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Antimicrobial Activity (In Vitro) of *Panchvalkal* Mentioned In *Himvan Agada:* An Ayurvedic Formulation

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ABSTRACT:
Nyagrodha (Ficus bengalensis Linn.), Udumbara (Ficus glomerata Linn.), Ashwatha (Ficus religiosa Linn.), Parisha (Thespesia populnea Soland Ex Correa) and Plaksha (Ficus infectoria Roxb.) the stem barks of these five plants known as Panchavalkal in Ayurveda (Sample A). This is found previously that there is a mention of the use of Shirish (Albizzia lebbeck Benth.) and Vetasa (Salix caprea Linn.) as a substitute of Udumbara and Parisha in the Panchavalkal (B) recognized by Arundatta the commentator of Ashtanga Hrudaya, which belong to different families, genera & species and with different active chemical. So this study has been planned to comparatively explore and evaluate the poly herbal combination Panchavalkal (A) and (B) with dissimilar set of herbs to assess their antimicrobial properties (Agar well diffusion method) against Candida albicans, Escherichia coli and Staphylococcus aureus using Methanolic extract of formulations in three dilutions (12.5, 25 & 50mg/ml). Gentamycin (antibacterial) and Griseofulvin (antifungal) used as standard drug. Antimicrobial activity found to be significant when compared between sample A & B at each concentration with related standard drug ZOIs. Statistical analysis shows that the P values are lower than 0.05 which confirms that comparisons of results are non-significantly differ with each other which suggest both the samples having same antimicrobial activity at each concentration.

Keywords – Ayurveda, Panchavalkal, Antimicrobial

INTRODUCTION:

The stem bark of five plants viz. Nyagrodha (Ficus bengalensis Linn.), Udumbara (Ficus glomerata Linn.), Ashwatha (Ficus religiosa Linn.), Parisha (Thespesia populnea Soland Ex Correa) and Plaksha (Ficus infectoria Roxb.) is known as Panchavalkal 1. Bark paste of Panchavalkal (A) with the help of Madhu (Honey) is locally applied on the skin in the case of Mandali Sarpa -Visa and also given in Shopha (Inflammation) Visarpa (Erysipelas) , Visphota (Erruptive boils) , Jvara (Pyrexia) and Daha (Burning sensation) etc.

Several references with different herbs as components of Panchavalkal have been found 2- 3. The commentator (Arundatta) says that 'Astanga Hrdayam' substitute ' SIRISA ' and some others ' VETASA ' in place of ' Parisa ' -Now - a - days ' Nyagrodha , Udumbara, Asvattha, Plaksha and Vetasa ' , these five are being used as ' Panchavalkala' (B) by majority 4.

In the Himavan agada (anti-poisonous formulation) used to treat the Mandali Sarpa Visha, there is this difference in the opinion of Panchavalkal. The Panchavalkal (B) combination of dissimilar herbs however shares similar pharmacological property as that of Panchavalkal (A) viz. Shophaghna (anti-inflammatory).
The prime basis for the combination of these five medicinal plants as Panchavalkal is very unusual. Further it is more interesting to substitution of plants which belong to different families, genera & species and with different active chemical constituents. Therefore the present study has been planned to explore the scientific rationale for the substitution of the medicinal plants. In addition we wish to validate the traditional Ayurvedic principles.
Therefore the present study has been planned to assess their antimicrobial properties.

MATERIALS AND METHODOLOGY:

Preparation of *Panchavalkala* sample A and B

a) Procurement and Authentication of raw drugs for preparation of Samples
b) Raw drugs required for preparation were procured form authenticated market dealer.
c) Procured drugs were authenticated in Dravyaguna Department, Uttaranchal Ayurvedic College, Dehradun
d) Drugs were powdered separately in *Khalwa Yantra* to get in *Yawakuta* form.
e) After pounding drugs are mixed (I part each) to prepare *Panchavalkala* A and B.

