

## Antimicrobial Activity of *Panchavalkal* Powder and Ointment

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**Abstract:** *Panchavalkala* is one of the ideal combinations for a vast range of therapeutics focused in Ayurveda like *Vranaropana*, *Shothahara*, *Graahi*, *Visarpahara*, etc. Researchers in recent and past evaluated anthelmintic, antimicrobial, wound healing etc. activities of these plants in combination and individual too. *Panchavalkal* is widely used in different form like *Kwath*, powder, ointment and *Malahara* etc. The advantages of different innovative *Kalpanas* as compare to traditional one have increased shelf life, ready to use, better acceptability and ease of application. The study planned with objective to evaluate in-vitro antibacterial activity of *Panchavalkal* powder and *Panchavalkal* ointment. The antimicrobial activity of *Panchavalkal* powder (traditional) and ointment (conventional) was assessed by adopting agar diffusion method in which different concentrations are incorporated into an agar medium. Replicator device was used to inoculate multiple specimens on to a series of plates with respective standard drugs. Here responses of organism to the trial drugs were measured and compared with respective standard drugs i.e. Chloramphenicol, Ciprofloxacin. Micro organisms studied were *Pseudomonas aeruginosa* (gram -ve), *Staphylococcus aureus* (gram +ve) and *Escherichia coli* (gram -ve). Antimicrobial activity of both the samples against three strains of bacteria was in between 10 to 13 that is less as compared to standard. Ointment is sensitive against *Staphylococcus aureus* and *Escherichia coli*. The drug is more sensitive for gram positive bacteria i.e. *S. aureus* than that of gram negative bacteria *E. coli*. Based on this it can be said that the drug *Panchavalkala* ointment has significant antimicrobial activity against both the strains at concentrations of 0.5 g and 1.0 g. *Panchavalkal* powder had found to exhibit antimicrobial action against *S. aureus*. It can be stated that antimicrobial action might increased significantly in *Panchavalkal* ointment. The increased additional activity might be due to concentrated *Panchavalkal* in ointment form that clearly proved the role of *Samskara*. This efficacy may also be due to the other ingredients such as sesame oil and bee wax that are the essential components in preparation of ointment. The study concluded that *Panchavalkal* ointment is better than *Panchavalkal* powder as antibacterial agent in cases of *Staphylococcus aureus* and *E. coli* infections and need to be evaluated clinically.

**Keywords:** Antimicrobial Activity, *Panchavalkal*, *S. aureus*, *P. Aeruginosa*, *E. coli*.

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### 1. INTRODUCTION

In Ayurveda various formulations such as solid dosage forms (pills, powders), liquid dosage forms (*Asavas*, *Aristhas*), and semisolid dosage forms (*Ghritas*, *Avlehas*, and *Paka*) uses for therapeutic purpose. Among all these formulations *Choorna* (power) *Kalpana* is one of the most important *Kalpana* as this form makes the traditional drug more stable for long-term storage and preserve, which can be used for internal and external purpose. *Malahara* is a unique *Kalpana* which is one among *Bahirparimarjana Chikitsa* (external applications). There are no references about this dosage form available in ancient literatures of Ayurveda. Later it gained more importance and was included in the pharmaceutical dosage forms. *Siktha Taila*, a compound of oil and bee wax is used as a base in most of the *Malahara*. In Ayurved classic *Panchavalkala* i.e. the bark of five trees viz. *Vaṭa* (*Ficus bengalensis* Linn), *Udumbara* (*Ficus glomerata* Roxb.), *Ashwattha* (*Ficus religiosa* Linn.), *Parisha* (*Thespesia populenoides* L.) and *Plakṣha* (*Ficus lacor* Buch Ham.) in mentioned in the treatment of *Vranashotha* (inflammations and abscess) and *Vrana* (wounds). All the ingredients present in the *Panchavalkala* has *Kashya rasa* (Astringent properties) which helps in the *Shodhan* (cleaning) of

