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Research Article

PREPARATION OF '*RASMANIKYA*' AND STUDY ITS ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

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ABSTRACT:

The Ancient Indian alchemy is dealing with *Parada* (mercury) ie. *Rasa*, Minerals, Metals and aquatic substances, all are generally considered in *Rasashastra*. These substances are catagarised as *Maharasa*, *Uparasa*, *Sadharanrasa*s and *Ratnas*, *Uparanta*, *Lauhas (Dhatu), Vishas*, *Upavishas* Etc. as per their quantitative, qualitative differences, with reference to its action on *Dhatu* and Body.

The segment of the *Rasashastra*, covers the exclusive studies and experiments of inorganic Pharmaceutical Preparations. Such Ayurvedic *Rasaushadhi*s plays very vital role in to improve the scope of *Ayturveda*. Raw materials being toxic, are not permited to use in their original form, without the process of *Shodhana*, is performed.

Such *Rasaushadhi* are processed to such extend, so that it can be consume in small dosage, for quicker and long effectiveness in various ailments. There are many types of techniques and concepts, were set in motion like *Marana*, *Parpati Kalpana*, *Kupipakwa rasaayans* and *Pottali kalpana* etc., with the help of such techniques and concept, the effective medicines can be prepared as per the parameter and procedure, mentioned in ancients *Granthas*, for effective implementation in curing of various ailments.

The present study was preparation of *Rasamanikya* and its antimicrobial activity done on these bacteria viz. Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalacti, Pseudomas aeuriginosa, Candida albicans, Asperagus niger, Trichophyton rubrum by Disc diffusion Method.

KEY WORDS: *Rasa Manikya*, anti Bacterial, Anti Fungal.

INTRODUCTION:

"Ayurveda" is the ancient science, which was developed by the various ages, by way of continuous trials, experiments and deliberate minute observations. The equal contribution of the follower's of the Ayurveda is unique in flourishing it. However, Ayurveda is ancient Science, it has been honored, respected and applied for the prevention and cure of human beings with purity and sacred. The Ayurvedic Medicines are proved to be more effacious in various diseases. It is pertinent to note that the various diseases have been treated with the help of metalics, Non Metallic and compound of Ayurvedic material medica.

First time it is mentioned by *Dundhukanath* by *Antar Dhoom* procedure. In this process purifide *Hritala* has been taken in an earthen *sharava*, and closed with another an earthen *sharava, seald* with *badari patrakalka* and placed on fire(*Bhrashti*, or *Chullika* etc.) by heating up to red color of the bottom after that allow for self cooling. From that *Sharava* collect the contents, which is *manikya varna* (ruby color) called *Rasamanikya*. The same found mentioned in *Rasendra Chintamani* (IX/42/128-131 P.376), *Rasendra Sarasangraha* (1/191 - 196), *Siddha Bheshaja Manimala* (4/50 - 67) The above reference mentions that the *Rasamanikya* is one of the form of *Haritala Satva*. (commentary of *Shri Gulraj* Sharma) *Rasatarangini* (11/85 - 87).

Aims:

- Preparation of *Rasmanikya and* Study of Anti-bacterial and anti-fungal activity of *Rasmanikya.*

Objectives:

- To prepare *Rasa Manikya*
- To study Anti Bacterial and Anti Fungal Activity

MATERIALS AND METHODS:

***Shodhan* of Raw *Hartal* in *Kushmand swaras* (kshipta method)**

Ref. *Rasendra Chintamani*, *Rasendra Sara sangraha*

Ingredients - *Ashudh Hartal, Kushmand Swarasa* and *Dadhi Amla*

Process – *Kshipta*

Procedure –

At first, *Ashudha Haritala* made in to small pieces with help of mortar and pestle, that Ashudha Haritala taken in a earthen pot and poured the fresh *kushmanda swarasa* in it.

In addition, every day changed with new juice for 7 days. Every day Wt. of Ph of juice of *Hartal,* Wt. of *Kushmand*, and volume of *Kushmsnd Swaras* used and time was noted. Along with this pH of both fresh *Kushmand swaras* and previous day's *swaras* was noted.

