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Research Article

ANTI BACTERIAL AND ANTI FUNGAL ACTIVITY OF SHODHITA MANASHILA PREPARED BY AGASHTHYA PATRA SWARASA

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Abstract

Physicians are unable to treat various bacterial and fungal infections appropriately due to hindrances like development of resistance, adverse effects, patient affordability etc. Suddha Manashila (purified realgar) mentioned in Ayurvedic Texts can be an ideal replacement for treating various infectious diseases. Assessment of its antibacterial and antifungal activity may provide scientific evidence for the study. Manashila purified by Agashthya patra swarasa (Leaf juice of *Sesbania grandiflora*) according to the classical reference was subjected to antibacterial and antifungal activity by cup plate method. Different concentration tested against bacteria like *Staphylococcus aureus*, *Pseudomonas auregenosa* and *E. coli* and fungi *Aspergillus niger*, *Cryptococcus neoformans*, *Candida albicans* and *Trycophytum rubrum*. Fluconazole and Benzathine Pencillin were taken as a standard for comparison. Suddha manashila (purified realgar) solutions in different concentrations showed a significant zone of inhibition against three strains of bacteria (16-28 mm) and four strains of fungi (14-27mm) when compared to Fluconazole (22 mm), Benzathine Pencillin (28 mm) & control.

Key words: Manashila; Bhavana; Benzathine Pencillin; Fluconazole; *Sesbania grandiflora*.

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INTRODUCTION

Disease and death have always attracted the attention of human mind. Ancient humans ascribed them to divine wrath and other supernatural forces. Invention of microscope exposed the world of micro-organisms to humans.[1] Out of these microorganisms; pathogenic organisms are the root cause of majority of diseases in human beings. Now a day's number of antimicrobial agents are discovered which are effective in various infections. Even though, physicians face many hindrances while treating such infections.[2] Emergence of Resistance to the conventional antimicrobial is a serious problem.[3] Other than this adverse effects and patient affordability are also difficult tasks for a physician.[4] The scenario is further made complicated by infections like HIV where most immunity itself suppressed. These all difficulties create a need for constant development of newer antimicrobial agents which are safe, cost effective to inhibit growth or kill organisms. Manashila (realgar) is one of the mineral drug used for krimi roga (infectious disease) both external as well as in internal administration.[5][6] Manashila[7] after shodhana (purification), mainly cures diseases like krimi (infectious disease), kushta (skin disease), kasa (cough), swasa (breathing problem) etc and has got wide range disease curing capacity which is a positive thing for us in today's era.[8][9][10]

MATERIAL AND METHODS

To evaluate the antibacterial and antifungal activity of Suddha Manashila (purified realgar) the following material were used.

Drugs

Suddha Manashila (Purified realgar), Fluconazole and Benzathine penicillin are used as trial drugs.

Micro organisms

Bacteria

The bacteria like *Staphylococcus aureus*, *Pseudomonas auregenosa*, *Escheria coli*, *Aspergillus niger* are used for this study.

Fungi

The fungi like *Candida albicans*, *Cryptococcus neoformans*, *Trycophytum rubrum* are used for this study.

Chemicals & solvents

The chemical and solvents used for this study are Nutrient broth, Nutrient agar, Surgical spirit, Potassium Hydroxide, Sodium Hydroxide and Distilled water

Equipments and Glasswares

Equipments like Water bath, Loops and loop holder, Loops and loop holder, Borer, Hot air oven, Inoculation hood, Autoclave and Incubator; the glassware like Petri dish, Conical flask, Test tubes, Beakers, Funnel and Stirrer were used for this study.

Pharamaceutical study

Raw manashila procured from Dorle & sons, Kohlapur, Maharashtra. Shodhana (Purification) procedures were carried out in AVS Ayurveda Mahavidhyalaya, Bijapur, Karnataka, India.

Preparation of Shodhita manashila

Manashila were purified by bhavana (Trituration) method with the media of Agashtya patra swarasa.