*Vrana*. Individual drugs also have *Shothahara* (anti-inflammatory), *Vedanasthapan* (pain reliever), *Vranaropak* (wound healing), *Stambhana* (striking action), *Raktashodhak* (hemostatic), etc properties. In the last few decades, there has been exponential growth in the field of herbal remedies. Pharmacopoeial preparations like *Avleha or Paka*, *Swarasa*, *Kalka*, *Hima*, *Phanta*, *Kwatha* and *Choorna* form are the backbone of Ayurvedic formulations. Today the Government of India (GOI) has formed stringent to regulate issues related to quality, safety, efficacy, and practice of herbal medicine with a unique holistic approach. Most of the times *Chikitsa* become unsuccessful due to inefficacy of medicaments which is mostly due to usage of impotent medicament with or without knowledge. As population is increasing, there is also an increasing demand for the medicaments. But at the same time, scarcity of drugs and also medicaments is arising. So it has become necessary to preserve the medicaments. Hence, there is an utmost need to develop new formulation with reference of classics and using different preserving techniques with a hypothesis that the final products are of higher potency, longer shelf life and good acceptance to meet the trend of fast moving people of the present era.

*Panchavalkala* is one of the ideal combinations for a vast range of therapeutics focused in *Ayurveda* like *Vranaropana*, *Shothahara*, *Graahi*, *Visarpahara*, etc.<sup>[2]</sup> Researchers in recent and past evaluated anthelmintic, antimicrobial, wound healing etc. activities of these plants in combination and individual too.<sup>[3]</sup> Keeping this in consideration, in present study first time an attempt has been made to convert the new formulation i.e. *Panchavalkala Malahara* (ointment) and other one is *Panchavalkala Choorna*.

## 2. MATERIALS AND METHODS

### 2.1. Collection of Plant Material

Stem bark of selected plants viz., *Panchavalkal* is the formulation with barks of five trees viz. *Vata* (*Ficus bengalensis* Linn), *Udumbara* (*Ficus glomerata* Roxb.), *Ashwatha* (*Ficus religiosa* Linn.), *Parish* (*Thespesia populenea* Soland ex corea.) and *Plaksha* (*Ficus lacor* Buch-Ham.) free from disease were collected from Gadhinglaj, Maharashtra (Table 1). The plants parts were dried in sunlight till it became moister free for 7 days.

### 2.2. Preparation of Panchavalkal Powder

*Panchavalkal* powder was prepared by adopting classical method of choorna kalpana.<sup>[4]</sup> Small pieces of *Panchavalkal* were dried completely and grinding of enough size (sieve number 10). The prepared *Panchavalkala* Choorna was kept in air tight container for antimicrobial activity.

### 2.3. Preparation of Panchavalkal ointment

- The ointment was prepared by following these stages:
- Preparation of *Panchavalkala kwatha*
- Preparation of *Panchavalkala taila*
- Preparation of *Panchavalkala* ointment

*Panchavalkal kwatha* was prepared by following specified classical guidelines.<sup>[5]</sup> Prepared *Panchavalkal* coarse powder was soaked in 16 times of water for overnight. Next day it was subjected to heat with continuous stirring and the quantity was reduced to 1/4<sup>th</sup> of the initial volume. The liquid was filtered through four folded clean cotton cloth and the filtrate was collected as *Panchavalkala Kwatha*.

*Panchavalkala taila* was prepared by guideline of *sneha Kalpana* given by AFI.<sup>[6]</sup> *Panchavalkal kalka* (one part) *tila taila* (4 parts) *Kwatha* (16 part) was used as *Drava Dravya* while preparing *Panchavalkala Taila*. Cotineuos heated and when *Siddhi lakshana* of *Sneha* like free from water contents, no cracking sound on fire, froth appearance, desired colour, odour and taste of drug were observed. Then the taila was collected for further use. *Panchavalkala* ointment was prepared by classical guideline of Rasatarangani.<sup>[7]</sup> Prepared *Panchavalkala tail* is added with 1/6<sup>th</sup> part of *Siktha* (Bee wax) and dissolved properly. Final product *Panchavalkal* ointment was collected and packed in air tight container for its antimicrobial activity

## Antimicrobial Activity of Panchavalkal Powder and Ointment

**Table1.** Composition of Panchavalkala: <sup>[8]</sup>

Drugs	Latin Names	Part used	Proportion
Vata	<i>Ficus bengalensis</i> Linn.	Bark	1 Part
Udumbara	<i>Ficus glomerata</i> Roxb.	Bark	1 Part
Ashwattha	<i>Ficus religiosa</i> Linn.	Bark	1 Part
Parisha	<i>Thespesia populnea</i> Soland. ex Correa.	Bark	1 Part
Plaksha	<i>Ficus lecor</i> Buch. Ham.	Bark	1 Part

### 3. ANTIMICROBIAL ACTIVITY

Micro organisms are broadly classified as bacteria, fungi, viruses, actinomytes, etc. These are causative factors in the manifestation of various infectious diseases. Detailed study of these factors, the ways in which they produce diseases in human body and information regarding diagnosis and treatment is essential.

