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Antibacterial and Antifungal activity of *ShodhitaManashila* Prepared by *BijapuraSwarasa*

B.Saravanan*

*Srijayendrasaraswathi Ayurveda College, Chennai, Tamilnadu, India

Abstract

Today physicians are facing problem to treat various type of infection, especially bacterial and fungal infections. The main reason for the problem is drug resistance, adverse reactions and patient affordability. *SuddhaManashila* (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 *bijapuraswarasa*(citrus medica)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 Aspergillusniger, Cryptococcus neoformans, Candida albicans and Trycophytumrubrum. Fluconazole and BenzathinePencillin were taken as a standard drug for comparison. *Suddhamanashila*(purified realgar) solutions in different concentrations showed a significant zone of inhibition against three strains of bacteria (16-27 mm) and four strains of fungi(14-22mm) when compared to Fluconazole (22 mm),BenzathinePencillin (28 mm) &control.

Keywords

Manashila, Bhavana, BenzathinePencillin, Fluconazole

Greentree Group

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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. Development of science and technology explained the theory of micro-organisms to the world[1].Microorganisms were the etiological factors of major health disorder in human being. Presently more number of antimicrobial agents was discovered to treat various types of infection[2].Even though, physicians were facing problem to treat various type of infection, due to that drug resistance and adverse reaction. Drug resistance of antimicrobial agent is a serious problem for physician.[3]Apart from that patient affordability also is a difficult task for physician[4].The scenario is further made complicated by infections like HIV where most immunity itself suppressed. Due to these all difficulties found a need to develop new antimicrobial agents which are safe, cost effective to inhibit growth or kill organisms.*Manashila*(realgar) is one of the mineral drug used *for krimiroga*(infectious disease)both external as well as in internal administration.[5,6]*Manashila*[7]after *shodhana*(purification), mainly cures diseases like *krimi*(infectious disease)[8], *kushta(*skin disease)[9], *kasa*(cough)*[10]*, *swasa*(breathing problem)[11]etc and has got wide range disease curing capacity which is a positive thing for us in today's .

MATERIALS AND METHODS

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

Materials:

I Drug:

1. *SuddhaManashila*(Purified realgar)- Purified *manashila* taken as atest drug
2. Fluconazole - Standard drug for fungal organism
3. Benzathine penicillin - Standard drug for bacterial organism
4. 10%koh solution - Control drug for both bacterial and fungal organism

2. Micro organisms

Selective bacterial and fungal organism's like Staphylococcus aureus, Pseudomonas auregenosa, Escheria coli and Candida albicans, Cryptococcus neoformans, Trycophytumrubrum, Aspergillusniger, are taken for this study.

Methods:

Pharmaceutical Study

Preparation of Shodhitamanashila- *Bjapuraswarasabhavana*(Trituration).

Preparation of *bijapuraswarasa* (citrus medica juice)

Principle: Squeezing

Ingredients: *bijapura*.(citrus medica)

Preparation of *Bijapuraswarasa*:

Principle: squeezing

Ingredients: *Bijapura*

Equipment: Squeezer, filter, measuring glass.

Procedure:

Properly cleaned *bijapura* was cut into two halves and squeezed. Seeds are removed by filtering.

Observation: Very light yellow coloured juice obtained

Shodhana of manashila:

Principle: *Bhavana*(Trituration)- 7 times

Ingredients: Raw *manashila-*, *Bijapuraswarasa*q.s.

Equipments: *Khalwayantra*

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

Antibacterial and antifungal activity was carried by cup-plate method.

Solubility Test:

To assess the percentage of solubility of *SuddhaManashila* in different solvents for carrying out antimicrobial activity.

Solubility test of *SuddhaManashila* was carried out in following solvents

1) Distilled water 2) Chloroform

3) Xylene 4) Toluene

5) Methanol 6) Ethanol

7) Carbon tetrachloride 8) Acetone

9) Benzene 10) Acetic acid glacial

11) 10% KOH 12) 10% NaOH

13) 6 N HCl 14) 5% NH_2SO_4

Procedure: A pinch of *ShuddhaManashila* 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 no. 1

Table 1 Shows Solubility test of Shuddhamanashila

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-**Shodhitamanashila

Percentage of Solubility:

Procedure: Five gms of sample was stirred with 100 ml of solvent, then 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 no 2

Table 2 Shows percentage of solubility

Solvents	SM
Distilled water	25%
10%NaOH	43.6%
10%KOH	76%

SM- Shodhitamanashila

EXPERIMENTAL STUDY

Identification of cultures was done under microscopic examination

Methods:

