Published online in http://ijam. co. in

ISSN: 0976-5921

Comparative Antimicrobial Potential of *Tribhuvana-Mishrana* and Its Ingredients Against Clinical Bacteria

Research Article

Vashisht Kiran[1*], Thakur Vivek [2], Kumar Deepak[3], Sharma Khemchand[4]

1. Ph.D Scholar, Department of Dravya Guna Vigyana, Faculty of Ayurveda, I.M.S, B.H.U, Varanasi, India
2. Post Graduate Scholar, P.G. Department of RS&BK , Uttarakhand Ayurveda University, Rishikul Campus, Haridwar
3. Assistant Professor, Department of Microbiology, IMS, BHU Varanasi, India.
4. Professor, Department of RS&BK, Uttarakhand Ayurveda University, Gurukul Campus, Haridwar

Abstract

There are many single drug and compound formulations prescribed for *Jwara (~ fever) in Ayurvedic classics. Tribhuvanakirti Rasa (TKR), Godanti Bhasma (GB) and Sudarshana Ghana Vati (SGV)* are widely used compound formulations that are separately indicated for the treatment of Jwara (~fever) in different dosage schedules. In Ayurvedic Formulary of India Tribhuvana-Mishrana (TM) has been chiefly indicated in treatment of Sarva Jwara (~ fever). Therefore, in this study comparative antimicrobial activity of TM and its individual ingredients viz. TKR, GB and SGV were evaluated against four fever-causing microbes such as gram-positive bacteria Staphylococcus aureus and the gram-negative bacteria Salmonella typhii, Escherichia coli and Pseudomonas aeruginosa. The aqueous extract of all the four samples (formulation as whole and the three individual ingredients separately) in a dose 125mcg/ml were tested for their antimicrobial activity against four different microbes by agar well diffusion method. The study shows that TM and its ingredients independently show significant antimicrobial activity. S. aureus was found to be most sensitive to SGV and moderately sensitive to TKR and TM while S. typhii was found to be most sensitive to TM as compared to others. TKR was found to be most effective against E.coli while SGV and TM both showed significant antimicrobial effect against E. coli. TM could provide an alternative to synthetic antibiotics against human microbial infections.

Keywords: *Tribhuvana-Mishrana, Tribhuvanakirti Rasa, Godanti Bhasma and Sudarshana Ghana Vati*, Antimicrobial activity.

Introduction

In *Ayurveda Jwara* (~ fever) is considered to be one of the first disease recognized to be affecting human beings.(1) Caraka has mentioned that no other disease is as severe, complicated and difficult for management as *Jwara* (~ fever).(2) According to current biomedical science, fever is a surrogate marker for disease activity in many infectious and inflammatory disorders. Fever is result of bacterial, viral and other infections also. It is caused by many microbes gram +ve and gram -ve bacteria such as *Staphylococcus aureus, Streptococcus agalactiae, Echerichia coli, Salmonella typhii* and *Pseudomanas aruginosa* etc. directly or indirectly. In *Ayurveda Kaphaja krimi* is also considered to be a cause of fever.(3) Whenever persons suffers from fever it is the common practice to take antipyretic medicines such as *Paracetamol, Nimusulide, Diclofenac Sodium* etc. but these medicines having various side effects like dyspepsia, ulceration, gastreointestinal bleeding, rashes, epigastric distress, heart burn, pruritis, etc. To avoid these side effects physicians in need of a safe and effective antipyretic medicine.

In *Ayurveda*, many of formulations have been advocated for correcting fever in various dosage forms. Herbomineral preparations like *Bhasma* and *Rasoushadhies* are stated to be very potent in eliminating diseases and also for rejuvenation purposes due to their small dose, quick effectiveness, tastelessness, and long shelf-life. There are many single drugs and compound formulations indicated for *jwara* (~fever) in the classics. *Tribhuvanakirti Rasa (TKR), Godanti Bhasma(GB) and Sudarshana Ghana Vati (SGV)* are separately indicated for the treatment of *Jwara* (~ fever) in different dosage schedule,. But in Ayurvedic Formulary of India a combination of these three medicines namely *Tribhuvana-Mishrana (TM)*(4) *(*herbomineral preparation) is chiefly indicated for the treatment of *Sarva Jwara* (~ fever).