In-vitro tests are used as screening procedure for new agents and for testing the susceptibility of individual isolates from infections to determine which of the available drug might useful therapeutically. In general any compound or drugs that inhibit the growth or cause the death of micro organisms are known as antimicrobial agents. Any drug that inhibits the growth of bacteria or fungi is said to possess bacteriostatic and fungistatic activity respectively. In the current study it is planned to check comparative antimicrobial activity of the *Panchavalkal* in powder and ointment form.

### 4. MATERIALS

- *Panchavalkala* powder
- *Panchavalkala* ointment

#### 4.1. Place of Study

The antimicrobial study has been performed on two samples one *Panchavalkala* powder and *Panchavalkala* ointment at VASU Research Centre, Vadodara. <sup>[9]</sup>

### 5. SENSITIVITY TESTING METHOD

#### 5.1. Microbial Load

The prepared drugs *Panchavalkala* powder and *Panchavalkala* ointment were assessed for their microbial load and any adulteration before going for activity. Those both were found pure and no any microbes isolated.

#### 5.2. Agar Diffusion Method (Cup Plate Method): <sup>[10]</sup>

In agar diffusion method different concentrations are incorporated into an agar medium. Replicator device was used to inoculate multiple specimens on to a series of plates with respective standard drugs. Here responses of organism to the trial drugs were measured and compared with respective standard drugs i.e Chloramphenicol, Ciprofloxacin.

### 6. MICRO ORGANISM

Following three bacteria were used

- *Pseudomonas aeruginosa* (gram -ve)
- *Staphylococcus aureus* (gram +ve)
- *Escherichia coli* (gram -ve)

#### 6.1. Standard Inoculum

Standardization of inoculums is essential to provide reproducible minimum inhibitory concentrations (MICs). It is determined by comparing the turbidity of the liquid medium to a standard that represents a known number of bacteria in suspension. Here inoculums prepared by comparing with Mc Farland standards. <sup>[11]</sup>

#### 6.2. Material Requirements

- Nutrient Agar (For bacterial cultivation)

- 12-15 hours (Overnight) young culture of micro organisms.
- Standard solution antibiotics.
- Different concentrations of trial drugs.
- Sterile petri dishes and sterile cork borer.
- Incubator and laminar air flow cabinet ( LAF)

### 6.3. Sample Preparation

For the preparation of test samples of ointment olegenous solvent was used for dissolution.0.5gm and 1gm samples of ointment were dissolved in 5ml Di methyl sulphoxide (DMSO) and filtered. The samples in the form of solution were then used for determination of antimicrobial activity.

0.5gm and 1gm *Choorna* of *Panchavalkala* was taken and were added with 5ml of Distilled water. Mixtures were stirred well and kept aside undisturbed to sediment. Supernant layers of the mixture was then decanted and filtered. Standard Chloramphenicol and Ciprofloxacin as per IP standard were taken as standard.

### 6.4. Culture Conditions

24 hours old cultures of all these organisms were inoculated in sterile broths and incubated till 0.5 Mac Farland standard turbidity with Barium sulphate suspension obtained and then used for assay.

### 6.5. Antimicrobial Assay

Sterile soyabean casein digest agar (25 ml per plate) was used in antibacterial activity. 20 ml sterile medium was poured aseptically in sterile plates and allowed to solidify. Then 0.5 ml of culture was inoculated in 5 ml sterile, melted, cooled medium and poured them on solidified agar plates aseptically. After solidification of medium, wells were made with the help of cup borer and 0.3 ml of sample was inoculated.

Two standard drug solutions were added into the well and other standard antibiotic discs were directly placed on agar surface and pressed with the help of sterile forceps aseptically. For diffusion purpose, plates were placed in refrigerator for 20-25 minutes. Then plates were incubated at 37<sup>0</sup> C for 24 hours. After incubation, zone of inhibition was measured with Himedia antibiotic zone scale- c. 0.3 ml of different extracts as well as Chloramphenicol (30mcg/disk) and Ciprofloxacin (5mcg/disk) standard antibiotic discs were used for the assay.