Table- 1: Ingredients of *Panchavalkal* [5] Sample A

Sr. no	Name of Ingredient of *Panchvalkala* sample A	Quantity
1.	*Nyagrodha (Ficus bengalensis* Linn.)	1 part
2.	*Udumbara (Ficus glomerata* Roxb.)	1 part
3.	*Asvattha (Ficus religiosa* Linn.)	1 part
4.	*Parisha (Thespesia populnea* Soland ex.Correa)	1 part
5.	*Plaksha (Ficus infectoria)*	1 part

Table- 2: Ingredients of *Panchavalkal* [6] Sample B

Sr. no	Name of Ingredient of *Panchvalkala* sample B	Quantity
1.	*Nyagrodha (Ficus bengalensis* Linn.)	1 part
2.	*Shirish (Albizzia lebbeck Benth.)*	1 part
3.	*Asvattha (Ficus religiosa* Linn.)	1 part
4.	*Vetasa (Salix caprea Linn.)*	1 part
5.	*Plaksha (Ficus infectoria)*	1 part

The sample of Panchavalkal (A) and (B) were later subjected to antimicrobial Activity. The following material and methods were used for analyzing both the sample i.e. Panchavalkal (A) and (B).

ANTIMICROBIAL ACTIVITY: [7-9]

Purpose - To lay down the procedure for Antimicrobial activity to be performed in formulation with reference of using standard culture.
Scope - It is applicable to the microbiology lab for Antimicrobial activity.

Responsibility- Microbiology personnel

Procedure:

1. Precaution taken during antimicrobial activity.
2. Glassware to be used shall be sterilized.
3. Media to be used shall be pre incubated
4. Microbial area should be sterilized before testing.

Test organisms used:

Use cultures of the following microorganisms *Candida* MTCC no 227, *Escherichia coli* MTCC No. 1687 and *Staphylococcus aureus* MTCC No. 737. The feasible microorganisms used in the experiment should not be more than five passages distant from the original MTCC culture or any other equivalent cultures.

Preparation of inoculums:

1. Prepare the inoculums.
2. To harvest the bacterial and fungal cultures, use sterile peptone saline, wash the surface growth, collecting it in suitable glassware, and adding sufficient sterile peptone saline to obtain a microbial calculation of approximately 1 × 108 colony forming units (CFU) per ml.
3. Determine the number of CFU per ml in each suspension, using the conditions of media and microbial recovery incubation times 72 hours to confirm the initial CFU per ml. 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:

1. For weighing media use Calibrated Balance, the glass wares and utensil are depyrogenated in oven at 250^0C for 60 min, weigh media carefully and dissolve in distill water, shake well and heat on hot plate for complete dissolve.
2. Check the water level of autoclave if necessary adjust level with DMW.
3. Load all prepare Media, carefully close the lid of autoclave, check power supply & run the autoclave for sterilization. After reach the temp. of 121^0C hold 15 min on this temp then off supply and release steam slowly.
4. Before testing switch on the U.V light of LAF, Pass Box & LAF room for 30 mins.
5. Open the lid of autoclave takes all the media on SS trays and sent to pass Box.
6. Enter in air lock and then secondary change room & change the dress and wear sterilize full dress, enter on LAF Room off the UV Light of LAF and switch on white light with airflow.
7. Sterilize hands & work bench with IPA 70%.
8. Test Procedure:
9. In vitro antibacterial activity of formulations was carried out by using the Agar well diffusion method.
10. This standard technique yields a zone of inhibition in mm outcome for the total of antibacterial or antifungal that is desired to inhibit development of specific microorganisms.
11. Methanolic extract was prepared for each formulation by cold maceration technique and methanol evaporated at lower temperature to retain the activity of extract.
12. The dilutions (12.5, 25 & 50mg/ml) of formulation extract in DMSO and Gentamycin Sulphate (5µg/ml) as antibacterial and Griseofulvin (25 mg/ml) as antifungal for positive reference standards /antibiotics were prepared in double distilled water.
13. 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.

