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# ANTIMICROBIAL ACTIVITY OF *GAGANAPARPATI* - AN INVITRO EXPERIMENTAL STUDY

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## ABSTRACT

The aim of the *Ayurveda* is to cure the disease and to maintain the healthy life of every individual. *Rasaushadhi* is the combination of herbal, mineral and herbo-mineral components. *Parpati Kalpana* is one among the *Parada Murchita Avastha* and the *Parpati Kalpana* is a unique method of pharmaceutical part of medicine. Micro- organisms are the major cause of the various infectious disorders, *Abhraka Bhasma* is considered to have the *Krimihara* property and the mercurial products show the synergistic action when combined with the other plants, metals and minerals. *Abhraka Bhasma* and *Kajjali* are the ingredients of the *Gaganaparpati*. Here an attempt is made to prepare, analyze and to evaluate the antimicrobial activity of *Gaganaparpati*.

**KEYWORDS:** *Parpati*, *Gaganaparpati*, Antimicrobial activity, Disc diffusion and Minimum Inhibition Concentration/Serial Dilution Method

## INTRODUCTION

Among the *Rasaushadhi*, the *Parpati* is mentioned as one among the *Murchita Avastha* of *Parada*. Among these, use of the *Parpati* in the *Kustha Roga* during the 5th century A.D by Siddha Nagarjuna[1], but in 11th century A.D *Parpati*was first introduced by Chakrapani Dutta in the context of *Grahani Chikitsa*[2].

In Rasendra Sara Sangraha- states that *Chikitsa Visheshagnya* Charaka, Shushruta *Maharshigan* in *Sadya Roga* the *Chikitsa Vidhan*is done but by considering the *Rasachiktsa* as supreme it can be given even in the *Asadhya Roga*[3].

*Krimi* are correlated to the microorganisms and these are the main cause of the various infectious disorders. In the present era there are many number of antimicrobial agents and antibiotics, but these have their own limitations. As the cause of the infection is due to the interaction between the natural defense mechanism of the body and the micro-organisms. These micro- organisms cause plenty of infectious diseases in the human beings[4].

However few of the anti-microbial agents have been discovered but due to the lack of availability, considering the cost and lack of awareness has lead to develop the newer anti-microbial agents. In the classical texts like Bhava Prakash- the *Abhraka* is considered to have the *Krimihara* property[5].

In the developing countries due to the various causes either directly or indirectly

are contributing to the various infectious disorders. So, there is a need of the anti-microbial agents which can cure or eradicate the disorders and this curing capacity is seen in many of the *Ayurvedic* formulations.

*Gaganaparpati* a formulation has been mentioned in the SiddhayogaSangraha with the unique method of preparation in the form of *Parpati*[6].

## MATERIALS AND METHOD:

To evaluate the antimicrobial activity of *Gaganaparpati*,following materials are used:

**Materials**

A) Drugs

*1. Gaganaparpati*

2. Brain Heart Infusion agar

B)Micro- organisms

Bacteria

a) *Staphylococcus aureus*

b) *Staphylococcus epidermedis*

c) *Klebsiella*

d) *E. coli*

Fungi

a) *Candida albicans*

b) *Aspergillus niger*

**Methods:**

The commonly used tests for the antimicrobial study are: Disk diffusion and Serial dilution/ Minimum Inhibition Concentration (MIC) methods.

Two gram positive (*Staphylococcus aureus* and *Staphylococcus epidermedis*), two gram negative (*Klebsiella* and *E. coli*) and two fungi (*Candida albicans* and *Aspergillus niger*) were selected for the study, because these micro- organisms occur large number in most natural environment.

**Disc diffusion method**[7]

In the present study brain heart infusion agar and the stock solution prepared with Dimethyl sulfoxide used as a culture media for bacteria and Sabouraud agar medium for fungi. These two are the basic media for the cultivation of the respective organisms. The *Gaganaparpati* sample was in the powder form. The following procedure is carried out at the Maratha Mandal's Central Research Laboratory, Belgaum.

**1. Temperature:** Bring agar plates to room temperature before use.

**2. Inoculation preparation:**

a. By using the loop or swab, transfer the colonies to the plates.

b. Visually adjust turbidity with broth to equal that of a 0.5 McFarland turbidity standard that has been vortexed. Alternatively, standardize the suspension with a photometric device.