Aim

In the present study the research plan is based on the hypothesis that *TM* and its ingredients viz. *TKR, GB and SGV* possess antimicrobial action which may be the course behind its therapeutics action in eradication of fever. So we have selected most commonly used drugs in *Ayurveda* having *Jwarghna* (antipyretic) and *Krimighna* (antimicrobial) properties, which has not been tested scientifically so far.

*Corresponding Author:
Vashisht Kiran
PhD Scholar, Department of Dravyaguna Vigyana,
Faculty of Ayurveda,
I.M.S, B.H.U, Varanasi, India
E-mail: vashishtkiran312@yahoo.com

Published online in http://ijam. co. in

ISSN: 0976-5921

International Journal of Ayurvedic Medicine, 2017, 8(1), 08-11

Objective

To evaluate the comparative antimicrobial activity of *TM* and its ingredients viz. *TKR*, *GB* and *SGV* against four fever causing bacteria.

Materials and Methods

Collection of materials

All the ingredients were procured from the Khari Baowli Market, Old Delhi, except leaves of *Nimba, Dronapushpi, Tulasi, Dhatura, Nirgundi* were collected from Rajaji national park Shyampur, Haridwar and rhizome of *Adraka* collected from local market of Haridwar authenticated by subject expert. These were cleaned properly to remove any type of contamination using distilled water.

Table-1: Ingredients of *TM*

S.no.	Ingredients	Ratio
1.	*Tribhuvanakirti Rasa (TKR)*	1 part
2.	*Godanti Bhasma* (GB)	1 part
3.	*Sudarshan Ghan Vati (SGV)*	2 part

Preparation of *TM*

For preparing *TM* and its ingredients viz. *TKR*, *GB* and *SGV,* processing were carried out in Department of *Rasa Shastra and Bhaishjya Kalpana,* Uttarakhand Ayurved University, Rishikul Campus, Haridwar.

Assessment of antimicrobial activity

The Antimicrobial activity of *Ayurvedic* drug *TM* and its ingredients viz. *TKR*, *GB* and *SGV* against bacterial strains were evaluated by using Agar well diffusion method. *Staphylococcus aureus*(MTCC No.3160), *Salmonella* typhi(MTCC No.733), *Escherichia coli* (MTCC No.901) and *Pseudomonas aeruginosa (*MTCCNo.424) were used in this study. All cultures were obtained from American type culture collection (ATCC), Microbial type culture collection(MTCC), clinical strain preserved at Department of Microbiology, Gurukul Kangri University, Haridwar.

Preparation of culture media

Mueller Hinton agar (MHA) (Hi Media Laboratories Ltd, Mumbai, India) was used as media for detection of antimicrobial activity. The MHA was prepared as per the instruction of the manufacturer. The molten media was poured into sterile Petri dishes to achieve a depth of 4mm.

Inoculum preparation

The bacterial strains were revived on nutrient agar media (Hi Media Laboratories Ltd, Mumbai, India). Inoculum was prepared in a test tube containing 1.5 ml of sterile nutrient broth by mixing 2-3 colonies of bacterial strain. Density of the suspension was adjusted to a density visually equivalent to 0.5 McFarland units.

Inoculation of media

A sterile cotton-wool swab was dipped into the suspension and surplus was removed by rotation of the swab against the wall of the tube above the fluid level. The MHA plate was inoculated by even streaking of the swab over the entire surface of the plate by rotating at 60^0 three times. Wells or cups of 6 mm size were made with sterile borer into inoculated MHA plates, and the lower portion was sealed with a little molten agar medium. 10 µl of *Ayurvedic* drug *TM* and its ingredients *TKR*, *GB* and *SGV* each were poured into different wells. Wells with 10 µl of ciprofloxacin and distilled water each were taken as positive and negative control.[5] The plates thus prepared were transferred in B.O.D incubators at 37°C for 18-24 hours. The zone of inhibition around the wells was measured and expressed in millimeters (Table -3). Antimicrobial activity was recorded if the zone of inhibition was greater than 8mm.[6]