14. Muller Hinton agar plates for bacteria were seeded with liquid culture of bacterial strains and allowed to stay at 37°C for bacteria and 25^0C for fungus.
15. The zones of growth inhibition around the wells were measured after 18 to 24 hours of incubation at 37°C for bacterial and 25^0C for 48 hours for fungal.
16. The compassion of the microorganism genus to formulation were resolute by measuring the sizes of inhibitory zones (including the diameter of well) on the agar plane with comparison to the standard antibiotic zones.

Diameter of Well- 10 mm

Vol. applied in each well- 150 µl

Control as DMSO and Positive control or Standard as Gentamycin and Griseofulvin

RESULT:

Table no- 3: Results of Antimicrobial Study (A)

ANTI MICROBIAL SENSTIVITY DATA SHEET for Sample A					
SR.NO.	**Strains**	**Std.**	**12.5 mg/ml**	**25 mg/ml**	**50 mg/ml**
1	E.coli ZOI in mm	16	23	25	26
2		18	24	26	26
3		18	23	26	27
1	Candida ZOI in mm	28	26	28	30
2		27	26	28	31
3		27	27	28	30
1	S. aureus ZOI in mm	26	16	18	27
2		28	16	18	26
3		28	16	17	27

Table no- 4: Results of Antimicrobial Study (B)

ANTI MICROBIAL SENSTIVITY DATA SHEET for Sample B					
SR.NO.	**Strains**	**Std.**	**12.5 mg/ml**	**25 mg/ml**	**50 mg/ml**
1.	E.coli ZOI in mm	16	20	23	24
2.		17	20	22	26
3.		17	20	23	26
1.	Candida ZOI in mm	28	23	25	28
2.		27	22	26	28
3.		27	22	26	28
1.	S. aureus ZOI in mm	27	17	21	22
2.		28	17	20	23
3.		28	16	21	23