Agasthya patra swarasa was obtained by squeezing method. For that fresh Agasthya patra (*Sesbania grandiflora* leaves) were collected from the herbal garden of AVS

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Ayurveda Mahavidhyalaya, Bijapur, Karnataka, India. The leaves were properly cleaned and good leaves were selected. Then the leaves made into kalka (paste) by pounding in khalwa yantra. Kalka (paste) is squeezed through white cotton cloth which is then filtered in percolator and swarasa (Juice) is collected. Dark green coloured juice obtained.

Shodhana of manashila

Procedure: Raw manashila(Realgar) powdered and sufficient quantity of Agasthya patra swarasa added till samyak plutha (Completely immersed). Bhavana (Trituraion) done till it gets dried. It completes one bhavana (Trituration). This procedure is done for 7 times.

Method of Antibacterial and antifungal activity

Antibacterial and antifungal activity was carried by cup-plate method[11] at AVS PGCRC, Bijapur .

Solubility Test:

To assess the percentage of solubility of Suddha Manashila in different solvents for carrying out antimicrobial activity.

Solubility test of Suddha Manashila was carried out in different media. They solvents are Distilled water, Chloroform, Xylene, Toluene, Methanol, Ethanol, Carbon tetrachloride, Acetone, Benzene, Acetic acid glacial, 10% KOH, 10% NaOH, 6 NHCl, 5% NH_2SO_4.

Procedure

A pinch of shuddha Manashila (purified) was added to test tube containing 1 ml of each solvent and shaken well. It was allowed to settle and solubility noted and graded as soluble, insoluble and sparingly soluble. This procedure was done separately for different solvent. The results are shown in the Table 1.

Percentage of Solubility

Procedure

5 g of sample + 100 ml of solvent were stirred well. It was kept for 24 hours and filtered through whatman filter paper no. 42. The filter paper along with residue was dried and weighed. Percentage of solubility was calculated as per the standard formula.

Initial weight of filter paper = F_1
Weight of filter paper with residue = F_2
Total insoluble matter = $F_3 = F_2 - F_1$
Percentage of insoluble matter = F3 x 100 /5
Percentage of soluble matter = 100 - % of insoluble matter. The results are shown in Table 2.

EXPERIMENTAL STUDY

Identification of cultures was done under microscopic examination

Methods

A. Preparation of solution

a) Preparation of control solution

100 ml of Distilled Water + 10 gm KOH pellets was mixed together to prepared control solution

b) Preparation of test solution

5 g samples of Suddha Manashila was added to 50ml of control solution, stirred well and filtered through Whatman filter paper no 42. Filtrate solution of Suddha Manashila was taken as 100% solution.1ml of 100% solution of Shodhitamanashila was added to 9 ml control → 10% solution. 2ml of 100% solution of Shodhita manashila was added to 8ml of control → 20% solution.

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Table 1: Solubility test of Shuddha manashila

Solvents	SM	Solvents	SM
Distilled water	S.S	Acetone	I.S
Chloroform	S.S	Benzene	I.S
Xylene	S.S	Glacial Acetic acid	S.S
Toluene	I.S	10%KOH	S.S
Methanol	S.S	10%NaOH	S.S
Ethanol	S.S	6NHCL	S.S
CCl_4	S.S	5%H2SO4	S.S

I.S- Insoluble S.S.- Sparingly soluble S- Soluble SM- Shodhita manashila

Table 2: Percentage of solubility

Solvents	SM
D.W	25%
10 % NaOH	43.6 %
10 % KOH	76%

SM- Shodhita manashila D.W-Distilled Water

Table 3: Zone of inhibition (in mm) in the Antimicrobial sensitivity test of SM

Antimicrobial sensitivity testing of 100%, 20%, 10% SM solution							
Test drugs	**Test Organisms and it zone of inhibition in mm**						
	S.A	**P.A**	**E.C**	**T.R**	**A.N**	**C.N**	**C.A**
I. 100% Solution	24	28	24	20	24	24	20
II 20% solution	20	22	22	18	20	22	18
III. 10% solution	18	20	20	16	19	20	17

SM- *Shodhita manashila*; S.A- *Staphylococcus aureus*; P.A- *Pseudomonas auregenosa*; E.C- *Escherichia coli*; T.R- *Trycophytum rubrum*; A.N- *Aspergillus niger*; C.N- *Cryptococcus neoformans*; C.A- *Candida albicans*; MM-millimeter