Antimicrobial Assay

The Agar well diffusion method for antimicrobial susceptibility testing was used. Strain of gram-positive bacteria *Staphylococcus aureus* (MTCC No.3160) and the gram-negative bacteria *Salmonella typhii* (MTCC No.733), *Escherichia coli* (MTCC No.901) and *Pseudomonas aeruginosa (*MTCCNo.424) were used in this study. (Table -2)

Results

Result indicated that drug *TM* and its ingredients were found having considerable antimicrobial activity against all four microbes but the zone of inhibition of our trial drug was found less as comparable to Ciprofloxacin *as* shown in Table – 3 .

Figure – 1: Inhibition zone observed in *Staphylococcus aureus, Salmonella typhii, Psuedomonas aeruginosa* and *E. coli* plates due to *GB, TKR, SGV,* and *TM*

Staphylococcus aureus

Salmonella typhii

Psuedomonas aeruginosa

Escherichia coli

Table–2 showing selected species of Microbes for experiments

Name of Microbe	Drug concentration	Well size
Staphylococcus aureus (MTCC No.3160)	100mcg/ml (Ciprofloxacin)	6 mm
	125mcg/ml (*Godanti Bhasma*)	6 mm
	125mcg/ml (*TribhuvanaKirti Rasa*)	6 mm
	125mcg/ml (*Sudarshana Ghana Vati*)	6 mm
	125mcg/ml (*Tribhuvana Mishrana*)	6 mm
Salmonella typhii (MTCC No.733)	100mcg/ml (Ciprofloxacin)	6 mm
	125mcg/ml (*Godanti Bhasma*)	6 mm
	125mcg/ml (*Tribhuvanakirti Rasa*)	6 mm
	125mcg/ml (*Sudarshana Ghana Vati*)	6 mm
	125mcg/ml (*Tribhuvana Mishrana)*	6 mm
Escherichia coli (MTCC No.901)	100mcg/ml (Ciprofloxacin)	6 mm
	125mcg/ml (*Godanti Bhasma*)	6 mm
	125mcg/ml (*Tribhuvanakirti Rasa*)	6 mm
	125mcg/ml (*Sudarshana Ghana Vati*)	6 mm
	125mcg/ml (*Tribhuvana Mishrana*)	6 mm
Pseudomonas aeruginosa (MTCCNo.424)	100mcg/ml (Ciprofloxacin)	6 mm
	125mcg/ml (*Godanti Bhasma*)	6 mm
	125mcg/ml (*Tribhuvanakirti Rasa)*	6 mm
	125mcg/ml (*Sudarshana Ghana Vati*)	6 mm
	125mcg/ml (*Tribhuvana Mishrana*)	6 mm

Table - 3 showing Antimicrobial activity of *TM* and its ingredients

Name of Microbe	Drug concentration	Zone of inhibition (ZOI)
Staphylococcus aureus (MTCC No.3160)	100mcg/ml (Ciprofloxacin)	24.06mm
	125mcg/ml (*Godanti Bhasma*)	12mm
	125mcg/ml (*TribhuvanaKirti Rasa*)	14mm
	125mcg/ml (*Sudarshana Ghana Vati*)	18mm
	125mcg/ml (*Tribhuvana Mishrana*)	14mm
Salmonella typhii (MTCC No.733)	100mcg/ml (Ciprofloxacin)	30.10mm
	125mcg/ml (*Godanti Bhasma*)	12mm
	125mcg/ml (*Tribhuvanakirti Rasa*)	12mm
	125mcg/ml (*Sudarshana Ghana Vati*)	12mm
	125mcg/ml (*Tribhuvana Mishrana)*	14mm
Escherichia coli (MTCC No.901)	100mcg/ml (Ciprofloxacin)	32.02mm
	125mcg/ml (*Godanti Bhasma*)	8mm
	125mcg/ml (*Tribhuvanakirti Rasa*)	14mm
	125mcg/ml (*Sudarshana Ghana Vati*)	10mm
	125mcg/ml (*Tribhuvana Mishrana*)	12mm
Pseudomonas aeruginosa (MTCCNo.424)	100mcg/ml (Ciprofloxacin)	26.35mm
	125mcg/ml (*Godanti Bhasma*)	12mm
	125mcg/ml (*Tribhuvanakirti Rasa)*	12mm
	125mcg/ml (*Sudarshana Ghana Vati*)	14mm
	125mcg/ml (*Tribhuvana Mishrana*)	14mm

