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Anti-Microbial Study of *Bhagottar Gutika*

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ABSTRACT

Modern science has made tremendous progress and acquired deep knowledge about Micro-organisms. But still infections caused by these, still ranks very high as a cause of death in the world. Even though there are number of Antibiotics present and new are being developed, the Micro-organisms are developing resistance towards these Antibiotics, hence there is need to find out a better medicine which can contradict all the Micro-organisms. In Ayurveda various formulations are mentioned and well documented, those are having the antimicrobial effect. This is the time to re-establish the effect of antimicrobial agents by proving it clinically or experimentally. Their effect will be evaluated by comparing with the standard drugs (Ciprofloxacin, Ampicillin,) which are in use now. The formulation taken for the study is *Bhagottar Gutika*. Its antimicrobial study will be done experimentally by adopting cup-plate diffusion method. The study does not require any investigation or intervention to be conducted on patients or animals.

Key words: *Bhagottar Gutika*, Ciprofloxacin, Ampicillin, Antimicrobial activity.

INTRODUCTION

National Health Service and Health Organization across the world are trying to reduce the use of antibiotics especially for condition that are not serious.^[1] Non-judicious use of antibiotics and corticosteroids in contemporary system of medicines during the present era has led to iatrogenic suppression of host immunity and birth of multi drug resistant traits of pathogens. This phenomenon in turn results in the recurrence of Respiratory Tract Infection (RTI).^[2] The WHO estimate these are around 1,70,000 death related to Maticillin - resistant

staphylococcus aureus (MDR)-TB per year. The biggest worry is new strains of bacteria may emerge that can't be effectively treated by any existing antibiotics.^[3] There are claims across the media that antibiotic resistance is a ticking time bomb with the daily expenses clamming superbug treat ranks alongside terrorism.^[4]

In Ayurveda, *Kasa* can co-relate with cough that is a clinical condition. In modern system of medicine, antibiotics, anti-histamines, bronchodilators, cough expectorants etc., are commonly used for the management of RTI. Hence there is a pressing need for establishment of new antimicrobial compound producing no or minimal side effects. Many *Krimihara* formulations have been extensively described in our Ayurvedic classics. *Bhagottar Gutika* is one among them which has been described in *Kasaadhikara* in *Bhaishajya Ratnavali*^[5] well-known text of Ayurveda. It is a herbo-mineral preparation and is considered as an ideal one, because exhibits faster and grater efficacy even in minute dose. By viewing its wide range of *Kasahara*, *Shwasahara*, *Krimighna* and *Kushtaghna* properties, the present study an effort will be made to evaluate Antimicrobial study of *Bhagottar Gutika*.

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OBJECTIVES OF THE STUDY

To evaluate the Anti-microbial activity of *Bhagottar Gutika*.

MATERIALS AND METHODS

Preparation of *Bhagottar Gutika*: Bhashajya Ratnavali 15/127-129

SN	Drugs	English/Botanical name	Quantity	Parts used
1	<i>Shuddha Parada</i>	Mercury	2g.	-
2	<i>Shuddha Gandhaka</i>	Sulphur	4g.	-
3	<i>Pippali Churna</i>	<i>Pipper longum</i>	6g.	<i>Phala</i>
4	<i>Haritaki Churna</i>	<i>Terminalia chebula</i>	8g.	<i>Phala</i>
5	<i>Bhibitaki Churna</i>	<i>Terminalia bellirica</i>	10g.	<i>Phala</i>
6	<i>Vasa Churna</i>	<i>Adhatoda vasica</i>	12g.	<i>Moola</i>
7	<i>Bharangi</i>	<i>Clerodendrum serratum</i>	14g.	<i>Moola</i>
8	<i>Babbula</i>	<i>Acacia Arabica</i>	QS	<i>Twak</i>
9	<i>Madhu</i>	Honey	QS	-

The Kajjali will be prepared by triturating *Shuddha Hingulottha Parada* and *Shuddha Gandhaka* and the *Churna* of *Pippali*, *Haritaki*, *Bhibitaki*, *Vasa* drugs will be prepared. Both are taken in above mention quantity and mixed well, then *Bhavana* will be given to the mixture with *Babbula Twak Kwath* for 21times, then by adding *Madhu* 1-1 *Masha Pramana* (size-1g.) *Gutika* was prepared in D.G.M. Ayurvedic Medical College, Gadag.

- Initial wt : 56 g.
- final wt : 63.20 g.

- Total weight gain during the procedure : 7.20 g.



