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Original Research Article

IN-VITRO ANTIBACTERIAL STUDY OF *PANCHAVAKTRA RASA* AND *TRIBHUVANA KIRTI RASA*

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ABSTRACT

Ayurveda is the science of life and aims at maintaining the health of Healthy individuals as well as treating the diseased ones. There are wide range of herbal and mineral drugs are used in Ayurvedic medicines. *Rasashastra* and *Bhaishajya Kalpana* is the branch of *Ayurveda* having mandate of formulation development and its use. There are potent drugs in Ayurveda that are being used for treatment of number of infectious diseases. In modern science the Antibiotics are used for the treatment of infectious diseases. But the Antibiotics has its limitations. In today's modern life, Antibiotic resistance have become an important threat in developing countries like India, because of significant death rate due to the development of resistance to the existing antimicrobial agents. So for this methods for antimicrobial activity testing can be used for drug discovery. Antibacterial activity of a classical herbo-mineral formulations such as

Panchavaktra Rasa (PR), *Tribhuvana Kirti Rasa* (TKR) were tested against certain pathogens such as Streptococcus pyrogenes, Escherichia coli, Staphyloccous aureus, Pseudomonas aeruginosa, and Salmonella typhi. It was in vitro study of antimicrobial activities in DMSO (Dimethyl Sulphoxide) dissolved samples were tested through well diffusion method and result shows antimicrobial activity in different bacteria were moderate to high in higher concentration.

KEYWORDS: *Panchavaktra Rasa, Tribhuvana Kirti Rasa,* Herbo-mineral, Antibacterial resistance, DMSO etc.

INTRODUCTION

'Prevention is better than cure' is a famous quote of medical science. Today's era is of Preventive approach, emphasis is given to preventive health, customized care, body–mind medicine and the use of natural products. Recent health-seeking behavior studies suggests that any societal model of healthcare based on only single system of medicine will become obsolete in the next two decades, unless it broadens out to judiciously combine with complementary systems of medicine. This obsoleteness will occur on account of the insufficiency of a single system to offer on its own, effective treatment for curative and preventive healthcare.

Rasayoga (medicines prepared with mercury and other metals or minerals) preparations are being used in fever of various etiological conditions, like infectious, inflammatory. It is clinically much effective. Therefore, we can assess the antimicrobial activity of such preparations in-vitro (i.e. culture and sensitivity Tests).

Panchavaktra Rasa and *Tribhuvana Kirti Rasa* are herbo-mineral solid dosage forms effective in the treatment of various types of fever.

MATERIALS AND METHODS

1) The ingredients of *Panchavaktra Rasa* and *Tribhuvana Kirti Rasa* were procured from the pharmacy attached with NIA, Jaipur, & identified or authenticated by the expert of the P.G. Department of *Dravyaguna Vigyan*, NIA, Jaipur.

2) The drug PR & TKR had been prepared in P.G. Department of *Rasashastra* & *Bhaishajya Kalpana*, as per the reference of *Rasa Prakash Sudhakar*[1] *and Yoga Ratnakara*[2].

3) This was an in vitro study which had been tested in DMSO (Dimethyl Sulphoxide) solution of the sample against five bacteria such as Staphyloccous aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Streptococcus pyrogenes employed by Well diffusion method.

PHARMACEUTICAL PREPARATION

A) PREPARATION THREE SAMPLES OF *PANCHAVAKTRA RASA*-

Following ingredients were taken in similar quantity i.e. 30 g. each.

1)	*Samguna Gandhaka Kajjali*	60 g.
2)	*Maricha Churna*	30 g.
3)	*Pippali Churna*	30 g.
4)	*Shuddha Tankana Churna*	30 g.
5)	*Suddha Vatsanabha Churna*	30 g.
	Total Wt.	180 g.

B) PREPARATION OF THREE SAMPLES OF *TRIBHUVANA KIRTI RASA*-

Following ingredients were taken in similar quantity i.e. 30 g. each.

