



Anti-Microbial Study of Shwasakuthar Rasa: In Vitro Study

Research article

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Abstract

Shwasa kuthar Rasa is well known drug used in Respiratory disorders in Indian System of Medicine. It is herbomineral formulation made up of *Parada, Gandhaka, Vatsanabha, Tankana, Manashila, Maricha, Pippali, Sunthi* by triturating in *Tambula patra Swarasa* and indicated in *Shwasa, Kasa, Mandagni*, disorders prominent in *Vata-Shleshma doshas*. These action of *Shwas kuthar rasa* may be due to its antimicrobial activity, so that in vitro antimicrobial activity was assessed by Agar Disc Diffusion method with reference to *staphylococcus aureus*. *In-vitro* tests are necessary to help determine initial dose-response data as well as to evaluate potential susceptibility and/or resistance of specific pathogens. *In-vitro* systems include the ability to control the number of bacteria, extent of antimicrobial-bacteria contact time, as well as the influence of various environmental factors, such as oxygen tension, pH, and temperature. Agar dilution methods are to determine the lowest concentration of the assayed *Shwas kuthar rasa* as antimicrobial agent (minimal inhibitory concentration, MIC) that, under defined test conditions, inhibits the visible growth of the bacterium being investigated. MIC values are used to determine susceptibilities of bacteria to drugs and also to evaluate the activity of new antimicrobial agents.

Key words: *Shwas kuthar rasa*, Minimal inhibitory concentration

Introduction

“Health for all by the year 2000” is both a goal and a process which engages each nation of the world to improve the health of its people. This concept calls for a level of health that will permit all people to lead a socially and economically productive life. The success of any health system depends on the ready availability

and use of suitable drugs on a sustainable basis. Medicinal plants have always played a key role in world of health. WHO has also done its share to further awareness of the importance of traditional medicines to the majority of world’s population, and to promote increased rational exploitation for all present and future peoples of the world of safe and effective practices, including the use of medicinal plants Since the discovery and use of sulpha drugs, antibiotics such as penicillin’s and synthetic drugs, there has been a dramatic decline in the popularity of medicinal plants in therapy. In a number of cases, these synthetic drugs have caused side effects or adverse reactions that were not fore seen in preclinical and clinical

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examinations. As a result, a resurgence of interest in the study and use of medicinal herbomineral formulation has been taken place during the last two decades. As a result of modern isolation techniques and pharmacological testing procedures, herbomineral formulation found their way into modern medicine as purified substances rather than in the form of galenical preparations. One of the major problems encountered with the use of natural products, is maintaining product uniformity. Furthermore, plants and metals and minerals are valuable for modern medicine in four basic ways¹

- They are used as sources of direct therapeutic agents.
- They serve as raw materials base for elaboration of more complex semi synthetic chemical compounds
- The chemical structures derived from plant sources, metals and minerals can be used as models for new synthetic compounds.

The systematic investigation of drugs used in indigenous medicines on modern scientific line has brought into prominence, the merits and qualities of certain drugs and it has been shown that, if brought into general use they may prove to be very valuable addition to the present armamentarium of the medical man to relieve the sufferings of humanity². *Shwas kuthar rasa*³ is an important formulation in Indian system of Medicine which is used in various disorders like *Shwas*, *Kasa*,

Mandagni, disorders prominent in *Vata-Shleshma doshas* as well as in pleurisy. Anti microbial agent are the prime source of these disorders specially pleurisy. No detailed Pharmacological studies have been done on *Shwas kuthar rasa*, but however some phytochemical and pharmacological studies were carried out on the plants, metals and minerals used in preparation of it like anti fungal activity of *gandhak rasayana*⁴, anti inflammatory activity of *Piper longum*⁵ and *Sunthi*⁶ but effect on whole compound formulation studied on microbial activity was known.

Procurement of Ingredients

The entire drug needed for preparation of *Shwas kuthar rasa* was collected from local market of Varanasi.