Microbial study: Determination of antibacterial activity of *Bhagottar Gutika* was carried out by cup-plate method at Microlabs, Institute of Research and Technology, Alamelumangapuram, Vellore-09, Tamilnadu.

Materials

Drugs

- Test drug: *Bhagottar Gutika*.
- Standard antibacterial drug : Ciprofloxacin, Ampicillin.

A) Micro organism

Gram +ve Bacteria : *Staphylococcus Aureus*
Staphylococcus albus

Gram -ve Bacteria : *Escherichiacoli* *Pseudomonas aeruginosa*

B) Chemicals & solvents : Nutrient broth, Nutrient Agar, Distilled water.

C) Equipments and glasswares:

Equipments: loops, loopholder, borer, hot air oven, Inoculation hood, autoclave, incubator, spirit lamp, mask and gloves.

Glasswares: petridish, conical flask, test tube, beaker, funnel, stirrer, round bottom flask.

Principle

The method depends on the diffusion of the drug from a cavity through the solidified agar layer of a Petri dish to an extent, such that growth of the added

micro organisms is prevented entirely in a circular area or zone around the cavity containing a solution of the drug.

The rate and degree of diffusion may be affected by concentration and type of salts, viscosity of solution, solubility, temperature etc.

Cup plate method^[6] (Disk diffusion method)

It provides a uniform surface growth of the bacterium and useful for bacteriophage typing and antibiotic sensitivity testing. The disk-diffusion agar method tests the effectiveness of antibiotics on a specific microorganism. An agar plate is first spread with bacteria, then paper disks of antibiotics are added. The bacteria is allowed to grow on the agar media, and then observed. The amount of space around every antibiotic plate indicates the lethality of that antibiotic on the bacteria in question. Highly effective antibiotics will produce a wide ring of no bacterial growth, while an ineffective antibiotic will show no change in the surrounding bacterial concentration at all. The effectiveness of intermediate antibiotics can be measured using their zone of inhibition. This method is used to determine the best antibiotic to use against a new or drug-resistant pathogen.

A. Preparation of solutions

a) Preparation of test solution: 1mg of BG was added to 1ml of DMSO, and this was diluted with 10%, 20%, 30% & 40% solution of test drug.

b) Preparation of standard solution

- 1mg Ciprofloxacin was dissolved in 10ml distilled water and used as standard drug for antibacterial activity. 1ml contains 100 micro gm/ml of drug.
- 1mg Ampicillin tablet was dissolved in 10ml distilled water and used as standard drug for antibacterial activity. 1ml contains 100microgm/ml of drug.

B. Preparation of inoculums

A loop full of the organisms was emulsified in 100ml sterile growth media under proper sterile conditions and incubated for 72 hrs at 37°C in incubator.

C. Preparation of Agar plates

5ml of inoculums prepared was added to 45ml of flask containing nutrient agar at 37°C. This was immediately poured into a dry sterile Petri dish to a depth of 5mm. The Petri dishes were placed on a leveled surface to ensure that the layers of medium are of uniform thickness. Allow the plates to solidify at room temperature for 12hrs. Incubate the agar plates at 35°C to check sterility. The surface of the agar layer was kept for dry before use. With the help of sterile borer (diameter 8mm) cylinders were made in agar plates. A uniform volume (i.e. 0.5ml) of test solutions of BG, standard drug Ciprofloxacin and Ampicillin were added to each cavity, sufficient enough to fill the holes. After 30min agar plates were incubated.

D. Incubation

After introduction of Test and Standard drugs, the plates were placed in a refrigerator at 8°C - 10°C for diffusion of drugs into the media. After two hours of cold incubation, petriplates are transferred to Incubator and maintained at 37°C ± 2°C for 24 hrs for bacteria. Zone of inhibition was measured using Zone of inhibition scale. The diameter of the circular zone is the measurement of the zone of inhibition.

