



## Antimicrobial activity of “Trinpanchmool” drugs

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### Abstract

*Trinpanchmool* drugs consisting of Kush (*Desmostachya bipinnata*), Darbh (*Imperata cylindrica*), Kas (*Saccharum spontaneum*), Sar (*Saccharum munja*) and Ikshu (*Saccharum officinarum*) are used in Ayurveda for treatment of a number of diseases and some of the drugs are traditionally used against microbial infections. The present study deals with the evaluation of antibacterial and antifungal activities of the hydroalcoholic extract of the drugs both individually and in combination. The antimicrobial activity (minimum inhibitory concentration and zone of inhibition) of *Trinpanchmool* drugs was evaluated against different micro-organisms including Gram positive bacteria, Gram negative bacteria and fungi by agar-well diffusion assay. The drugs of *Trinpanchmool* showed promising antimicrobial activity against different microorganisms. Gram positive and Gram negative bacterias were more sensitive than the fungal strains. The drugs in combination are more active than the individual extracts against maximum micro-organisms tested. Kush was inactive against *S. aureus* and *E. coli*, and Ikshu against *S. aureus* upto 2 mg/ml concentration of the extracts. *Trinpanchmool* drugs exhibited antimicrobial activity against Gram Positive bacteria, Gram Negative bacteria and fungal strains which is in support of the ethnomedical uses of the drug.

**Keywords:** Antimicrobial activity, *Trinpanchmool*, agar-well diffusion assay.

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### INTRODUCTION

A number of Indian medicinal plants have been used for thousands of years in the traditional system of medicine (Ayurveda). This system of medicine has given great emphasis to the promotion of health. In Ayurveda group of drugs known as “*Trinpanchmool*” includes Kush (*Desmostachya bipinnata*), Kas (*Saccharum spontaneum*), Darbh (*Imperata cylindrica*), Sar (*Saccharum munja*) and Ikshu (*Saccharum officinarum*) of gramineae family and is prescribed for the treatment of various ailments [1, 2]. Ayurvedic pharmacopoeia of India also prescribes the use of decoction of the roots of the plants for treatment of a number of diseases [3-5]. Some

literature shows the use of *Trinpanchmool* drugs in some microbial infections [6-8]. Hence, the present study was designed to evaluate the antimicrobial activity of the hydroalcoholic extracts of the roots of the above plants individually and in combination.

## MATERIALS AND METHODS

### Plant material collection and authentication

The drugs were collected from Banaras Hindu University campus, Varanasi and authenticated by Dr. V.K. Joshi, Dean of Faculty of Ayurveda, Institute of Medical Science, B.H.U., Varanasi and also through National Botanical Research Institute (NBRI), Lucknow. A Voucher specimen of all the plants has been preserved in the Department of Pharmacognosy, College of Pharmacy, IFTM, Moradabad for further references. The roots were separated, washed, dried under shade and coarsely powdered.

### Preparation of Extracts

The coarsely powdered materials were kept separately with a solvent mixture of alcohol and water (50:50) for seven days. Then filtered, extract was dried on water bath and the hydroalcoholic extracts were kept in desiccator until further use.

### Microorganisms

The test micro-organisms used for the antimicrobial activity were *Bacillus subtilis* (ATCC 6633), *Micrococcus flavus* (ATCC 10240), *Staphylococcus aureus* (NCTC 7447), *Enterococcus faecalis* (MTCC 459), *Streptococcus pyogenes* (NCTC 10869), *Staphylococcus albus* (ATCC 11631), *Streptococcus thermophilus* (MTCC 1938), *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (ATCC 10662), *Salmonella typhi* (NCTC 5760), *Vibrio cholerae* (ATCC 14104), *Candida albicans* (ATCC 10231), *Neurospora crassa* (MTCC 159) and *Aspergillus parasiticus* (MTCC 2796).