1)	*Shuddha Hingula Churna*	30 g.
2)	*Shuddha Vatsanabha Churna*	30 g.
3)	*Shunthi Churna*	30 g.
4)	*Maricha Churna*	30 g.
5)	*Pippali Churna*	30 g.
6)	*Shuddha Tankana Churna*	30 g.
7)	*Pippalimoola Churna*	30 g.
	Total Wt.	210 g.

Study Design and Protocol: In-vitro antimicrobial zone inhibition study has been carried out.

Study conducted at: S R Labs & Research Centre, 230/20, sector 23, Haldighati Marg, PratapNagar, Sanganer, Jaipur- 302033. (AYUSH DTL/03)

Step A: Processing of sample:

Samples prepared by dissolving *Panchavaktra Rasa* and *Tribhuvana Kirti Rasa* in DMSO in10 mg/ml concentration.

Step B: Antimicrobial Susceptibility was tested by applying Well Diffusion Method: Agar well-diffusion method was followed to determine the antimicrobial activity. In thismethod 100 µl of test bacterial subculture was prepared in sterile both medium. For this in anappend of tube, taken 100µl sterile broth medium and few colonies of microbial culture leftinside tube.

Selection of micro-organisms: As mentioned earlier the *Panchavaktra Rasa* and *Tribhuvana Kirti Rasa* preparation is mainly used for *Jwara Rogadhikar*. Hence the following gram-positive, gram-negative organisms were selected for the study.

Table No. 1 Showing bacterial strain with their MTCC No.

S. No.	Species	Gram Positive / Negative	MTCC No.	Diseases
1.	Streptococcus pyogenes[3]	Gram positive	442	Pharyngitis, otitis media, Erysipelas, impetigo, pyoderma etc.
2.	E. coli[4]	Gram negative	1687	Pyogenic infections, ulcer formation, septicaemia etc.
3.	Staphylococcus aureus[5]	Gram positive	737	Pyogenic granuloma, superficial and deep infections etc.
4.	Pseudomonas aeruginosa[6]	Gram negative	7925	Nasocomial infections, suppurative otitis media, eye infections etc.
5.	Salmonella typhi[7]	Gram negative	733	Enteric fever, Food poisoning, Septicemia etc.

Preparation of Inoculums:

- Inoculums was prepared as per SOP.
- Harvested the bacterial and fungal cultures, used sterile peptone saline, surface growth washed, collecting it in suitable glassware, and adding sufficient sterile peptone saline and obtain a microbial count of about 1 × 108 colony-forming units (CFU) per ml.
- The value of CFU per ml in each suspension was counted, using the conditions of media and microbial recovery incubation times 72 hours to confirm the initial CFU perml. This value serves to calibrate the size of inoculums used in the test. The bacterial and yeast suspensions are to be used within 24 hours of harvest, but the fungal preparation may be stored under refrigeration for up to 7 days.

Preparation of media:

- Media was weighed on Calibrated Balance, the glass wares and utensil are depyrogenated in oven at 250^0C for 60 min, weighed media carefully and dissolved in distilled water, shook well and heated on hot plate for complete dissolve.
- Water level of autoclave was checked timely and adjusted with DMW if necessary.
- Loaded all prepare Media, carefully closed the lid of autoclave, checked power supply & ran an autoclave for sterilization. After 121^0C temp. is reached hold 15 min on this temp then off supply and released steam slowly.
- Before testing, U.V light of BIOSAFETY CABINET, Pass Box & BIOSAFETY CABINET room were switched on for 30 mins.
- Opened the lid of autoclave, took all the media on SS trays and sent to pass Box.
- Entered in air lock and then secondary change room & change the dress and wear sterilizefull dress, enter on BIOSAFETY CABINET Room off the UV Light of BIOSAFETY CABINET and switch on white light with airflow.
- Sterilized the hands & worked bench with IPA 70%.