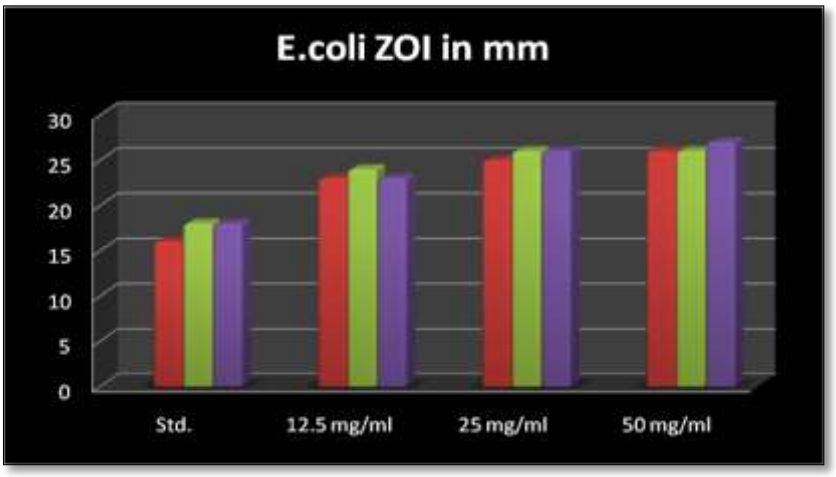
Std. is Gentamycin 5 ppm

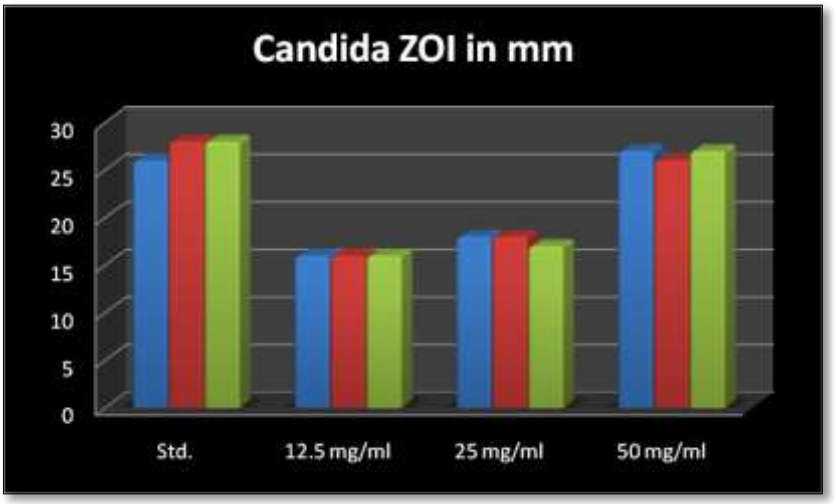
And Griseofulvin (25 mg/ml)

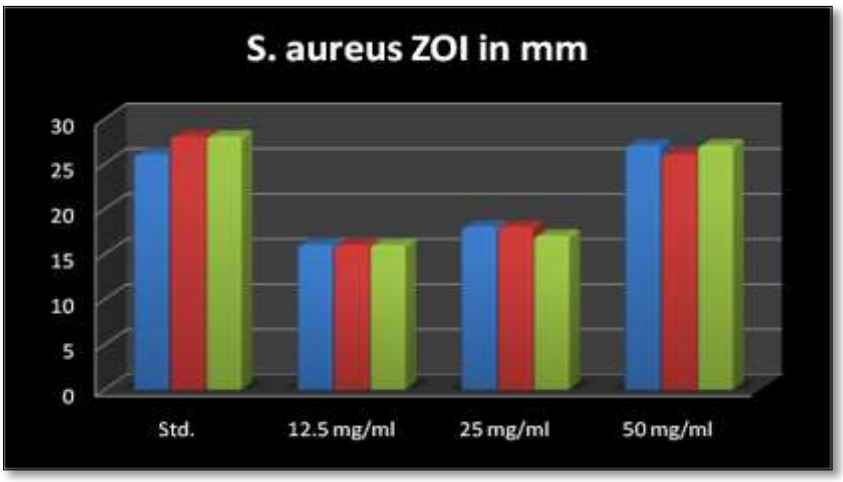
Control is DMSO Blank for all study

ZOI is Zone of Inhibition

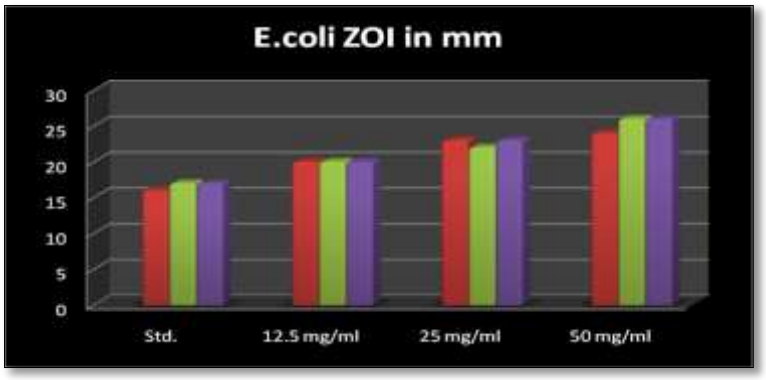
Graph -1: Showing anti microbial sensitivity data sheet for sample 'A'