Table 4: Zone of inhibition (in mm) in the Antimicrobial sensitivity test of SM

Antimicrobial sensitivity testing of Control and Standard solution							
Test drugs	**Test Organism & it zone of inhibition in mm**						
	S.A	**P.A**	**E.C**	**T.R**	**A.N**	**C.N**	**C.A**
I. Control Solution (10% KOH)	16	12	10	10	16	12	16
II Fluconazole solution	--	--	--	20	22	22	20
III. Benzathine Penicillin solution	28	28	27	--	--	--	--

SM- Shodhita manashila; S.A- *Staphylococcus aureus*; P.A- *Pseudomonas auregenosa*; E.C- *Escherichia coli*; T.R- *Trycophytum rubrum*; A.N- *Aspergillus niger*; C.N- *Cryptococcus neoformans*; C.A- *Candida albicans*; MM-millimeter.

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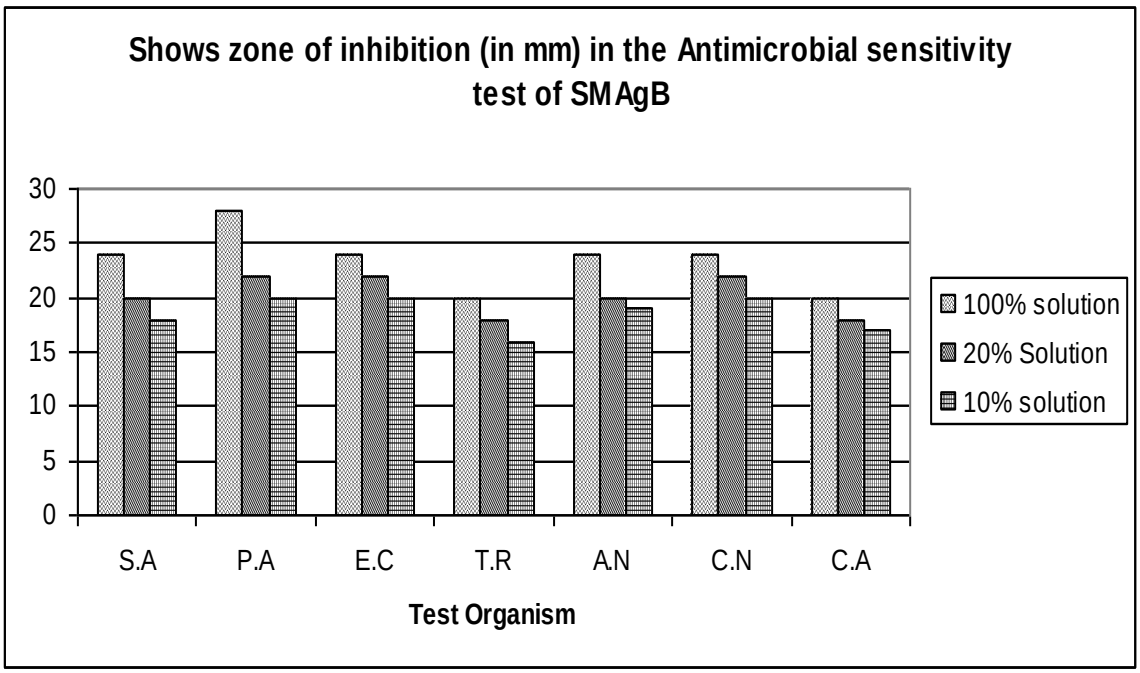
Graph 1: Zone of inhibition (in mm) in the Antimicrobial sensitivity test of Shodhita manashila



SMAgB- Shodhita manashila by Agasthya patra swarasa; S.A- *Staphylococcus aureus*; P.A-*Pseudomonas auregenosa*; E.C- *Escherichia coli*; T.R- *Trycophytum rubrum*; A.N- *Aspergillus niger*; C.N- *Cryptococcus neoformans*; C.A- *Candida albicans*; MM-millimeter.

c) Preparation of standard solution

1. 250 mg of Benzathine penicillin powder was dissolved in 100 ml distilled water used as standard drug for antibacterial activity.

2. 100 mg Fluconazole tablet was dissolved in 100ml distilled water and used as standard drug for antifungal activity.