A. Preparation of solution:

a) Preparation of control solution

Standard solution prepared by mixing of 100 ml of DistilledWater and 10 gm KOH.

b) Preparation of test solution

SuddhaManashila 5gm was added to 50ml of control solution, stirred well and filtered by What man filter paper no 42. Filtrate solution was taken as 100% solution.10% solution was prepared by mixing of 1ml of 100% solution and 9 ml control solution. .20% solution was prepared by mixing of 2ml of 100% solution and 9 ml control solution.

c) Preparation of standard solution

1. Benzathine penicillin 50 mg was taken as a standard drug for bacteria. Standard solution was prepared by mixing Benzathine penicillin 250 mg and100 ml distilled water.

2. Fluconazole was taken as a standard antifungal drug. Standard solution prepared by mixing of Fluconazole100 mg and100 ml distilled water.

B. Preparation of Growth Media:

Nutrient broth was used for the preparation of growth media .Nutrient broth 13 gm was dissolved in 1000ml 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:

Micro organisms were 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 28gm 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:

Prepared inoculums 0.5ml 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.The petridish 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. Test solution 0.5ml of 3 different concentrations of Suddhamanashila and standard drug Fluconazole and Benzathine Penicillin were added to each cavity of agar plate. Then these agar plates were incubated at 37^0c for 72hrs after 30 minutes. Zone of inhibition was measured by using mm scale. The diameter of the circular zone is considered zone of inhibition of particular micro organism.

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) 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 *bijapuraswarasa* according to the classical reference mentioned in *rasatharangini*[12,13,14]*shodhitaManashila* had

tested against selective bacterial and fungal organisms.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.*Shodhitamanashila* different concentration like 10%, 20% and 100% solutions were prepared[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

Different concentrations of *ShodhitaManashila* had shown sensitive zone of inhibition towards bacterial and fungal microorganisms.In bacterial micro-organism, 10% concentration of *Shodhitamanashila* showed 16 mm of zone of inhibition against Staphylococcus aureus, 22 mm zone of inhibition against Pseudomonas auregenosa and 18 mm zone of inhibition against E.coli. In fungi, 14mm of zone of inhibition against T.rubrum, 18mm zone of inhibition against A.niger and C.neoformans and 16mm zone of inhibition against C.albicans.*Shodhitamanashila* in a 20% concentration solution showed 18 mm of zone of inhibition against Staphylococcus aureus, 24 mm zone of inhibition against Pseudomonas auregenosa and 20 mm zone of inhibition against E.coli. In fungi, it showed 16mm of zone of inhibition against T.rubrum, 20 mm zone of inhibition against A.niger and C.neoformans, 18 mm zone of inhibition against Candida albicans .*Shodhitamanashila* in a 100% solution shown 27 mm zone of inhibition against Pseudomonas auregenosa, 26 mm zone of inhibition against E.coli and staphylococcus aureus. In fungi, it showed 22 mm zone of inhibition against A.niger and C.neoformans, 20 mm zone of inhibition against C.albicans and 18 mm of zone of inhibition against T.rubrum.Benzathine Penicillin standard drug for bacterial organism taken for this study shown sensitive zone of inhibition towards all bacterial organisms, high sensitive zone of inhibition 28mm noted against Staphylococcus aureus and Pseudomonas auregenosa, and 27 mm zone of inhibition against E. coli was also noted. Fluconazole standard drug for fungal organism taken for this study shown sensitive zone of inhibition towards all fungal organisms , high sensitive zone of inhibition 22mm

noted against Aspergillus Niger and Cryptococcus Neoformans, and 20 mm zone of inhibition noted against T.rubrum and C.albicans. Control solution taken for these study 10% KOH shown 16 mm zone of inhibition against Staphylococcus Aureus, 12 mm zone of inhibition against Pseudomonas auregenosa and 10 mm zone of inhibition against E.coli among bacteria.In fungi it shown 16 mm of zone of inhibition against A.niger and Candida albicans , 12 mm zone of inhibition against C.neoformans and 10 mm of zone of inhibition againstT.rubrum . The results are shown in the table no3.