Test Procedure:

- In vitro antibacterial activity of formulations was carried out by using the Agar well diffusion method.
- This classic method yields a zone of inhibition in mm result for the amount ofantibacterial that is needed to inhibit growth of specific microorganisms.
- Sample prepared as each purified formulation (10 mg/ml) were dissolved in DMSO.
- For the determination of zone of inhibition (ZOI), bacterial strain was taken and as a standard antibiotic and control DMSO for comparison of the results.
- The dilution (10 mg/ml) of formulation in DMSO and Gentamycin (5 µg/ml) as antibacterial as positive reference standards /antibiotics were prepared in double distilled water.
- Muller Hinton agar plates for bacteria were seeded with liquid culture of bacterial strains and allowed to stay at 37°C for 24 hours.
- The zones of growth inhibition around the wells were measured after 18 to 24 hours ofincubation at 37°C for bacterial.
- The sensitivity of the microorganism species to formulation were determined by measuring the sizes of inhibitory zones (including the diameter of well) on the agar surface with comparison to the standard antibiotic zones.

Diameter of Well - 8mm Vol.

applied in each well - 100 µl

Sample conc. - 10 mg

Control as DMSO and Positive control or Standard as Gentamycin 5 ppm.

RESULT

Table No. 2: Showing antimicrobial activity of PR:

S. No.	Micro-organism	Zone of inhibition (mm).				
		Gentamycin 5ppm		Test (DMSO) 10 mg/ml	Test (in DMSO) 10 mg/ml	Average
		1	2	1	2	
1.	Streptococcus pyogenes	40	38	10	9	9.50
2.	Escherichia coli	22	24	13	14	13.50
3.	Staphylococcus aureus	25	27	20	22	21
4.	Pseudomonas aeruginosa	28	29	13	14	13.50
5.	Salmonella typhi	26	28	16	15	15.50

Table No. 3: Showing antimicrobial activity of TKR

S. No	Micro-organism	Zone of inhibition (mm).				
		Gentamycin 5 ppm		Test (in DMSO) 10 mg/ml	Test (in DMSO) 10 mg/ml	Average
		1.	2.	1.	2.	
1.	Streptococcus pyogenes	39	37	10	11	10.50
2.	Escherichia *coli*	23	24	14	15	14.50
3.	Staphylococcus aureus	25	24	16	18	17

4.	Pseudomonas aeruginosa	30	29	14	16	15
5.	Salmonella typhi	24	26	12	11	11.50

Note: Diameter of the zone of inhibition is given.