B. Preparation of Growth Media

Nutrient broth was used for the preparation of growth media. Nutrient broth 13 g was dissolved in 1000 ml of distilled water, boiled for 15min and allowed to cool. 100ml was then transferred to each conical flask and sterilized in autoclave at 15 lbs pressure (i.e. 121^0C) for 20min.

C. Preparation of inoculums

A loopful of the organisms was emulsified in 100ml sterile growth media under proper sterile conditions and incubated for 72 hrs at 37^0c in incubator.

D. Preparation of Agar Media

Nutrient agar was used for the preparation of agar media .Nutrient agar 28g was dissolved in 1000ml of distilled water. It was boiled for 20min and allowed to cool. Then it was sterilized in autoclave at 15lbs pressure for 20min.

E. Preparation of Agar plates:

5ml of inoculums prepared was added to 45ml of flask containing nutrient agar at 37^0 C. This was immediately poured into a dry sterile petridish to a depth of 5mm.

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Figure 1: Pharmaceuticla study

Figure 2: Experimental study

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The petridishes were placed on a leveled surface to ensure that the layers of medium are of uniform thickness. Allow the plates to solidify at room temperature for 12hrs. Incubated some plates at 35^0C to check sterility. The surface of the agar layer was kept dry before use. With the help of sterile borer (diameter 8mm) cylinders were made in agar plates. A uniform volume (i.e 0.5ml) of test solutions 3 different samples of Suddha manashila and standard drug Fluconazole and Benzathine Penicillin were added to each cavity, sufficient enough to fill the holes. After 30min agar plates were incubated at 37^0c for 72hrs. Zone of inhibition was measured after 72hrs using mm scale. The diameter of the circular zone is the measurement of the zone of inhibition.

Interpretation of Results

A) In General

Results were interpreted by measuring the zone of inhibition shown by samples on test organisms.

a. Sensitive (S) Zone – Diameter wider than 8mm.
b. Intermediate (I) Zone – Diameter between 6mm to 8mm.
c. Resistant (R) Zone – No zone of inhibition or diameter less than 6mm.

B) With control Group

According to the zone size of the control group 3 categories of sensitivity of test strain can be interpreted.

a. Sensitive Zone – The zone size of the test strain measured as described above should be equal to or more than 3mm than that of control strain.
b. b) Intermediate Zone – The zone size of the test strain measured is equal to control strain or within 3mm range of control strain.
c. Resistant Zone – The zone of test strain is smaller than control strain.

Manashila was purified by agasthya patra swarasa according to the classical reference mentioned in rasa tharangini.[12][13][14] Shodhita Manashila was undertaken for antibacterial and antifungal study and tested against bacteria like *Staphylococcus aureus*, *Pseudomonas auregenosa* and *E. coli* and fungi like *Trycophytum rubrum*, *Aspergillus niger*, *Cryptococcus neoformans* and *Candida albicans*. The purified Manashila was subjected for solubility test in different solvents.[15] The sample of manashila was sparingly soluble in KOH, NaOH, Distilled water, CCl_4, Glacial acetic acid. 10% KOH taken as a solvent for this study. 10%, 20% and 100% solutions were prepared from shodhita manashila.[16] Then each samples were subjected to antibacterial and antifungal activity in comparison with control and standard drug Fluconazole and Benzathine Penicillin.[17][18][19]

OBSERVATON AND RESULTS

Shodhita Manashila had shown sensitivity towards all organisms. In bacteria, 0.5ml of 10% Shodhita manashila solution showed 18 mm of zone of inhibition against *Staphylococcus aureus*, 20 mm against *Pseudomonas auregenosa* and 20 mm against *E. coli*. In fungi, it has shown 16 mm of zone of inhibition against *T. rubrum*, 19 mm against *A. niger*, 20 mm against *C. neoformans* and 17 mm against *C. albicans*. In bacteria, 0.5ml of 20% Shodhita manashila solution showed 20 mm of zone of inhibition against *Staphylococcus aureus*, 22 mm against *Pseudomonas auregenosa* and 22 mm against *E. coli*. In fungi, it has shown 18 mm of zone of inhibition against *T. rubrum*, 20 mm against *A. niger*, 22 mm against *C. neoformans* and 18 mm against *Candida albicans*. In bacteria, 0.5ml of 100% Shodhita manashila solution showed 24 mm of zone of inhibition against *Staphylococcus aureus*, 28 mm against

