



A COMPARATIVE STUDY ON PRATHAMA AND DWITEEYA SINDHOORADHYA MALAHARA WITH SPECIAL REFERENCE TO ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Rasatarangini, one of the esteemed treatises of *Ayurvediya Rasasastra* has mentioned numerous *malahara* (ointment) preparations with herbal and herbo-mineral origin. The term *malahara* itself mean those which remove *mala* (impurities) from *vrana* (ulcers), *vidradhi* (abscesses), *twak vikara* (skin diseases) etc. Acharya Sadananda Sharma, author of *Rasatarangini* has mentioned *Sindhooradhyah malahara* a preparation with potent action on *vranshodhana ropana* (wound cleansing and healing) and *bhootasangha prashamana* (antimicrobial) properties. Acharya has mentioned two *malahara* preparations named as *Prathama* and *Dwiteeya Sindhooradhyah malahara* with only difference in single drug. Both preparations are indicated in skin manifestations like *pama* and *kandu*, which may be due to invasion of some external organisms or microbes. WHO has declared that antimicrobial resistance is one of the top 10 global public health threat facing humanity and thus the search for ayurvedic antimicrobials are progressing day by day. Since it is a topical antibiotic preparation, with very minimal ingredients proving its efficacy will be of great use for the society. The present study aims at the preparation and comparison between the antimicrobial activity of the two *Sindhooradhyah malahara* named as *Prathama* and *Dwiteeya* respectively. The main findings are, antimicrobial study by both agar well diffusion and macro broth dilution showed that more action in *Prathama Sindhooradhyah malahara*. Among the 4 tested strains, significant results are seen in case of *Streptococcus pyogenes* and *Candida albicans* whereas *Dwiteeya Sindhooradhyah malahara* had no action in the tested strains of microbes.

Keywords: *Sindhooradhyah malahara*, *Prathama*, *Dwiteeya*, antimicrobial, skin manifestations.

1. INTRODUCTION

Malahara is a dosage form, which has its roots in Unani system of medicine. References regarding *malahara* are present only in textbooks written after 15th century AD. Even though there are many *lepas* mentioned in ayurvedic classics, *malahara* was first introduced into ayurvedic pharmaceuticals by *Yogaratanakara* [1].

Rasatarangini, one of the greatly admired textbook of *Rasasastra* deals with wide number of *malahara kalpanas*. *Sindhooradhyah malahara* [2] is one such preparation with very minimal ingredients and with vast clinical applications. *Prathama* and *Dwiteeya Sindhooradhyah malahara* are two formulations having *bhootasangha prashamana*, *vranshodhana ropana* properties. It also has action on the skin manifestations like *pama* and *kandu*.

The word *vrana* can be linked to ulcer or wound. An

ulcer [3] is a break in the continuity of an epithelial surface. Chronic wounds are that which fails to heal and most of the chronic wounds are contaminated by microbes. Researches conducted on ulcers shed light on the fact that bacteria might be involved in and contribute to lack or delayed healing of the wound [4]. There is currently a great concern over rising resistance of many bacteria to most of the contemporary antibiotics. Here arises the importance of ayurvedic formulations with innate *bhootagghna* (antimicrobial) and *vrana ropana* (wound healing) properties.

Antimicrobial resistance continues to grow quickly among the key microbial pathogens such as *Staphylococcus*, *Streptococcus*, *Escherichia coli* etc. all around the world. WHO has declared that antimicrobial resistance is one of the top 10 global public health threat facing humanity [4]. Conventional antibiotics are becoming increasingly

ineffective as drug-resistance spreads globally leading to more difficulty in treating the infections and death. Ayurveda has a wide range of antimicrobial preparations which are yet to be explored. In the present study A comparative study on Prathama and Dwiteeya Sindhooradhya malahara W.S.R to its antimicrobial activity in order to prove their efficacy the preparations are tested on standard strains of microbes like *Staphylococcus aureus*, *Streptococcus pyogens*, *Candida albicans* and *Aspergillus niger*. Other objective were to compare the zone of inhibition by agar well diffusion method and to compare the Minimum Inhibitory Concentration (MIC) by macro broth dilution technique.