## 7. RESULTS AND DISCUSSION

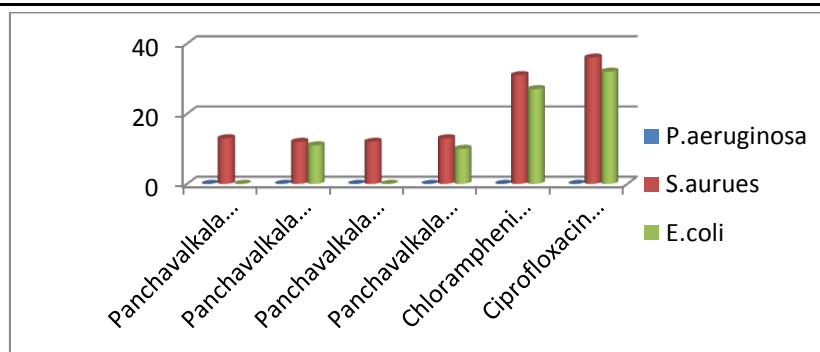
Zone of inhibition (ZOI) for 0.5gm and 1.0 gram is similar that are 13 mm for *Staphylococcus aureus*. No Zone of inhibition (ZOI) for *Pseudomonas aeruginosa* and *Escherichia coli* was found. Antibacterial activity of *Panchavalkal* ointment at two different concentrations is observed that zone of inhibition in between both concentrations i.e. 0.5gm and 1.0 gm was insignificant with 12 mm and 13 mm respectively against *Staphylococcus aureus*. Zone of inhibition against *Escherichia coli* was found to be 11 mm and 10 mm with 0.5gm and 1.0 gm respectively. No zone of inhibition (ZOI) was observed against *Pseudomonas aeruginosa*. (Table -2)

**Table 2.** Antimicrobial activity of *Panchavalkala* formulations

(Powder: 0.5g and 1.0g of the sample dissolved in 5ml D/W), (Ointment: 0.5g and 1.0g of the sample dissolved in 5ml DMSO

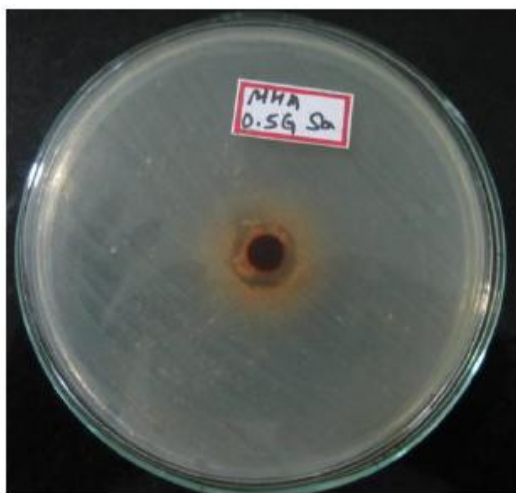
Test drugs	Microbes and ZOI		
<i>Panchavalkala</i> Forms	<i>P.aeruginosa</i>	<i>S.aurues</i>	<i>E.coli</i>
0.5g <i>Choorna</i>	No ZOI	13mm	No ZOI
1.0g <i>Choorna</i>	No ZOI	13mm	No ZOI
0.5g ointment	No ZOI	12mm	11mm
1.0g ointment	No ZOI	13mm	10mm
Standard Antibiotics	<i>P.aeruginosa</i>	<i>S.aurues</i>	<i>E.coli</i>
Chloramphenicol 30mcg/disk	No ZOI	31mm	27mm
Ciprofloxacin 5mcg/disk	No ZOI	36mm	32mm

D/W: Distilled Water; DMSO: Di Methyl Sulphoxide; *P.aeruginosa*: *Pseudomonas aeruginosa*; *S.aurues*: *Staphylococcus aureus*; *E.coli*: *Escherichia coli*; ZOI: Zone of Inhibition.