**3. Inoculation of Agar plate:**

a. Within 15 min of adjusting the inoculum to a Mcfarland 0.5 turbidity standard, dip a sterile cotton swab into the inoculum and rotate it against the wall of the tube above the liquid to remove excess inoculums.

b. Swab entire surface of the agar plate three times, rotating plates approximately $60^0$ between streaking to ensure even distribution. Avoid hitting side of the petriplate and creating aerosols.

c. Allow inoculated plate to stand for atleast 3 minutes but not longer than 15 min before making wells.

**1. Stock solution preparation:**

Prepare the stock solution weighing 10mg of compound (*Gaganaparpati*- powder form) and dissolve it in 1ml of Dimethyl sulfoxide.

**2. Addition of compound into plate:**

a. Take a hallow tube of 5mm diameter, heat it. Press it on above inoculated Agar plate and remove it immediately by making a well in the plate. Likewise, make five well on each plate.

b. With the help of micropipette add 75µ, 50µ, 25µ, 10µ, and 5µ in each well.

**3. Incubation:**

a. Incubate plates within 15 mins of compound application

b. Invert plates, and stack them no more than five high

c. Incubate for 18 - 24 hours at $37^0$c in incubator.

**1. Reading plates:**

a. Ready plates only if the lawn of growth is confluent or nearly confluent.

b. Measure diameter of inhibition zone to nearest whole millimetre by holding the measuring device.

**Minimum Inhibition Concentration (MIC)[8]/ Serial dilution method:**

**Preparation of inoculums for bacteria:**

Approximately 4 to 5 well isolated colonies of the below mentioned bacterial strain were transferred to sterile Brain Heart Infusion Broth. The turbidity of the suspension is adjusted to match Mc Farland standards.

**Minimum Inhibition Concentration Test (Aerobic)-**

1. 9 dilutions of each drug have to be done with Brain Heart Infusion Broth for Minimum Inhibition Concentration.

2. In the initial tube 20microliter of the drug was added into the 380microliter of Brain Heart Infusion Broth.

3. For dilutions 200micrpliter of Brain Heart Infusion Broth was added into the next 9 tubes separately.

4. Then from the initial tube 200microliter was transferred to the first tube containing 200microliter of Brain Heart Infusion Broth. This was considered as 10-1 dilution.

5. From 10-1 diluted tube 200microliter was transferred to second tube to make 10-2 dilution.

6. The serial dilution was repeated up to 10-9 dilution for each drug.

7. From the maintained stock cultures of required organisms, 5microliter was taken and added into 2ml of Brain Heart Infusion Broth.

8. In each serially diluted tube 200microliter of above culture suspension was added.

9. The tubes were incubated for 24 hours and observed for turbidity.

**Application of the solutions:**

The pH was maintained at 7.4. In each serially diluted tube 200microliter of the above culture suspension was added.

**Incubation:**

The bacterial cultures were incubated at $37^0 \pm 2^0$ for 24 hours.