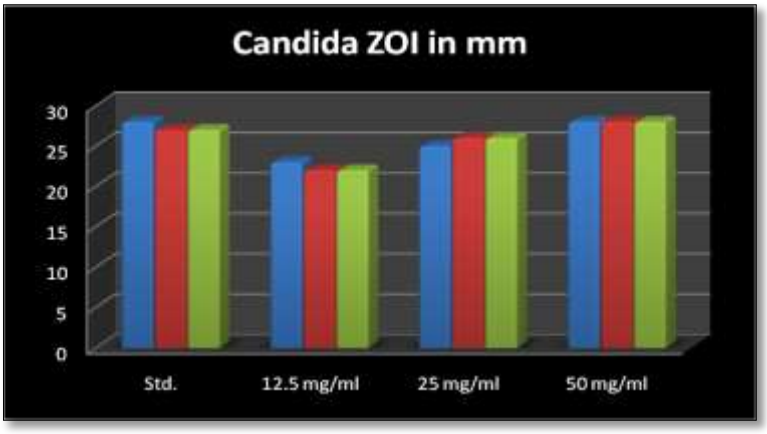


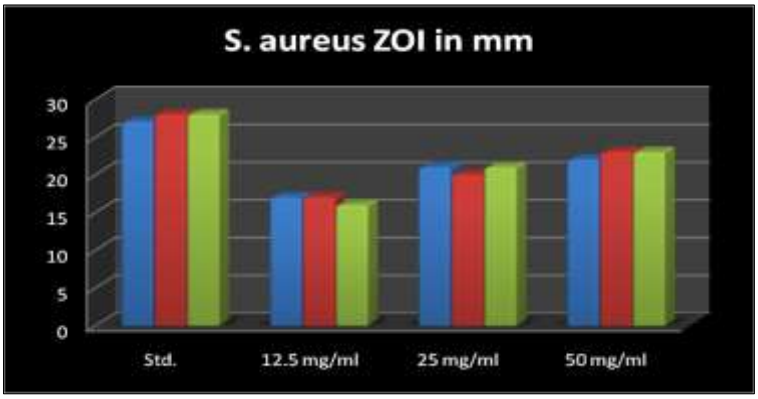




Graph- 2: Showing anti microbial sensitivity data sheet for sample 'B'







DISCUSSION:

For the present study *E. coli and S. aureus* are selected as above *Panchavalkal* are advidsed in *Vrana* condition and as it is opined by research scholars that they are the most common organisms for secondary infections in wounds (*Vrana*)

The selected formulation also used for various ailments related to yoni and Candida albicans is commonest causative organism for various vaginal infections

In present study antimicrobial activity was performed on methanolic extract for each sample. Extract was dissolved in DMSO 50mg/ml and susceptibility against different microbes checked by well diffusion method.

3 different concentration applied 12.5, 25 and 50 mg/ml in DMSO. Bacterial and fungal strains used were *E. coli, S. aureus* and *Candida ablicans*. Zone of inhibition (Zone of inhibition) were measured around the each well to compare with standard Gentamycin 5 ppm and test solution at different concentrations. Biostatistics principles applied to compare the means of different concentration and standard drugs at each microbial strain for both the samples A & B simultaneously. RM ANOVA (Repeated Measures Analysis of variance)

was performed by latest version of Graph Pad Prism 6 software on different values in ZOI at P value 0.05 to check the significant and non-significant difference between the antimicrobial results. Related table with all the microbes with different concentration for analysed P values.

Table no- 5: Result shown

Microbes	12.5 mg/ml	25 mg/ml	50 mg/ml
E. coli	0.001	0.0005	0.0007
S. aureus	0.0001	0.001	0.0021
Candida albicans	0.0009	0.0178	0.0048

All the measures found non-significant differences when compared between sample A & B at each concentration with related standard drug ZOIs.

CONCLUSION:

Antimicrobial activity found to be significant when compared between sample A & B at each concentration with related standard drug ZOIs. Statistical analysis shows that the P values are lower than 0.05 which confirms that comparisons of results are non-significantly differ with each other which suggest both the samples having same antimicrobial activity at each concentration.

Images of Antimicrobial activity

Sample A against *S. aureus*

Sample B against *S.aureus*

Sample A against *E. coli*

Sample B against *E. coli*

Sample A against Candida albicans

Sample B against *Candida albicans*

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