Shodhan of Raw *Hartal* in *Dadh*i *(Kshipta* method)

Ref. *Rasendra Chintamani, Rasendra Sara sangraha*

Equipment - S.S. vessel, Trey, Measuring glass, etc.

Ingredients - Ashudh Hartal and Dadhi Amla

Process – *Kshipta*

Procedure –

At first, *Shodhit Haritala* (in *Kushmand Swaras*) made in to small pieces with help of mortar and pestle, that *Shodhit Haritala* taken in a earthen pot and poured the fresh *dadhi* on it.*Dadhi* which is used for *Shodhana* was prepared one day before from *Godugdha.*

In addition, every day changed with new *dadhi* for 7 days. Every day Wt. of *Hartal,* pH of *dadhi*, pH of *dadhi* used for *hartal shodhan* of previous day and volume of *dadhi* used and time are noted

Preparation of *Rasmanikya*:

(Ref*: Resendrachintamani*)

(Closed *sharava*) Method:

Apparatus –

1. Earthen *sharavas* 2 no.s (Equal size)
2. Cloth
3. Mud. (Multani mitti)
4. *Badari patras*
5. Iron wire
6. Knife

Heating device – LPG gas

Procedure –

- At first, two equal sizes of earthen *sharavas* had taken.
- The *shodhita Haritala* spread over one *sharava*. In addition, other *sharava* with a hole in the centre, placed above the *shodhita Haritala*, in a pattern that they would form a *samput*. Two s*haravas* sealed with *mudsmered* cloth. After that, it has allowed for cooling for six hrs.
- Atfter that, sharava *sumpata* placed over gas burner.
- *Badari Patra kalka* was prepared from *badari patras*.
- *Rasmanikya* is prepared in five batches.

Precautions:

- *Shodhit Hartal* used for preparation of *Rasmanikya* was taken in small size particals (*tandulakruti*)
- *Madhyamagni* was maintain continuously during procedure

Disc diffusion Method

Materials:-

i) Drugs :

1. *Rasmanikya* (test drug)

ii) Micro organisms

Bacterial microorganisms:

staphylococcus aureus
streptococcus pyogenes
streptococcus agalacti
pseudomonas aeuriginosa

Fungal microorganisms:

candida albicans
asparagus niger
trichophyton rubrum

C) Chemicals & solvents

Nutrient broth
Ethyl acetate
Distilled water
Surgical spirit
Methanol
Chloroform
Double Distilled water
Propylene glycol

Equipments and Glassware's

Equipments	Glassware's
1) Water bath	1) Distillation apparatus
2) Loops and loop holder	2) Petri dish
3) Borer	3) Conical flask
4) Hot air oven	4) Test tubes
5) Inoculation hood	5) Beakers
6) Autoclave	6) Funnel
7) Incubator	7) Stirrer.
8) Digital balance	

Methods:

Inoculums preparations:-

1. By using a loop or swab, colonies were transferred to the plates.
2. Visually adjust turbidity with broth to equal that of a 0.5 McFarland turbidity standard that has been vortexed. Alternatively, standardize the suspension with a photometric device.

Inoculation Of Agar plate:-

1. Within 15 min of adjusting the inoculum to a McFarland 0.5 turbidity standard, sterile cotton swab was dip into the inoculum and rotated it against the wall of the tube above the liquid to remove excess inoculum.
2. Entire surface of agar plate was swabed three times; plates were rotated approximately 60º between streaking to ensure even distribution.
3. Inoculated plates were allowed to stand for 3-5 minutes but no longer than 15 min before making wells.

Addition of compound into plate :-

1. Hollow tube of 5mm diameter was taken. And it had pressed above inoculated Agar plate and it was removed immediately by making wall in the plate. Likewise 5 wells were made in the plate.
2. 75µl, 50 µl, 25 µl, 10 µl and 5 µl of compound was added into the respective wells on each plate.

Incubation :-

1. Plates were incubated within 15 min of compound application.
2. Plates were inverted and stack them no more than five high.
3. Incubation was done at 37-38^0 C for 14-15hrs

Reading plates :-

1. Plates were read only if growth of the lawn is confluent or nearly confluent.
2. Daimeter of inhibition zone was measured by measuring device.