Precautions

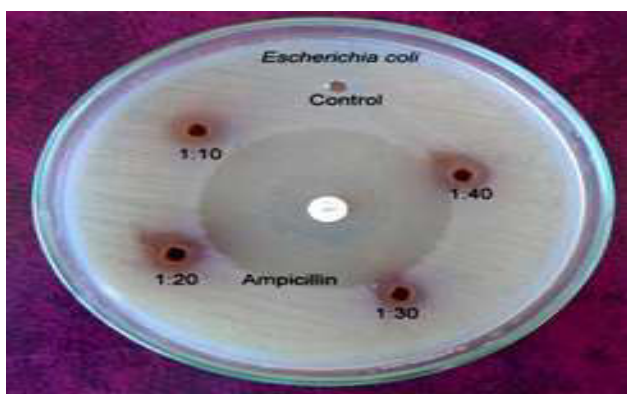
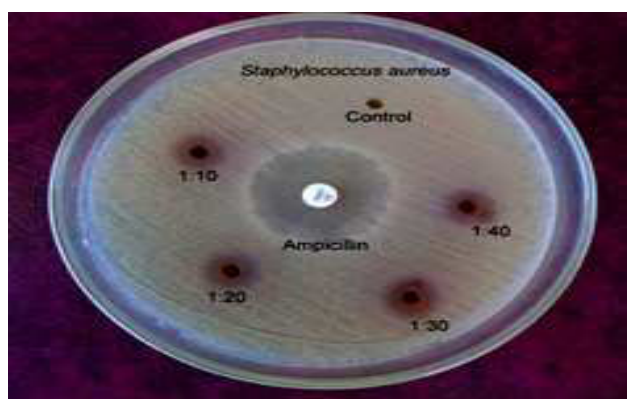
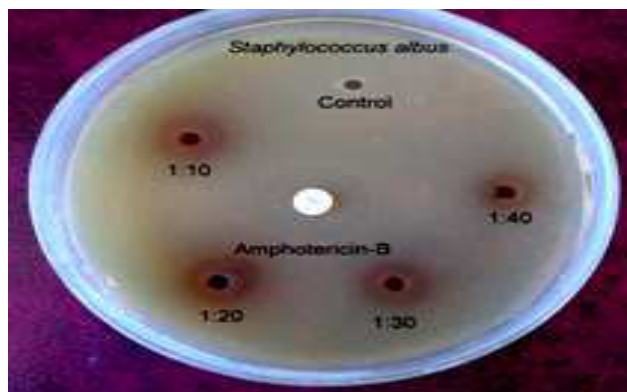
1. pH of all the media was accurately maintained for normal ions uptake by microorganisms.
2. Petridish, conical flask etc. were properly sterilized by autoclaving at 15lbs/sq inch for 15 minutes.
3. Activity was conducted by using gloves & mask and it was carried in the Laminar airflow.
4. Zone of inhibition was recorded by placing the petriplates on colony counter.

INTERPRETATION OF RESULTS

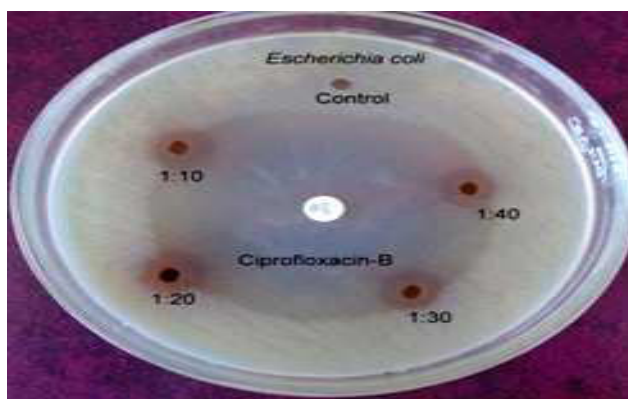
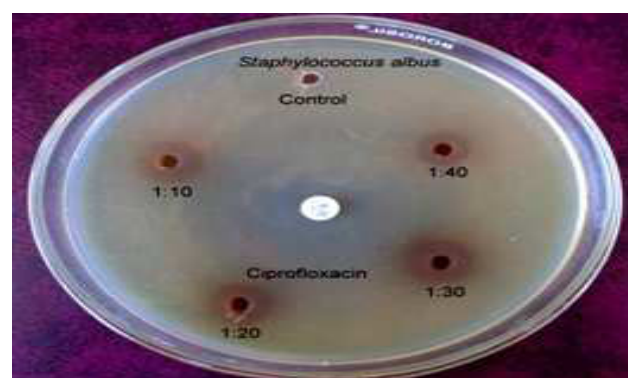
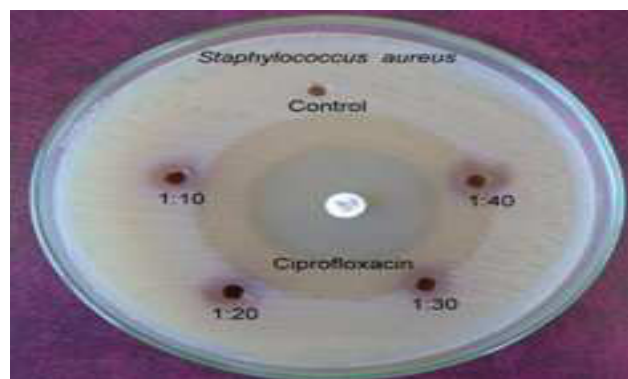
The interpretation was done on the basis of the zone of inhibition. The zone of inhibition was calculated in the millimeter (mm).

