

METHOD OF *MARANA*

Tilaparni

Bark of Ficus Religiosa

Kumari (Aloe Vera)

Godanti Chakrika

Samputikaran of Godanti

Godanti Bhasm

Hartala Bhavana

Hartala Chakrika

Hartala Bhasm

H.Godanti Bhavana

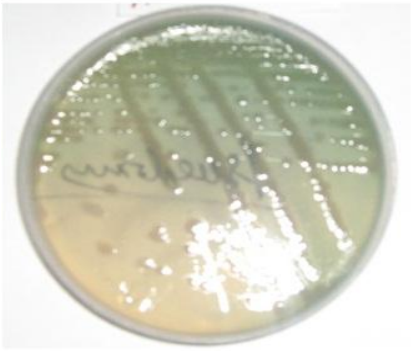
H.Godanti Chakrika

Hartalagodanti Bhasm

Cultured Plates of Bacterial Strains

Streptococcus Pneumonia

Klebsiella Pneumoniae



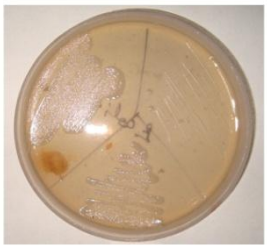
Pseudomonas Aeruginosa

Staphylococcus Aureus

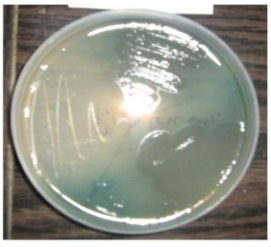
Inhibition of Strains against *Hartala Bhasm* and *Hartalagodanti Bhasm*

Hartala bhasm

S. Pneumonia



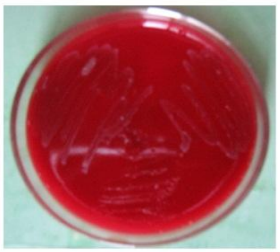
K. Pneumoniae



P. Aeruginosa

S. Aureus

Hartalagodanti Bhasm



S. Pneumonia

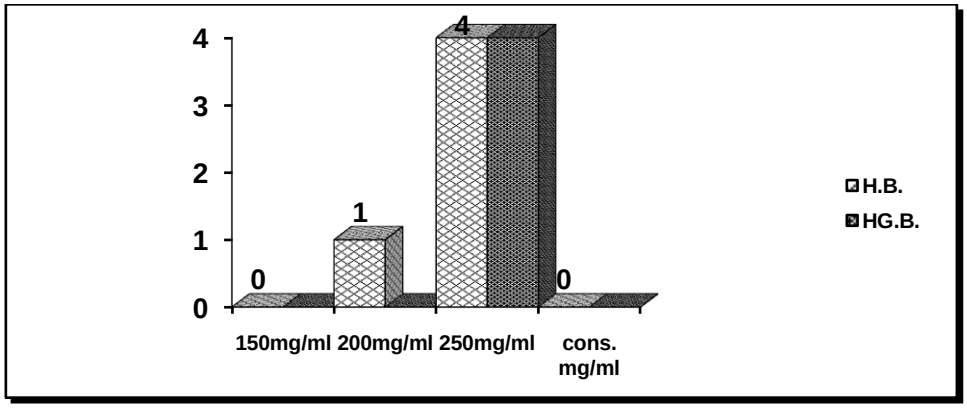
K. Pneumoniae

P. Aeruginosa

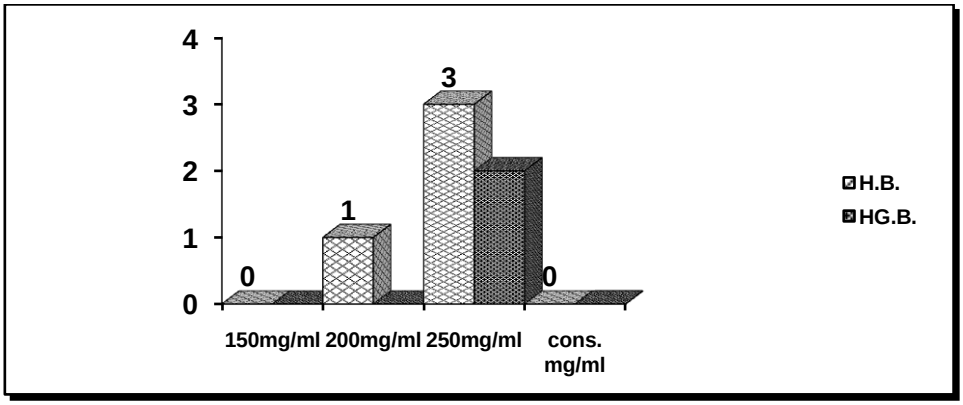
S. Aureus

Graphical Presentation of Inhibition of Strains in Different Concentration of *Haratala Bhasm* (H.B.) and *Haratalagodanti Bhasm* (HG.B.)

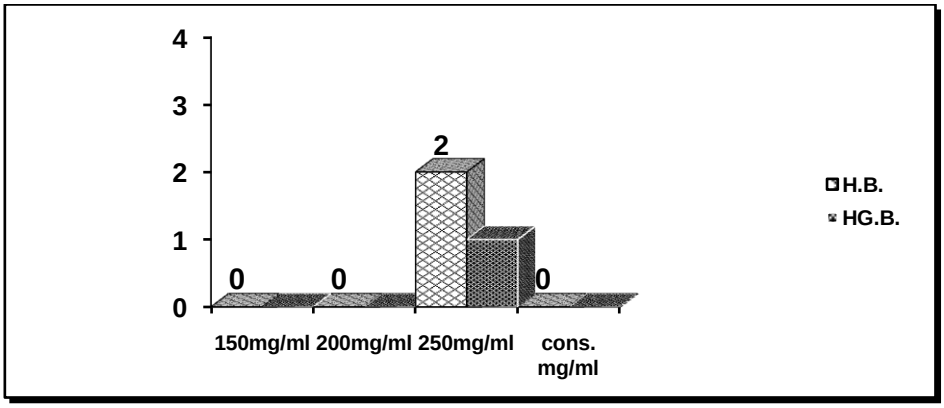
A. Streptococcus Pneumoniae



B. Klebsiella Pneumoniae



C. Pseudomonas Aureginosa



D. Staphylococcus Aureus