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Pseudomonas auregenosa and 24 mm against *E. coli*. In fungi, it has shown 20 mm of zone of inhibition against *T. rubrum*, 24 mm against *A. niger*, 24 mm against *C. neoformans* and 20 mm against *C. albicans*. Standard drug, Fluconazole was sensitive to all fungal organisms and highly sensitive against *Aspergillus niger* and *Cryptococcus neoformans*, where zones of inhibition were 22 mm where as 20 mm against *T. rubrum* and *C. albicans*. 0.5ml of control solution of 10% KOH showed 16 mm of zone of inhibition against *Staphylococcus aureus*, 12 mm against *Pseudomonas auregenosa* and 10 mm against *E. coli* among bacteria. In fungi it has shown 10 mm of zone of inhibition against *T. rubrum*, 16 mm against *A. niger*, 12 mm against *C. neoformans* and 16 mm against *Candida albicans*. The results are shown in the Table 3.

DISCUSSION

All three samples of different concentrations of shoditha Manashila had shown sensitivity towards all organisms. 10% solution of shodhia manashila has shown high sensitivity towards *Plasmodium auregenosa* and *E. coli* of 20 mm of zone of inhibition. Among fungi it has shown high sensitivity towards *Cryptococcus neoformans* of 20 mm of zone of inhibition. 20% solution of Shodhita manashila has shown high sensitivity towards *Plasmodium auregenosa* and *E. coli* of 22 mm of zone of inhibition. Among fungi it has shown high sensitivity towards *Cryptococcus neoformans* of 22 mm of zone of inhibition.100% solution of shodhita manashila has shown high sensitivity towards *Plasmodium auregenosa* of 28 mm of zone of inhibition. Among fungi it has shown high sensitivity towards *Cryptococcus neoformans* and *Aspergillus niger* of 24 mm of zone of inhibition. Different concentrations of shoditha manashila zones of inhibition were significant in comparison to control. Sample Shodhita manashila had shown sensitive against all organisms this may be due to the presence of elements like Arsenic and Sulphur which might have contributed to antibacterial and antifungal activity. Agashthya patra swarasa is used for bhavana of Manashila which also contributes to the antibacterial and Anti-fungal activity. Manashila due to it katu tikta rasa (Pungent and bitter in taste), ushna veerya (hot in potency), lekhanahara (scrapping) properties destroys kledamsa (moisture) in the body which is responsible for growth of micro-organisms. It is one type of Prakruthi vighatha Chikitsa (opposite of nature). As already been described shodhita manashila consists of arsenic and Sulphur along with other herbal ingredients. Sulphur has been associated as an important constituent in Sulphonamides, which are used as antimicrobial agents. These groups of drugs have been proved to act by inhibiting Folic acid metabolism in the susceptible bacteria and preventing their growth. Arsenic and Sulphur has been detoxified with ancient process mentioned in Rasashastra text, so that the irritant and toxic effect of Arsenic and Sulphur is reduced. At the same time Bhavana dravya agasthyapatra have attributed additional therapeutic properties and proved to have antimicrobial activity. Phyto-chemicals like steroids and tannins were present in shodhita manashila plays vital role in wound healing. Tannins are astringent cardio tonic and also useful in skin eruptions boils and diarrhea. Steroids regulate carbohydrate and protein metabolism and possess strong anti-inflammatory action. They also influence the electrolyte and water balance of the body.

CONCLUSION

All three samples of different concentrations of shoditha Manashila had shown sensitivity towards all organisms. Benzathine Penicillin and Fluconazole act only as antimicrobial agents and may produce adverse effects on human beings but shodhita manashila not only act as antimicrobial agent but have additional properties like rejuvenation and promotes positive health and vigor by increasing the

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immunity, thus making the body resistant against disease causing factors. Shodhita manashila has demonstrated significant antibacterial and antifungal activity, which gives further scope for experimental and clinical study on various microorganisms.

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