### Antimicrobial activity

The antimicrobial activities of the various extracts and combination of all five were evaluated by means of agar-well diffusion assay [9-11] with some modifications. Twenty five milliliters of different media were poured into sterile petri dishes. Cell suspensions of different microorganisms were prepared and evenly spread onto the surface of different media (Table 1) using sterile swab sticks. Once the plates had been aseptically dried, 10 mm wells were bored using a sterile cork borer. Extracts (5 µl) of different concentrations (0.1-2.0 mg/ml) were placed into the wells, left for one hour at room temperature for diffusion and the plates were incubated at 37<sup>0</sup>C for 24 h for bacterial strains, 48 h for fungi at 25<sup>0</sup>C. Antimicrobial activity was evaluated by determining the minimum inhibitory concentration (MIC) and measuring the zone of inhibition against the test organisms at MIC. The tests were performed in triplicate.

## RESULTS

Table 2 showed that the MIC of the extracts of *Trinpanchmool* drugs were in the range of 0.1-2.0 mg/ml against maximum strains and the extract of Kush and Ikshu against *S. aureus*, and Kush against *E. coli* did not produce any activity upto the highest concentration (2.0 mg/ml). All the extracts have a MIC of 0.1 mg/ml against both *M. flavus* and *P. aeruginosa*. Kasa and Darbh have highest MIC value (2.0 mg/ml) against *E. coli*.

The zone of inhibition of different extracts at MIC against different micro-organisms was in between 5.0-11.0 mm. The combination of the extracts was more active than individual drugs against *B. Subtilis*, *M. flavus*, *S. aureus*, *E. faecalis*, *E. coli* and *P. aeurogenosa*. The drugs in combination were most active against *M. flavus* and *P. aeurogenosa* with a zone of inhibition of 11.0 mm (Table 3).

**Table 1. Different media against various microorganisms used for antimicrobial activity**

Microorganisms	Media
<i>Bacillus subtilis</i> (ATCC 6633)	Brain Heart Infusion Broth
<i>Micrococcus flavus</i> (ATCC 10240)	Brain Heart Infusion Broth
<i>Staphylococcus aureus</i> (NCTC 7447)	Brain Heart Infusion Broth
<i>Enterococcus faecalis</i> (MTCC 459)	Brain Heart Infusion Broth
<i>Streptococcus pyogenes</i> (NCTC 10869)	Brain Heart Infusion Broth with 5% defibrinated human blood
<i>Staphylococcus albus</i> (ATCC 11631)	Brain Heart Infusion Broth
<i>Streptococcus thermophilus</i> (MTCC 1938)	MRS Broth
<i>Escherichia coli</i> (NCTC 10418)	MacConkey Broth
<i>Pseudomonas aeurogenosa</i> (ATCC 10662)	Nutrient Broth
<i>Salmonella typhii</i> (NCTC 5760)	Brain Heart Infusion Broth
<i>Vibrio cholerae</i> (ATCC 14104)	Brain Heart Infusion Broth
<i>Candida albicans</i> (ATCC 10231)	Yeast Extract Peptone Dextrose
<i>Neurospora crassa</i> (MTCC 159)	Yeast Extract Peptone Dextrose
<i>Aspergillus parasiticus</i> (MTCC 2796)	Yeast Extract Peptone Dextrose