2. MATERIAL AND METHOD

2.1. Preparation of the test drugs: Prathama and Dwiteeya sindhooradhya malahara

The test drug samples of prathama and dwiteeya sindhooradhya malahara was prepared from Rasasala, Department of Rasasashtra and Bhaishajyakalpana, MVR Ayurveda Medical College, Parassinikkadavu, Kannur. The ingredients of both the malahara are listed below:

Table 1: Ingredients of prathama sindhooradhya malahara

Drug	English name	Quantity
Sikta taila	Beewax and sesame oil	3 karsha (36g)
Shudha tankana	Purified borax	½ karsha (6g)
Girisindhura	Red lead / red oxide of lead	½ karsha (6g)

In the same yoga, when Shudha tankana is replaced by sarjarasa (resin of *Shorea robusta*) it become dwiteeya sindhooradhya malahara. The ointments are prepared by mixing all the finely powdered ingredients in prepared sikta taila and triturating till it forms a homogenous mixture.

The prepared samples are tested against standard strains of *Staphylococcus aureus*, *Streptococcus pyogens*, *Candida albicans* and *Aspergillus niger* from reputed institute, Carekeralam, Thrissur.

2.2. Antimicrobial evaluation of prathama and dwiteeya sindhooradhya malahara

2.2.1. Agar well diffusion method [5, 6]

A loopful of bacterial culture was transferred from

working stock slants to 5ml of MHB/YEPD/PDB and incubated at 37°C/ 25°C till getting a visible turbidity equivalent to 0.5 MacFarland unit. MHA/MGYPA plates were prepared in sterile petridishes and allowed to solidify. a sterile swab was taken and dipped it into the broth culture. The swab was gently squeezed against the inner wall of the tube in order to remove excess fluid in the swab. A lawn of growth of the test organism was made by swabbing on the plate and allowed the plate to dry for 5 minutes.

PDA plates were prepared by pouring 25ml media inoculated with 0.1ml culture into sterile petri plate. Using sterile Cork Borer of 8mm diameter, wells on the swabbed agar plates were prepared. 100µl of sample concentrations were added to the well using micropipette. Kept solvent and standard drug control along with the sample. The plates were kept in the biosafety cabinet till complete diffusion of sample occurs and after that incubate the MHA plates at 37°C for 48 hours, MGYP plates at 25°C for 48 hours and PDA plates 25°C for 5 days. The plates were observed for zone of inhibition and the results were recorded.

2.2.2. Method: macro broth dilution [6]

A loopful of bacterial culture was transferred from working stock slants to 5ml of MHB/YEPD/PDB and incubated at 37°C/ 25°C till getting a visible turbidity equivalent to 0.5 MacFarland unit. Place sterile tubes in a rack and label them. 0.5ml of sterile broth (MHB/YEPD/PDB) was added to each tube. 0.5ml of the sample was added to the first tube and contents were mixed thoroughly and transferred its 0.5ml to the next tube. THE dilution process continued till the last tube and discarded 0.5ml from the last tube, so that the concentration of the sample was reduced by half in each dilution. Then 0.1 ml of relevant cultures having turbidity equivalent to McFarland 0.5 standard was inoculated. Kept blank (Broth and culture) and media control along with the samples. The rack was shaken gently to mix the tube contents and place the tubes in the incubator at 37°C/25°C for 24-48 hours/5 days. Each of the tube was examined for the presence or absence of turbidity to determine the MIC of the sample. MIC is the lowest concentration which inhibits the visible growth of the target organism.

2.3. Observations

Table 2: antimicrobial test result of agar well diffusion method of *Prathama sindhooradhyalahara*

Sl No	Test organism	Test Result (zone of inhibition in mm)		Test method
		Sample (50% in hexane)	Standard Drug	
1	<i>Staphylococcus aureus</i>	NZ, NZ	Streptomycin (1000ppm)-23,23	CKL/MB/MOA-044
2	<i>Streptococcus pyogenes</i>	14,14	Streptomycin (1000ppm)-23,23	
3	<i>Candida albicans</i>	16,16	Nystatin (1000ppm)-32,32	
4	<i>Aspergillus niger</i>	NZ, NZ	Nystatin (1000ppm)-18,17	