**Graph1.** Antimicrobial activity of Panchavalkala Powder and Ointment

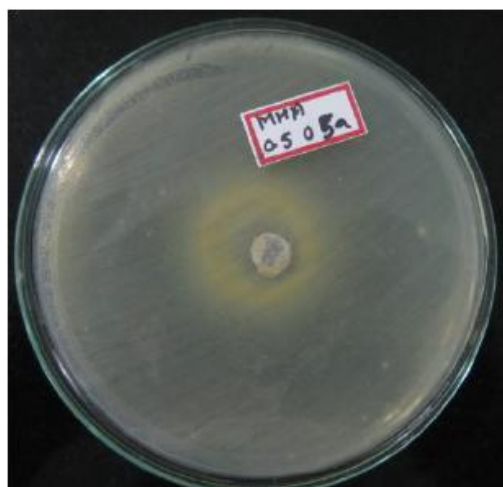
*Panchavalkala* ointment showed ZOI for *Staphylococcus aureus* 12 mm & 13 mm at 0.5g and 1gm concentration respectively (Figure-1a & b) and *Panchavalkala* powder showed ZOI for *Staphylococcus aureus* 13 mm & 13 mm at 0.5g and 1gm concentration respectively (Figure-2a & b). *Panchavalkala* ointment showed ZOI for *Escherichia coli* 11 mm & 10 mm at 0.5g and 1gm concentration respectively (Figure-3 a & b) but *Panchavalkala* powder did not show ZOI for *Escherichia coli* (Graph-1). *Panchavalkala* ointment and powder are having less antimicrobial activity in comparison to standard antibiotics (Chloramphenicol and Ciprofloxacin). But the *Panchavalkala* ointment is more effective than *Panchavalkala* powder.



**Figure-1 a:** 0.5 gm Choorna showing ZOI of 13mm against *S.aureus*



**Figure-1 b:** 1 gm Choorna showing ZOI of 13mm against *S.aureus*



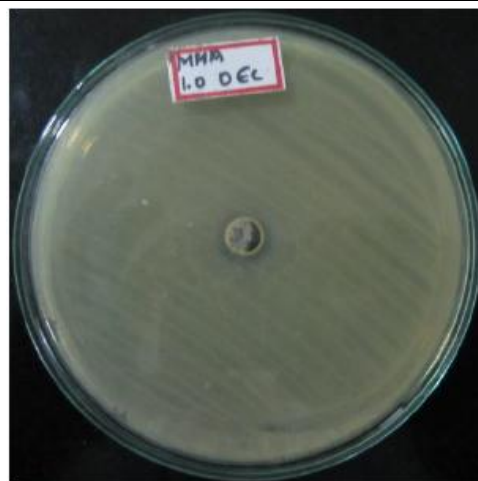
**Figure-2 a:** 0.5 gm ointment showing ZOI of 12mm against *S.aureus*



**Figure-2 b:** 1 gm ointment showing ZOI of 13mm against *S.aureus*



**Figure-3 a:** 0.5 gm ointment showing ZOI of 11mm against *E.coli*



**Figure-3 b:** 1 gm ointment showing ZOI of 10mm against *E.coli*

Ointment is sensitive against *Staphylococcus aureus* and *Escherichia coli*. The drug is more sensitive for gram positive bacteria i.e. *S. aureus* than that of gram negative bacteria *E. coli*. Based on this it can be said that the drug *Panchavalkala* ointment has significant antimicrobial activity against both the strains at concentrations of 0.5 g and 1.0 g. *Panchavalkala Choorna* had found to exhibit antimicrobial action against *S. aureus*. It can be stated that antimicrobial action might increased significantly in *Panchavalkala* ointment.

Antimicrobial activity of both the samples against 3 strains of bacteria was in between 10 to 13 that is less as compared to standard. Antibacterial property of *Panchavalkala* ointment was found similar as that of *Panchavalkala* powder against *Staphylococcus aureus*. *Panchavalkala* ointment showed additional antibacterial property against *E. coli* which was not exhibited by *Panchavalkala* powder. The increased additional activity might be due to concentrated *Panchavalkala* in ointment form that clearly proved the role of *Samskara*. Previous clinical research work carried out on *Panchavalkala* ointment and cream in the management of wounds showed encouraging results which support this in-vitro study.<sup>[12-13]</sup> This efficacy may also be due to the other ingredients such as sesame oil and bee wax that are the essential components in preparation of ointment. The study supports that ointment is better than the conventional powder form in case of external application as antibacterial agent in cases of *Staphylococcus aureus* and *E. coli* infections.

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