SM- Shodhitamanashila

Table 3 Shows zone of inhibition (in mm) in the Antimicrobial sensitivity test of SM

TEST DRUGS	TEST ORGANISM						
	S.A	P.A	E.C	T.R	A.N	C.N	C.A
100% SOLUTION	26	27	26	18	22	22	20
20% SOLUTION	18	24	20	16	20	20	18
10% SOLUTION	16	22	18	14	18	18	16

SM- Shodhitamanashila S.A-Staphylococcus aureus
P.A- Pseudomonas auregenosa E.C- Escherichia coli
T.R-Trycophytumrubrum A.N- Aspergillusniger
C.N-Cryptococcus neoformans C.A- Candida albicans MM-millimeter

Table 4 Shows zone of inhibition (in mm) in the Antimicrobial sensitivity test of SM

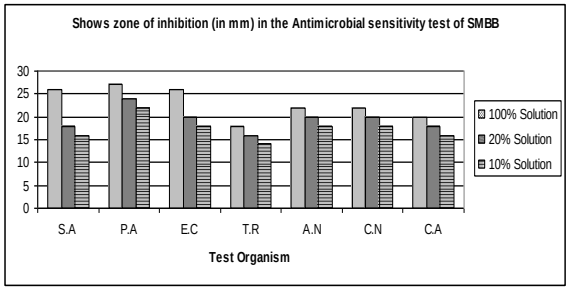
TEST DRUGS	TEST ORGANISM						
	S.A	P.A	E.C	T.R	A.N	C.N	C.A
CONTROL SOLUTION	16	12	10	10	16	12	16
B.PENICILLIN SOLUTION	28	28	27	-	-	-	-
FLUCONAZOLE SOLUTION	-	-	-	20	22	22	20

SM- Shodhitamanashila S.A-Staphylococcus aureus
P.A- Pseudomonas auregenosa E.C- Escherichia coli
T.R-Trycophytumrubrum A.N- Aspergillusniger
C.N-Cryptococcus neoformans C.A- Candida albicans MM-millimeter

RESULTS AND DISCUSSION

Three different samples of *shodithaManashila* showed sensitivity against all bacterial and fungal organisms.

Bar diagram Shows zone of inhibition (in mm) in the Antimicrobial sensitivity test of SM



SMBB -

Shodhitamanashilabijapuraswarasabhavitha

S.A-Staphylococcus aureus P.A-Pseudomonas auregenosa

E.C- Escherichia coli T.R-Trycophytumrubrum

A.N- Aspergillusniger C.N-Cryptococcus neoformans

C.A- Candida albicans MM-millimeter

Shodhitamanashila in a 10% concentration showed high sensitivity towards Plasmodium Auregenosa, zone of inhibition of 22mm noted against this bacterial microorganism. In fungi it showed high sensitivity towardsAspergillus Niger and Cryptococcus neoformans where zone of inhibition of 18mm.*ShodhitaManashila* in a 20% concentration showed high sensitivity towards Plasmodium Auregenosa, 24 mm of zone of inhibition noted against these bacterial organism. Among fungi it shown high sensitivity towardsAspergillus Niger and Cryptococcus neoformans, 22 mm of zone of inhibition was noted against this fungal. *ShodhitaManashila* in a 100% concentration showed high sensitivity towards Plasmodium Auregenosa zone of inhibition of 27mm noted against these bacterial organisms. Among fungi it shown high sensitivity towards Aspergillusniger and Cryptococcus neoformans zone of inhibition were noted 22mm against these fungal organism. For three different concentrations of *shodithamanashila* significant zones of inhibition were obtained against bacterial and fungal organism compared to control group. *Shodhitamanashila* had shown sensitive zone of inhibition against all bacterial and fungal organisms due to the presence of organic and inorganic elements. *Manashila* has elements like Arsenic and Sulphur which might have been contributed for this antibacterial and antifungal activity. *Bhavana*dravya used for this study *bijapuraswarasa* also contributes for this antibacterial and Anti-fungal activity. *Manashila* had properties like *katutiktarasa*, *ushnaveerya*, lekhanahara. These properties restrict the growth of micro-organisms in the human body.*Shodhitamanashila* consists of arsenic and Sulphur along with other herbal ingredients which also helps to restrict the growth of micro organisms. Sulphur has 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 *Bhavanadravyabijapura* have attributed additional therapeutic properties and proved to have antimicrobial activity. Phyto-chemicals like steroids and tannins were present in *shodhitamanashila* 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 different concentration of *ShodhitaManashila* samples shown sensitive shown of inhibition towards all bacterial and fungal organisms.Standard drug BenzathinePenicillin and Fluconazole showed sensitive zone of inhibition against all bacterial and fungal organism. Standard drug taken for this study may produce adverse effects towards humans but *ShodhitaManashila* will not produce any adverse effect to the humans, instead ~~of that~~ it will improve the human health by its additional property of *rasayana* etc. This experimental study provides the scientific data for antimicrobial property of *shodhitamanashila* prepared by *bijapuraswarasa*..

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