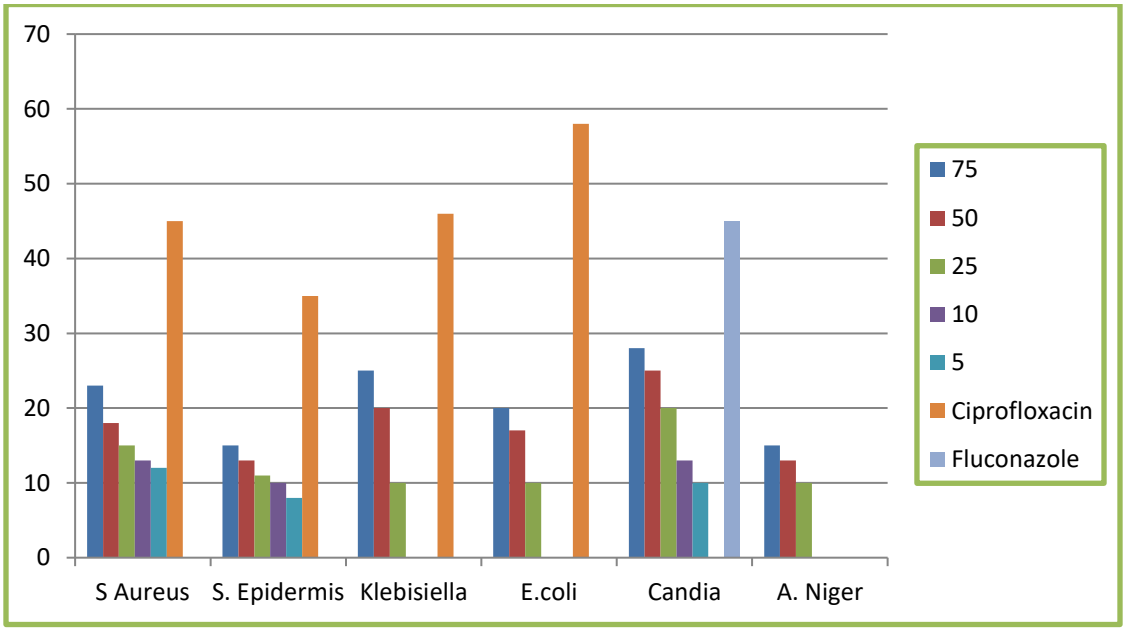
**Readings of MIC and interpretation of the results:**

The tubes were incubated for 24 hours and observed for turbidity that is the cloudiness indicates that bacterial growth has not been inhibited by the concentration of drug present in the medium.

## RESULT:

**Disc Diffusion Method**

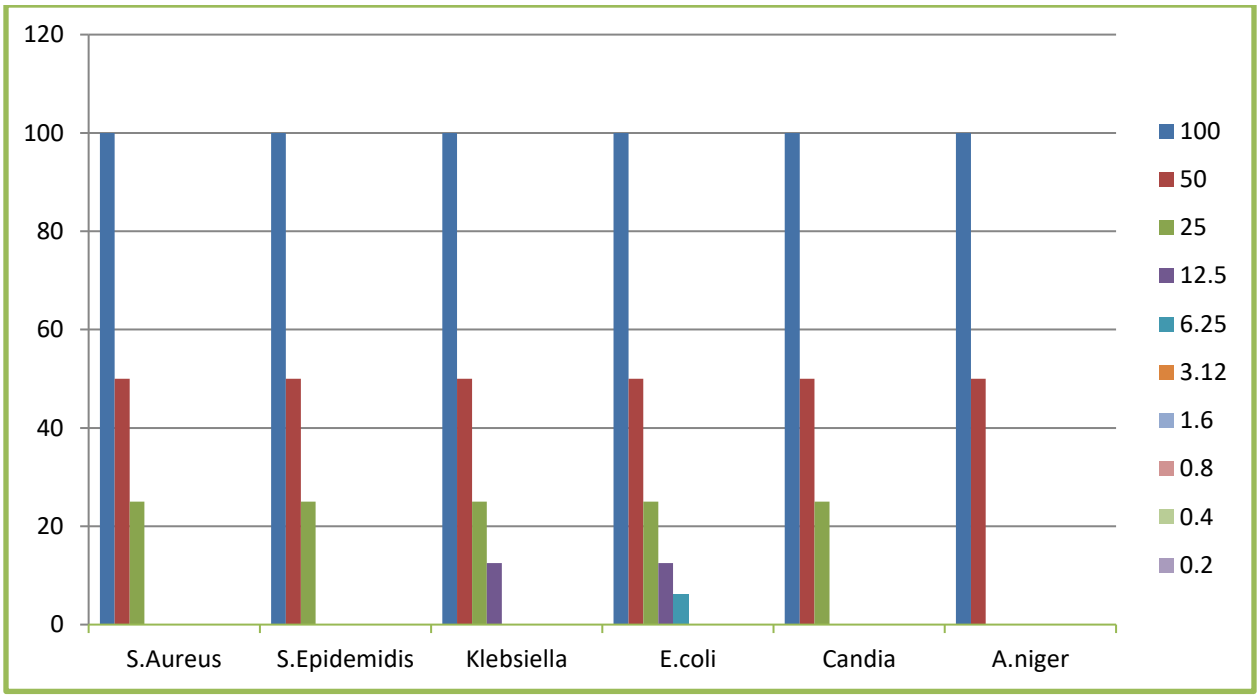
| Organisms | 75µl/ml | 50µl/ml | 25µl/ml | 10µl/ml | 5µl/ml | Ciprofloxacin | Fluconazole |
|---|---|---|---|---|---|---|---|
| S. aureus | 23mm | 18mm | 15mm | 13mm | 12mm | 45mm | - |
| S. epidermis | 15mm | 13mm | 11mm | 10mm | 08mm | 35mm | - |
| Klebisiella | 25mm | 20mm | 10mm | R | R | 46mm | - |
| E. coli | 20mm | 17mm | 10mm | R | R | 58mm | - |
| Candia | 28mm | 25mm | 20mm | 13mm | 10mm | - | 45mm |
| A.niger | 15mm | 13mm | 10mm | R | R | - | 28mm |