Pharmaceutical process of *Shwas kuthar rasa*

Plant material like *Maricha*, *Pippali* and *Sunthi* were dried in sunlight and made in to fine powder. *Parada*, *Gandhaka*, *Manashila* and *Vatsanabh* was used after shodhan process (Details are mentioned in table 1). *Kajjali* was prepared with *Sudha Parada* and *Sudha Gandhaka* by trituration process. Then the powder, which was prepared from *Sunthi*, *Maricha* and *Pippali* was incorporated in *Kajjali* and mixed uniformly. The above materials was triturated with *Adrak swarasa* for three days and made in to vati equal to one ratti⁷.

Table 1: Showing details about ingredients of *Shwas kuthar rasa*

S. No.	Name of Drugs	Drugs used for Shodhana	Process of Shodhana
1	Parada	Lime, Saindhav, Nistush Rasona	Mardana and Prakshalana
2	Gandhaka	Go-Dugdha	Swedana
3	Manashila	Adhraka	Bhawana
4	Vatsanabha	Go- Mutra	Swedhana

Materials and Methods

In vitro study of *Shwas kuthar rasa* was studies against staphylococcus

aureus by using Agar disc diffusion method. In this study Ciprofloxacin was used as standard drug.



Agar Disc Diffusion method

In vitro antibacterial study of *Shwas kuthar rasa* was performed by disc diffusion method. The agar diffusion test⁸, was used for measuring the effect of an antimicrobial agent against bacteria grown in culture. A filter-paper disk, impregnated with the *Shwas kuthar rasa* was placed on the surface of the agar.

The *Shwas kuthar rasa* diffuses from the filter paper into the agar. The concentration of the *Shwas kuthar rasa* was highest next to the disk, and decrease as distance increase from the disk. Different strains of *Staph. aureus* were grown on plate using Mueller Hinton as growth media⁹. Mueller Hinton agar plate was used for susceptibility testing for all organism, except those which need blood or altered blood for growth. For preparing the test plate, prepare the medium as described on manufacturers instruction,

cool down at 50 °C, pour in large size petri dishes to a uniform depth of 4 mm, then allow the plates to solidify at room temperature. Just prior to use we dried the culture plates in an incubator at 35 °C with lid partly open then inoculate the plates within 15 minutes after preparing the inoculum. With a wax pencil we divided the plate in to section according to number of no. of groups in study and labelled the plate with identification number. A Dip sterile cotton swabs rub gently over the plate in several direction to obtain uniform distribution of inoculums. Press the disc in to agar to ensure complete contact. Inoculate the plates overnight at 37 °C, under appropriate condition, suitable for staphylococous aureus. Following inoculation, we measured the diameter of zone of inhibition of growth by using of callipers.

Result

Out of four strains, *Shwas kuthar rasa* showed effect on three strains. In one strains *Shwas kuthar rasa* do not shows any activity. Details about observation are mentioned in Table 2 - 3.

Table2: showing results of *Shwas kuthar rasa* on organism by agar disc diffusion method

S.No.	Organism	Dilutions of <i>Shwas kuthar rasa</i> in different concentrations			
		300 mg/dl	400 mg/dl	500 mg/dl	600 mg/dl
1	<i>Staph. aureus</i> 1 st strain	M	M	M	M
2	<i>Staph. aureus</i> 2 nd strain	S	S	S	S
3	<i>Staph. aureus</i> 3 rd strain	M	M	M	M
4	<i>Staph. aureus</i> 4 th strain	R	R	R	R

S: Sensitive (Inhibition zone > 12 mm), M: Moderate Sensitive (Inhibition zone 7-12 mm), R: Resistant (Inhibition zone <7 mm)

Table 3: showing results of Ciprofloxacin (Standard drug) on organism by agar disc diffusion method

S.No.	Organism	Dilutions of Ciprofloxacin in different concentrations			
		300 mg/dl	400 mg/dl	500 mg/dl	600 mg/dl
1	<i>Staph. aureus</i> 1 st strain	S	S	S	S
2	<i>Staph. aureus</i> 2 nd strain	S	S	S	S
3	<i>Staph. aureus</i> 3 rd strain	S	S	S	S



4	<i>Staph. aureus</i> 4 th strain	S	S	S	S
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S: Sensitive (Inhibition zone > 12 mm)