NZ- No Zone

Table 3: antimicrobial test result of *Dwiteeya Sindhooradhyalahara* by agar well diffusion method

Sl No.	Test organism	Test Result (Zone of inhibition in mm)		Test method
		Sample (50% in hexane)	Standard Drug	
1	<i>Staphylococcus aureus</i>	NZ, NZ	Streptomycin (1000ppm)-23,23	CKL/MB/MOA-044
2	<i>Streptococcus pyogenes</i>	NZ, NZ	Streptomycin (1000ppm)-23,23	
3	<i>Candida albicans</i>	NZ, NZ	Nystatin (1000ppm)-32,32	
4	<i>Aspergillus niger</i>	NZ, NZ	Nystatin (1000ppm)-17,17	

NZ-No Zone

Table 4: antimicrobial test result of *Prathama sindhooradhyalahara* by macro broth dilution method

Sl No	Test organism	Test result							Test method	
		Sample						Blank		Media control
		25%	12.5%	6.25%	3.12%	1.56%	0.78%			
1	<i>Staphylococcus aureus</i>	G	G	G	G	G	G	G	NG	CKL/M BMOA-047
2	<i>Streptococcus pyogenes</i>	NG	NG	NG	NG	NG	G	G	NG	
3	<i>Candida albicans</i>	NG	NG	G	G	G	G	G	NG	
4	<i>Aspergillus niger</i>	G	G	G	G	G	G	G	NG	

G- Growth, NG- No Growth

Table 5: antimicrobial test result of *Dwiteeya Sindhooradhyalahara* by macro broth dilution method

Sl No	Test organism	Test result							Test method	
		Sample						Blank		Media control
		25%	12.5%	6.25%	3.12%	1.56%	0.78%			
1	<i>Staphylococcus aureus</i>	G	G	G	G	G	G	G	NG	CKL/MB MOA-047
2	<i>Streptococcus pyogenes</i>	G	G	G	G	G	G	G	NG	
3	<i>Candida albicans</i>	G	G	G	G	G	G	G	NG	
4	<i>Aspergillus niger</i>	G	G	G	G	G	G	G	NG	

G- Growth, NG- No Growth

3. RESULTS

In agar well diffusion technique for *Prathama Sindhooradhyalahara*, no zone of inhibition is seen for *Staphylococcus aureus* (Fig.1) and *Aspergillus niger* (Fig. 2) whereas mild action is seen in case of *Streptococcus*

pyogenes (Fig. 3) and *Candida albicans* (Fig. 4). In a 50% sample prepared in hexane, *Streptococcus pyogenes* showed the zone of inhibition with a diameter of 14mm and that of the standard drug, Streptomycin showed 23 mm. Under similar conditions, *Candida albicans* produced a

zone of inhibition with 16mm diameter whereas the standard drug Nystatin produced 32mm diameter zone. When comparing with the standard drug the action of *Prathama Sindhooradhyah malahara* is mild.

Under the macro broth dilution technique for *Prathama Sindhooradhyah malahara*, Minimal Inhibitory Concentration (MIC) for *Streptococcus Pyogens* was found to be 1.56% and that of *Candida albicans* was 12.5% (Fig. 5, 6). Since there is no action in other two strains, MIC were not observed in *Staphylococcus aureus* and *Aspergillus niger* (Fig.7, 8).

A sample of *Dwiteeya Sindhooradhyah malahara* was also tested under similar circumstances and found no zone of inhibition in all the 4 strains (Figs. 9-12). Thus, when comparing both the *malahara* (ointments), *Prathama* variety has better action compared to the *Dwiteeya* variety.

Under the macro broth dilution technique for *Dwiteeya Sindhooradhyah malahara* no MIC of the sample was observed against all the tested pathogens (figs. 13-16). There was growth in all the 4 strains with different possible dilutions.