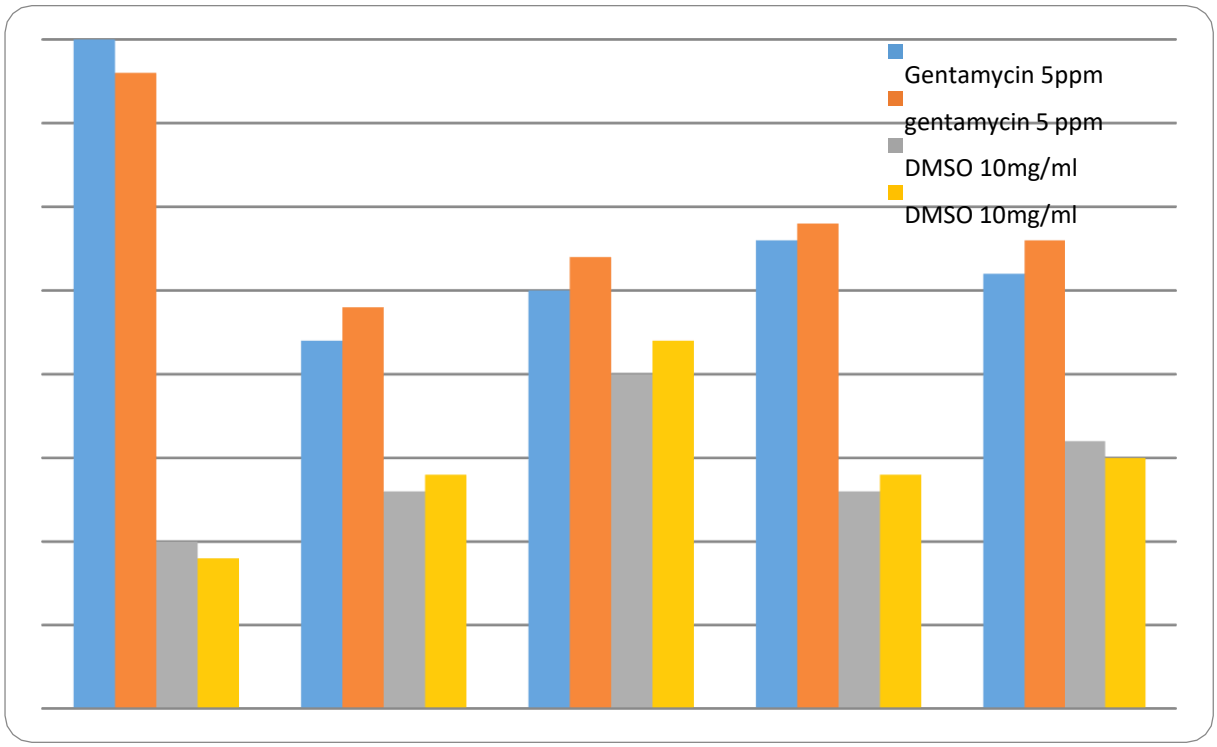


Fig. 1: Graph showing antibacterial activity of PR

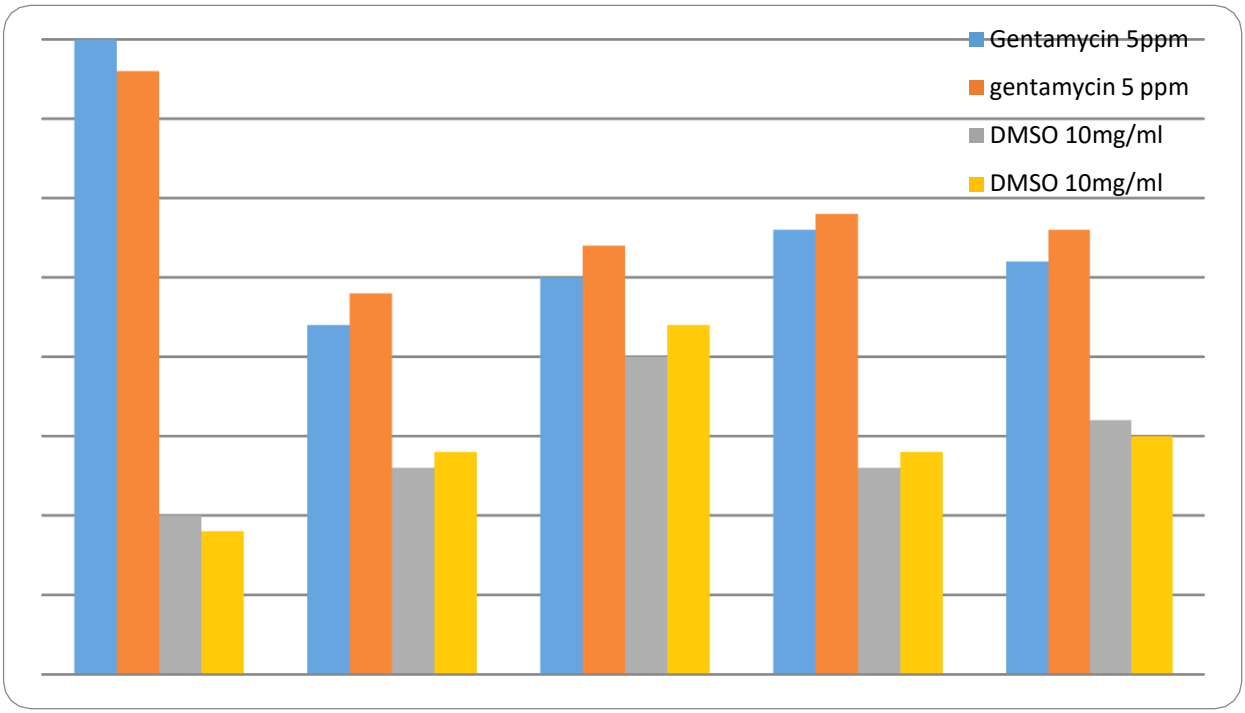
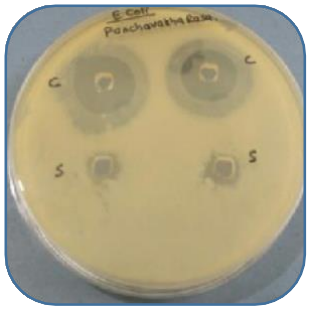