The zone of inhibition of the trial drug BG was compared to the standard drugs ciprofloxacin, Ampicillin and the efficacy was assessed.

Shows zone of inhibition (in mm) of 10%, 20%, 30% and 40% solutions of BG and standard drug Ampicillin.



Shows zone of inhibition (in mm) of 10%, 20%, 30% and 40% solutions of BG in comparison with Control and Standard drug (Ciprofloxacin)



OBSERVATIONS AND RESULTS

- Four solutions of BG, i.e. 10%, 20%, 30% and 40% were tested against Gram +ve Staphylococcus aureus, Staphylococcus albus and Gram -ve Escherichia coli, Pseudomonas aeruginosa bacteria for antimicrobial activity.
- Ampicillin and Ciprofloxacin are used as the standard drug for antibacterial activity, the dosage used for the test was 100µg/ml.
- 0.5ml of each of the solutions i.e. 4 test solutions (10%, 20%, 30%, & 40%), 1 control drug solution (DMSO) and 2 standard drug solutions (Ampicillin & Ciprofloxacin) were injected into the bore, having the maximum capacity 0.5ml.
- Zone of inhibition (in mm) of 10%, 20%, 30%, & 40% solutions of BG against micro organisms (4 bacteria's) in comparison with control drug and standard drugs, Ampicillin are showed in table 21 and ciprofloxacin are showed in table 22.
- From the results, the Gram positive bacteria Staphylococcus aureus, Staphylococcus alba and Gram negative Escherichia coli, Pseudomonas aeruginosa have shown less sensitivity to BG than that of Ciprofloxacin and Ampicillin. Variations in the response of the micro-organisms may be due to the % of trial drug diffusion, which is much necessary to exhibit the drug action is questionable. It is clear that BG has shown significant results against Gram positive and Gram negative bacteria.

Table 2: Shows zone of inhibition (in mm) of 10%, 20%, 30% and 40% solutions of BG and standard drug Ampicillin.

S N	Microorganism	Control	1:10	1:20	1:30	1:40	Ampicillin
1	Staphylococcus aureus	-	10	10	12	10	25
2	Staphylococcus albus	-	13	12	10	09	12

3	E.coli	-	12	10	09	12	35
4	Pseudomonas aeruginosa	-	12	11	08	07	15

Table 3: Shows zone of inhibition (in mm) of 10%, 20%, 30% and 40% solutions of BG in comparison with Control and Standard drug Ciprofloxacin.

S N	Microorganism	Control	1:10	1:20	1:30	1:40	Ciprofloxacin
1	Staphylococcus aureus	-	10	08	08	10	25
2	Staphylococcus albus	-	11	11	11	09	30
3	E.coli	-	12	12	09	09	34
4	Pseudomonas aeruginosa	-	11	10	10	09	38

DISCUSSION

Antimicrobial activity is a technique in which response of an organism to a particular antimicrobial agent can be established. In the present study cup plate method was selected and carried in Microlabs, Institute of Research and Technology, Alamelumangapuram, Vellore-09, Tamil Nadu.

Two gram +ve, two gram -ve bacteria were selected for the study. Microorganisms occur in large number in most natural environments. These are the major causative factors for many infectious diseases like respiratory disorders, diarrhea, dysentery, skin disorders etc.

Each kind of microorganism has specific growth requirements most of the microbes can be grown in culture medium in the laboratory. In the present study Nutrient broth is chosen as a culture media for bacteria. Growth of organism was confirmed by turbidity of the media.

Agar universally used as a solidifying agent, which have not been replaced by any other agent from 100

years. Tested drug BG, standard antimicrobial drug Ciprofloxacin and Ampicillin were used at 100 micro gm/ml and 100 micro gm/ml Concentrations respectively. Results are expressed by determining the zone of inhibition measuring in mm by using Scale.

CONCLUSION

The trial drug BG has been proved to be anti microbial during the experimental study conducted by the cup plate method. BG showed susceptible sensitivity against the Gram positive and Gram negative bacteria.

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