Published online in http://ijam. co. in

ISSN: 0976-5921

International Journal of Ayurvedic Medicine, 2017, 8(1), 08-11

Table - 4 Showing Antimicrobial activity of *TM* and its ingredients

	Zone of Inhibition in mm.(ZOI)					
Pathogens	**Control**	**Ciprofloxacin**	***GB***	***TKR***	***SGV***	***TM***
S. aureas	6	24.06	12	14	18	14
S. typhi	6	30.1	12	12	12	14
E. coli	6	32.02	8	14	10	12
Pseudomonas	6	26.35	12	12	14	14

Discussion

In Indian system of medicine; drug can be given as such in powder form to the patients with some specific vehicle according to the disease. Therefore, for evaluating the antimicrobial activity of four samples in powder form, 'Well method' was employed. The aqueous extract of all the samples in a dose 125mcg/ml were tested against four microbes namely S.*aureus, S.typhi, E.coli* and *P. aeruginosa.* Microbial spores were provided by the department and bacterial sensitivity was checked by Agar well diffusion method. Inhibition zone was measured in four different planes. Zone of inhibition more than 8 mm was considered as significant antimicrobial activity. The results of study showed that *TM* and all its ingredients were showed significant antimicrobial activity against all four microbes.

The inhibition zone around the well containing *GB* against *S.aureus*, *S.typhii* and *P.aeruginosa* were found 12mm and E. coli was 8mm. This showed that *GB* was found equally effective against in all three microbes but less effective against E.coli (8mm)

The inhibition zone around the well containing *TKR* against S.aureus and E. coli was 14mm; *S.typhii* and P.aeruginosa were 12mm. This showed that *TKR* posses good antimicrobial activity against all four microbes.

The inhibition zone around the well containing *SGV* against *S.aureus* was found 18mm, *S.typhii* and *E.coli* were12mm and *P.aeruginosa* was 14mm. This showed that *Sudarshana Ghana Vati* possessed significant antimicrobial activity against *S. aureus* which was to some extent comparable to standard drug (ciprofloxacin) and equally effective against in all three bacterias *S.typhi, E.coli, P.aeruginosa.*

The inhibition zone around the well containing TM against S.aureus, S. typhii, P. aeruginosa was 14mm and E. coli was 10mm. This shows that *TM* equally effective against all three bacterias *S. aureus S.typhi, Pseudomonas aeruginosa* and less effective against *E.coli*

It was very clear that *TM* and SGV were found having significant antimicrobial activity in comparison to *GB* and *TKR*.

Conclusion

The Aqueous extracts of *TKR*, *GB*, *SGV* and *TM* (Agar Well-diffusion method) showed significant effect against all the given microbial species. Viz. *Staph. aureus, E.coli, Pseudomonas aeruginosa* and *Salmonella typhi.*

References

1. Pandey Kashinath and Chaturvedi Gorakhnath, Charaka Samhita, Nidanasthana, 1/16, Chaukhamba Sanskrit Sansthan, Varanasi, 2005, page610
2. Pandey Kashinath and Chaturvedi Gorakhnath, Charaka Samhita, Nidanasthana, 1/35, Chaukhamba Sanskrit Sansthan, Varanasi, 2005, page615
3. Pandey Kashinath and Chaturvedi Gorakhnath, Charaka Samhita, Vimanasthana, 7/12, Chaukhamba Sanskrit Sansthan, Varanasi, 2005, page727
4. Anonymous, The Ayurvedic Formulary of India, Vol.III, Group-15, Government of India, Page322
5. Hammer K. A, Carson C. F. and Riley T. V, Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology, 1999, Vol.86, page 985-990.
6. Baveja C. P., Text book of Microbiology, Unit- 3, chapter- 23, 2nd addition, Arya publication,2000, page174- 179.

*****