Table 4: showing Minimum inhibitory concentration values of *Shwas kuthar rasa* on organism in different concentration

S.N	Micro organism	600 mg/ml	300 mg/ml	150 mg/ml	75 mg/ml	37 mg/ml	18.5 mg/ml	9.25 mg/ml	4.5 mg/ml	2.25 mg/ml
1	<i>Staph. aureus</i>	S	S	S	S	S	S	S	R	R
2	<i>E.coli</i>	S	S	S	S	S	S	S	R	R
3	<i>Pseudomonas</i>	S	S	S	S	R	R	R	R	R
4	<i>S.typhimerum</i>	S	S	S	S	S	S	S	S	R
5	<i>Morganella</i>	S	S	S	R	R	R	R	R	R
6	<i>Shingella</i>	S	S	S	S	S	S	R	R	R
7	<i>Kleb. Pneumoniae</i>	R	R	R	R	R	R	R	R	R
8	<i>Serratia</i>	S	S	R	R	R	R	R	R	R

S: Sensitive (Inhibition zone > 12 mm), R: Resistant (Inhibition zone < 7 mm)

Discussion

Shwas kuthar rasa prepared by Parada, Gandhaka, Vatsanabh, Tankana, Manashila, Maricha, Pippali, Sunthi by triturating in Tambula patra Swarasa. Mercurial products show synergistic action when combined with other Plants, metals and minerals. In this preparation Pippali¹⁰, Sunthi¹¹, and Gandhak⁴ were reported as antibacterial effect. By keeping these thing we studied antibacterial activity of *Shwas kuthar rasa* in different species bacterial as well as same bacteria with different strains and using ciprofloxacin as standard drug.

In Agar diffusion method, if the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. Thus, the size of the zone of inhibition is a measure of the compound's effectiveness: the larger the clear area around the filter disk, the more effective the compound. In above study we found that zone of inhibition for 2nd strains of

Staph. aureus was > 12 mm, this shows that *Shwas kuthar rasa* was significantly effective against 2nd strains of *Staph. aureus*. Zone of inhibition for 1st and 3rd strain of bacteria was in between 7-12 mm, this indicate that *Shwas kuthar rasa* was moderately effective against 1st and 3rd strain of bacteria but Standard drug showed more than 12 mm of zone of inhibition, this indicate that standard drug was effective against all strains of bacteria. Our test drug was effective but Standard drug was more effective. We further studied that effect of dilution of *Shwas kuthar rasa* on different species of bacteria to find minimum inhibitory concentration. We find that minimum inhibitory concentration of test drug towards *S.typhimerum*, *Pseudomonas*, *Morganella*, *Shingella*, *Serratia*, *Staph. aureus* and *E.coli* was 4.5 mg/ml, 75 mg/ml, 150 mg/ml, 18.5 mg/ml, 300 mg/ml and 9.25mg/ml respectively. *Kleb. Pneumoniae* was inhibited by any concentration of *Shwas kuthar rasa*.



Conclusion

Shwas kuthar rasa effective against only three strains of *Staph. aureus*. and one strain did not show any effect of it. This shows that *Shwas kuthar rasas* was effective against only third strain of *Staph. aureus*. *Shwas kuthar rasa* shows different minimum inhibitory concentration on different species of bacteria like *S.typhimerum*, *Pseudomonas*, *Morganella*, *Shingella*, *Serratia*, *Staph. aureus* and *E.coli* was 4.5 mg/ml, 75 mg/ml, 150 mg/ml, 18.5 mg/ml, 300 mg/ml and 9.25 mg/ml respectively.