**Results:** Minimum Inhibition Concentration/Serial Dilution Method

| Organisms | 100 µg/ml | 50 µg/ml | 25 µg/ml | 12.5 µg/ml | 6.25 µg/ml | 3.12 µg/ml | 1.6 µg/ml | 0.8 µg/ml | 0.4 µg/ml | 0.2 µg/ml |
|---|---|---|---|---|---|---|---|---|---|---|
| S. aureus | S | S | S | R | R | R | R | R | R | R |
| S.epidemidis | S | S | S | R | R | R | R | R | R | R |
| Klebsiella | S | S | S | S | R | R | R | R | R | R |
| E.coli | S | S | S | S | S | R | R | R | R | R |
| Candia | S | S | S | R | R | R | R | R | R | R |
| A.niger | S | S | R | R | R | R | R | R | R | R |

**Note: S-** Sensitivity**, R-** Resistance




## DISCUSSION:

*Gaganaparpati*, one among the *Parpati* preparation found explained in the Siddhayoga Sangraha by Yadavji Trikamji Acharya and indicated in the conditions like *Mandagni, Pandu Roga, Kshaya, Kasa, Swasa and Grahani.* This consists of *Kajjali* (*Shuddha Parada* and *Shuddha Gandhaka*) and *Abhraka Bhasma*. *Kajjali* is having the qualities like *Rasayana*, immunomodulator, *Yogavahi*, acts as catalyst. *Abhraka Bhasma* is having the *Sarvavyadhihara* and *Krimihara* property.

**In Disc diffusion method-** Bacteria *Staphylococcus aureus* shown the sensitivity even at the 5µg/ml with the12mm of zone of inhibition, *Staphylococcus epidermidis* shown the sensitivity at the 5µg/ml with the 08mm of zone of inhibition, *Klebsiella* shown the sensitivity till the 25µg/ml with the 10mm of zone of inhibition, *E. coli* shows the sensitivity till the 25µg/ml with the 10mm of zone of inhibition. Fungus *Candida albicans* shown the sensitivity till the 5µg/ml with the 10mm of zone of inhibition, *Aspergillus niger* shown the sensitivity till the 25µg/ml with the 10mm of zone of inhibition.

**In Minimum Inhibitory Concentration (MIC)-** based on turbidity the interpretation was done. Ten graded dilution of *Gaganaparpati* were done starting from the 100µg/ml to 0.2µg/ml. *Gaganaparpati* shown sensitivity upto 6.25µg/ml for the *E. coli*, 12.5 µg/ml for *Klibesilla*, 25µg/ml for the *Staphylococcus aureus, Staphylococcus epidermidis* and *Candida albicans*. 50µg/ml for *Aspergillus niger* and later became resistant. Here the *Gaganaparpati* was found sensitive to all the micro- organisms but found more sensitive for *E. coli* at 6.25µg/ml.

## CONCLUSION:

From the results it is concluded that, *Gaganaparpati* is having significant antimicrobial Here the *Gaganaparpati* was found sensitive to all the micro- organisms but found more sensitive for E. coli at

6.25µg/ml. *Gaganaparpati* one among the *Parpati Kalpana* of *Chaturvidha Rasayana.* It is having ingredients as *Kajjali* and *Abhraka Bhasma*, both being best anti-microbial in nature.

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