Images showing agar well diffusion method for various microbes tested in prathama sindhooradhyah malahara



Fig.1: *Staphylococcus aureus*

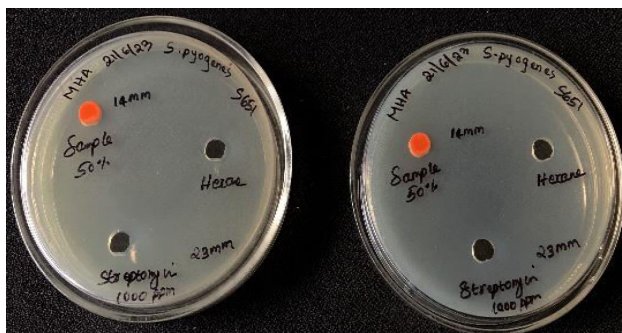


Fig. 3: *Streptococcus pyogenes*



Fig.2: *Aspergillus niger*



Fig. 4: *Candida albicans*

Images showing macro broth dilution test results of prathama sindhooradhyah malahara against various microbes.



Fig. 5: *Streptococcus pyogenes*



Fig. 6: *Candida albicans*

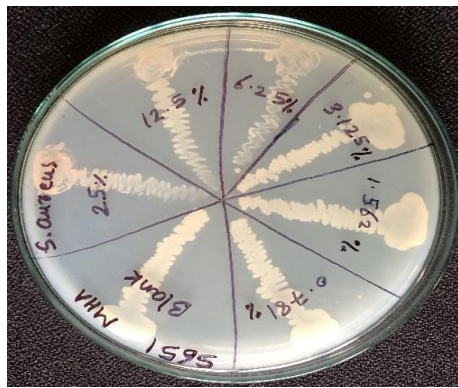


Fig. 7: *Staphylococcus aureus*

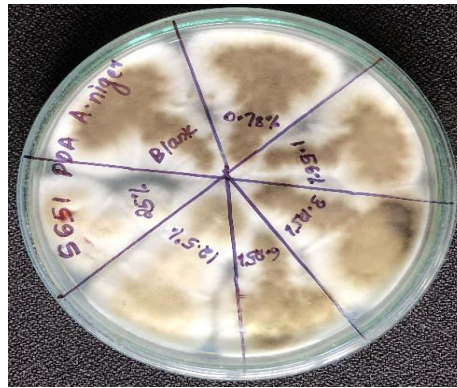


Fig. 8: *Aspergillus niger*

Images showing agar well diffusion method for various microbes tested in Dwiteeya sindhooradhy malahara



Fig. 9: *Aspergillus niger*



Fig. 10: *Candida albicans*



Fig. 11: *Staphylococcus aureus*



Fig. 12: *Streptococcus pyogenes*

Images showing macro broth dilution technique for dwiteeya sindhooradhy malahara against various microbes