**Table 2. Determination of MIC of Trinpanchmool drugs**

Microorganisms	MIC (mg/ml)					
	Kush	Kas	Sar	Darbh	Ikshu	Combination
<i>Bacillus subtilis</i> (ATCC 6633)	0.4	0.4	0.3	0.4	0.1	0.1
<i>Micrococcus flavus</i> (ATCC 10240)	0.1	0.1	0.1	0.1	0.1	0.1
<i>Staphylococcus aureus</i> (NCTC 7447)	-	0.5	0.1	0.5	-	0.5
<i>Enterococcus faecalis</i> (MTCC 459)	0.2	0.2	0.1	0.2	0.4	0.1
<i>Streptococcus pyogenes</i> (NCTC 10869)	0.1	0.1	0.1	0.3	1.0	0.5
<i>Staphylococcus albus</i> (ATCC 11631)	0.1	0.1	0.3	0.3	1.0	1.0
<i>Streptococcus thermophilus</i> (MTCC 1938)	0.5	0.5	0.3	0.2	1.0	1.0
<i>Escherichia coli</i> (NCTC 10418)	-	2.0	0.3	2.0	1.0	1.0
<i>Pseudomonas aeurogenosa</i> (ATCC 10662)	0.1	0.1	0.1	0.1	0.1	0.1
<i>Salmonella typhii</i> (NCTC 5760)	0.2	0.1	0.5	0.3	0.2	0.3
<i>Vibrio cholerae</i> (ATCC 14104)	0.2	0.1	0.3	0.3	0.2	0.2
<i>Candida albicans</i> (ATCC 10231)	1.0	1.0	0.3	0.5	1.0	1.0
<i>Neurospora crassa</i> (MTCC 159)	1.0	0.5	1.0	0.5	0.5	1.0
<i>Aspergillus parasiticus</i> (MTCC 2796)	0.5	1.0	0.3	0.5	1.0	1.0

**Table 3. Determination of zone of inhibition of *Trinpanchmool* drugs**

Microorganisms	Zone of Inhibition (mm)					
	Kush	Kas	Sar	Darbh	Ikshu	Combination
<i>Bacillus subtilis</i> (ATCC 6633)	6	6	7	7	8	10
<i>Micrococcus flavus</i> (ATCC 10240)	9	9	9	9	9	11
<i>Staphylococcus aureus</i> (NCTC 7447)	-	7	7	7	-	8
<i>Enterococcus faecalis</i> (MTCC 459)	9	9	9	9	8	10
<i>Streptococcus pyogenes</i> (NCTC 10869)	9	9	9	8	6	7
<i>Staphylococcus albus</i> (ATCC 11631)	9	9	6	7	6.5	7.5
<i>Streptococcus thermophilus</i> (MTCC 1938)	6	7	7.5	8	7	8
<i>Escherichia coli</i> (NCTC 10418)	-	7	6	7	7	8
<i>Pseudomonas aeruginosa</i> (ATCC 10662)	9	9	9	9	9	11
<i>Salmonella typhi</i> (NCTC 5760)	9	9	8	8	8	8
<i>Vibrio cholerae</i> (ATCC 14104)	8	9	8	8	8	9
<i>Candida albicans</i> (ATCC 10231)	6	6	8	7	5	5.5
<i>Neurospora crassa</i> (MTCC 159)	6	7	6	7	7	6
<i>Aspergillus parasiticus</i> (MTCC 2796)	8	6	7	6	6	7

## DISCUSSION

Some pathogenic bacteria are commonly resistant to many antibiotics and in many cultures, some infectious diseases are known to have been treated with herbal remedies. Present study demonstrated the antimicrobial activity of *Trinpanchmool* drugs (Kush, Darbh, Kas, Sar and Ikshu). Ikshu contains carbohydrates, amino acids, vitamins, flavonoids etc [2]; Sar mainly contains carbohydrates [12]; Kas contains proteins, glycosides, flavonoids etc [12]; Darbh mainly contains 5 triterpenoids viz. cylindrin, arundorin, ferneon, soburneol and simiareneol [3, 13, 14]; and Kush contains terpenoids, flavonoids etc [4]. The antimicrobial activity of the individual drugs may be due to some of the above phytoconstituents(s). Further, the enhanced activity of the combination of the drugs may be by synergistic action of the chemical constituents.

## CONCLUSION

*Trinpanchmool* drugs (Kush, Darbh, Kas, Sar and Ikshu) showed antibacterial and antifungal activities, and the effect is more when the drugs were tested in combination. However, it will be interesting to isolate the constituents from individual drugs responsible for antimicrobial activity.

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