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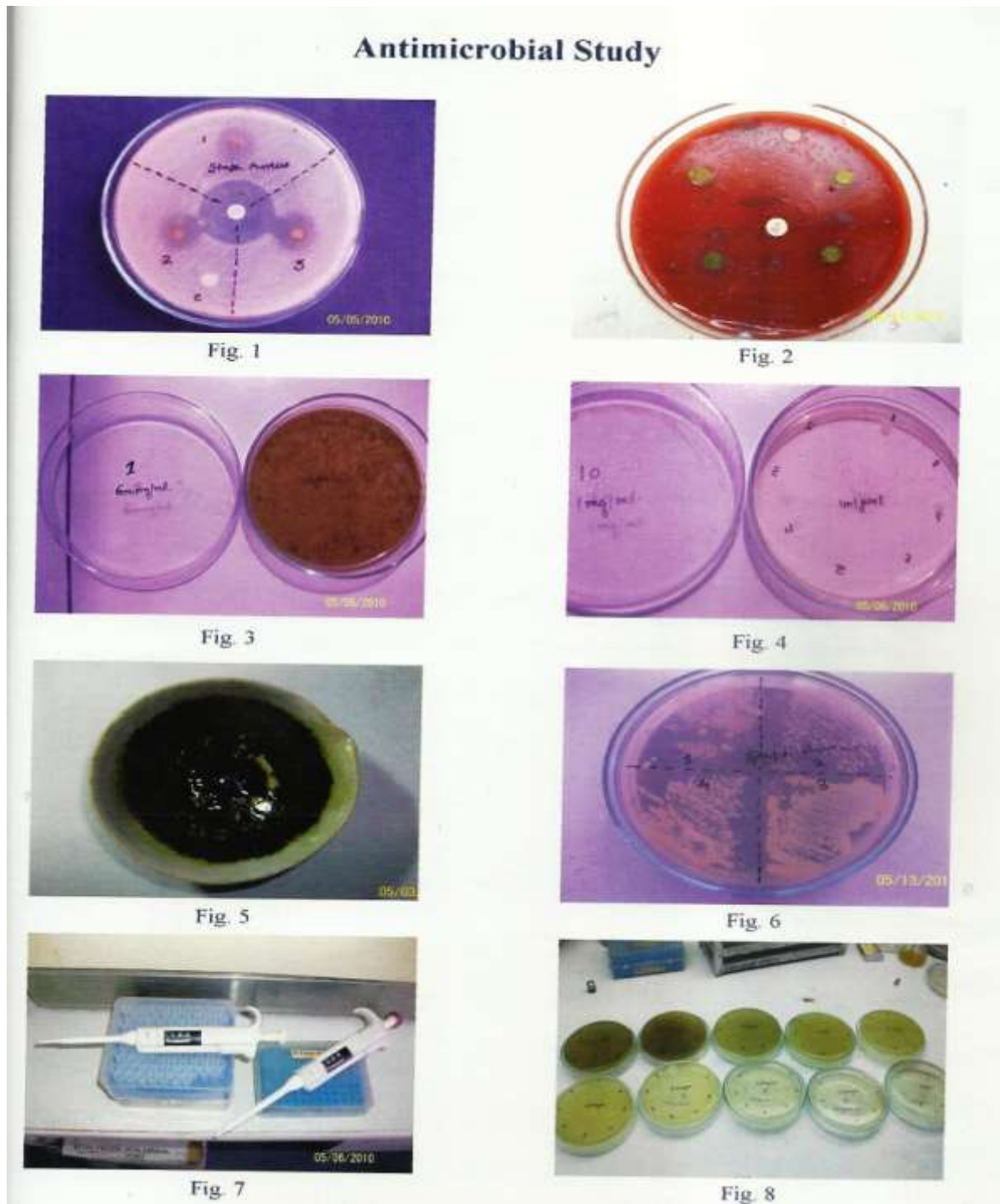


Fig.1 : Drugs Showing anti microbial activity against *Staph. aureus* compared with Ciprofloxacin

Fig.2: Drugs Showing anti microbial activity against *Streptococcus pneumoniae*

Fig.3: Drugs Showing anti microbial activity against different bacteria's at conc of 600mg/ml

Fig.4: Drugs Showing least anti microbial activity at conc. of 1mg/ml

Fig 5: Methanolic extract prepared for the antimicrobial study

Fig 6: Different strains of *Staph. aureus* grown on culture media

Fig .7: Micropipettes used in study

Fig. 8 : Different petridishes having different drug conc use for antimicrobial study