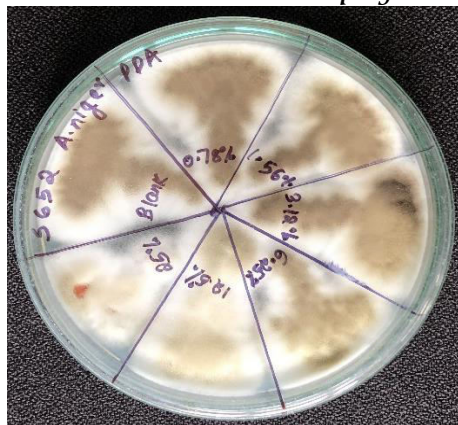


Fig. 13: *Aspergillus niger*

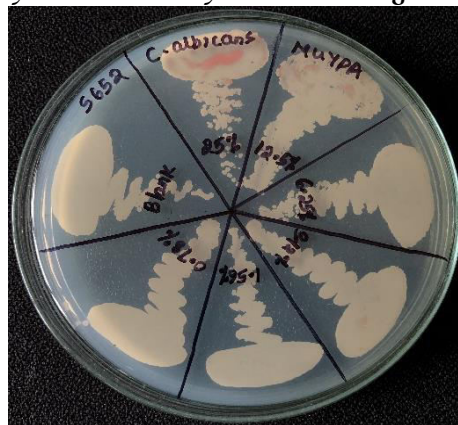


Fig. 14: *Candida albicans*

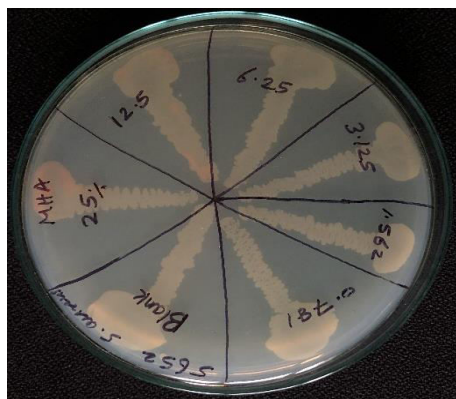


Fig.15: *Staphylococcus aureus*



Fig. 16: *Streptococcus pyogenes*

4. DISCUSSION

Antimicrobial study was carried out in two methods that is agar well diffusion and macro broth dilution techniques. Agar diffusion method involves the analysis of zone of inhibition around the antimicrobial agents on an agar plate. It is usually done to determine the susceptibility or resistance of bacteria to specific antibiotics. The circular areas in the agar plates showed the area where the bacterial growth is inhibited. These are the zones where bacteria or microorganisms were unable to grow due to the antibiotic action of the applied preparation. No zone in the report showed that the bacteria tested are resistant to the antibiotic and it did not have any effect in preventing their zone.

Macro broth dilution test is carried out to determine the Minimum Inhibitory Concentration (MIC) of an antimicrobial agent against a specific microorganism. MIC is the lowest concentration of the antimicrobial agent that prevents visible growth of the bacteria after a defined incubation period. If there is visible growth in the liquid medium at a particular concentration of the antimicrobial agent, it suggests that the concentration of the antibiotic is not sufficient to inhibit the growth of the specified microorganism and thus higher concentration is required. So, MIC is the highest dilution or concentration at which no growth is observed. Macro broth dilution test help in choosing the most appropriate antibiotic and its proper dosing. An antibiotic with a low MIC against a specific pathogen is considered more effective, as it can achieve bacterial inhibition at lower concentrations which reduce the risk of antibiotic resistance and improving better treatment outcomes.

The selection of microbes is based on the rationale that most of the organisms causing skin infections are bacteria, fungi and viruses. Certain parasites like lice and mites also cause certain skin issues. But in our

geographical area the major causative microbes are bacteria and fungi. So two strains of bacteria and fungi are selected for the study. Due to safety constraints and technical challenges viral strains were not chosen in this study. The common bacterial skin pathogens are *Staphylococcus aureus* and *Streptococcus pyogenes*. Thus they are selected for this study. In the fungi group *Candida albicans* and *Aspergillus niger* are the common causative agents.

The results of the studies give an insight into the antimicrobial action of the formulation in these pathogens. When both the samples are compared *Prathama Sindhooradhya malahara* showed better antimicrobial action to *Dwiteeya Sindhooradhya malahara*. This may be because of the presence of *tankana* in *Prathama Sindhooradhya malahara*. The antimicrobial action of *tankana* (*borax*) is already proven through various research works carried out in the field. The ayurvedic term *krimighna* or *bhootasangaprasamana* is a boundless concept and cannot be limited to the term antimicrobial. The concept of *bhuta* or extraordinary powers is beyond our knowledge perception. A study conducted in the Stanford University accentuates the fact that more than 99% of the microbes inside us are unknown to science. The Survey of DNA fragments circulating in the blood suggests the microbes living within us are vastly more diverse than previously known. Thus, these *malaharas* might have action in a wider sense or cannot be confirmed merely by studying four strains of microbes.

5. CONCLUSION

Antimicrobial study by both agar well diffusion and macro broth dilution showed that more action in *Prathama Sindhooradhya malahara*. Among the 4 tested strains, significant results are seen in case of *Streptococcus pyogenes* and *Candida albicans*.

Dwiteeya Sindhooradhya malahara had no action in the tested strains of microbes.

Further more detailed study with large spectrum of microbes is necessary to confirm the antimicrobial action. This is a preliminary study and it might pave way to further detailed animal and clinical trials to know the exact action and result.

The same study can be carried out by increasing the microbial load, as it is impossible to conclude the antimicrobial action just by analyzing four strains. To be more specific further clinical trial is a necessary. Since *Sindhooradhya malahara* is a formulation which is widely in use, clinical study result will serve as an evidence-based result. Shelf-life study is another area which is least explored, so it can also be conducted and compared between the two malahara.

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Conflicts of interest

There is no conflict of interest

Source of funding

None declared

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