OBSERVATION AND RESULTS:

Table No. 1 Observations Of *Shodhan* of *Hartal in kushmand Swaras*

Day	Ashuddha Hartal		Volume of swaras		Shodhit Hartal	
	color	wt	initial	final	wt	color
1	Golden yellow	500 gms	950ml	930 ml	505gms	Golden yellow
2	Golden yellow	505gms	950ml	940 ml	510gms	Golden yellow
3	Golden yellow	510gms	950ml	940 ml	510 Gms	Golden yellow
4	Golden yellow	510gms	950ml	942 ml	507gms	Bright yellow
5	Golden yellow	507gms	950ml	945 ml	510 gms	Bright yellow
6	Golden yellow	510gms	950ml	945ml	512 gms	Bright yellow
7	Golden yellow	512gms	950ml	945 ml	515 gms	Bright yellow
8	Golden yellow	512gms			515 gms	

Table No. 2: Observations Of *Shodhan of Hartal in dadhi*

Day	*Ashuddha Hartal*		Volume of *dadhi*		*Shodhit Hartal*	
	Color	Wt	Initial	Final	color	Wt
1	Bright Yellow	504 gms	250ml	220 ml	Bright yellow	510 gms
2	Bright Yellow	510 gms	250ml	220 ml	Bright yellow	510 gms
3	Bright Yellow	510 gms	250ml	225ml	Bright yellow	512 gms
4	Bright yellow	512 gms	250ml	228ml	Dull Yellow	512 gms
5	Bright yellow	512 gms	250ml	230ml	Dull Yellow	515gms
6	Bright yellow	515 gms	250ml	230ml	Dull Yellow	515gms
7	Bright yellow	515 gms	250ml	235ml	Dull Yellow	515 gms
8	Bright yellow				Dull Yellow	515gms

Table No. 3. Observations of Preparation of *Rasmanikya*:

Batch No.	*Shodhit hartal*		Time			*Agni*	Temp	*Rasmanikya*		Loss Wt.
	color	Wt	Starting	Completion	Total			Wt.	color	
1.	Golden yellow	100 gms	2:05pm	2:21pm	16 mins	*Madhyam*	116^{0}C	78gms	Ruby	22 gms
2.	Golden yellow	100 gms	2:40pm	2:57pm	17 mins	*Madhyam*	118^{0}C	82gms	Ruby	18 gms
3.	Golden yellow	100 gms	3:10pm	3:26pm	16 mins	*Madhyam*	115^{0}C	75gms	Ruby	25 gms
4.	Golden yellow	100 gms	3:50pm	4:08pm	18 mins	*Madhyam*	116^{0}C	85gms	Ruby	15 gms
5.	Golden yellow	100 gms	4:30pm	4:45pm	15 mins	*Madhyam*	120^{0}C	82gms	Ruby	18 gms
					16.4 mins		117^{0}C	80.4 gms		19.6 gms

Table No. 4 Observations of Anti-bacterial and anti-fungal activity of *Rasmanikya* by **disc diffusion** method:

Serial No.	Micro-organism	Diameter of Inhibition zone of Rasa Manikya 75µl
	***Bacterial Micro-organisms**:*	
1.	Staphylococcus aureus	14mm
2.	Streptococcus pyogenes	24mm
3.	Streptococcus agalacti	25mm
4.	Pseudomonas aeuriginosa	13mm
	***Fungal Micro-organisms**:*	
5.	Candida albicans	23mm
6.	Asparagus niger	24mm
7.	Trichophyton rubrum	25mm

DISCUSSION:

- The Historical review of *Hartal* and *Rasa Manikya* was done.
- Various methods of *Hartal shodhana* and *Rasa Manikya prepation* was discussed.
- Accordingly the *Rasamanikya* preparation method discussed.
- Discussion done on *Krumi* and anti micro organism done.
- Discussion on anti microbial disc diffusion method was done.
- Preparation of media for anti-microbial and preparation of agar for antimicrobial study was considered during discussion.

CONCLUSION:

- Antimicrobial activity of Rasamanikya significantly seen in above mentioned microorganisms but specifically more in Streptococcus agalacti and slightly less in other examined bacterias.
- Antifungal activity of *Rasamanikya* significantly seen in above mentioned fungi but specifically more in Trichophyton rubrum and slightly less in other examined Fungi.

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