Fig. 2: Graph showing antibacterial activity of TKR

S. pyogens



E. coli

S. aureus

P. aeruginosa

S. typhi

Fig. 3: showing antibacterial activity of *Panchavaktra Rasa*

S. pyogens

E.coli

S. aurens

P. aeruginosa

S. typhi

Fig. 4: showing antibacterial activity of *Tribhuvana Kirti Rasa.*

DISCUSSION

Positive (standard) control as well as negative controls were used for the anti-microbial study. The dilution (10 mg/ml) of formulation in DMSO and Gentamycin 5 ppm. As antibacterial as positive reference standards /antibiotics were used. Zone of inhibition was measured as diameter of zone, including discs. The results were positive against all the microbes as all microbes found to be susceptible against both samples of *Panchavaktra Rasa* and *Tribhuvana Kirti Rasa.*

Moderate zone of inhibition was observed against Staphylococcus aureus (MTCC 737), E.coli(MTCC 1687) and Pseudomonas aeruginosa (MTCC 7927) in 10mg/ml concentration .Low zone of inhibition was there against Streptococcus ayogenes (MTCC 442) and Salmonella typhi (MTCC 733). Comparatively, almost same zone of inhibition as compare to standard drug in both *Panchavaktra Rasa* and *Trtibhuvana Kirti Rasa* moderately sensitive and quiet effective anti-microbial activity.

All *Dravyas* in both formulations have *Krimighna* properties like *Vatshanabha*, *Tankana*, *Kajjali*, *Hingula* etc. because of the active constituents of these *Dravyas* shows potential antimicrobial activity on microbes. So it can be stated that the formulation have an antimicrobial potential and as both formulation have same ingredients except the *Shunthi* and *Vatsanabha* in TKR and *bhawana dravyas* also exhibits antimicrobial effect on microbes*;* both formulation has been shown quite similar antimicrobial potential against these selected microbes.

CONCLUSION

- Antibacterial activity of 3 samples of PR and TKR suggests moderate susceptibility of all selected microbes against both samples of *Panchavakatra Rasa* and *Tribhuvana Kirti Rasa* as compared to standard drug gentamycin.
- Antibacterial activity of both PR and TKR were comparable to each other; Maximum zone of inhibition by PR and TKR was shown against S. aureus (21,17) and minimum against S. pyogens (9.50, 10.50). Zone of inhibition shown by TKR and PR was 13.50, 14.50 against
  E. coli (13.50, 15) against P. aureginosa and (15.50, 11.50) against S. typhi respectively.
- On the basis of results obtained in antimicrobial study pharmacological efficacy of both formulations can be further evaluated experimentally and clinically.

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