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ANTIMICROBIAL ACTIVITY OF SHEETAMSHU RASA

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ABSTRACT

The present study was designed to examine in vitro antimicrobial and antifungal activities of Sheetamshu rasa. The major constituents of the product were determined as arsenic compounds. The antimicrobial activity of Sheetamshu rasa was tested against gram-positive and gram negative organisms and fungi using Kirby-Bauer and Stokes' disc diffusion method. Sheetamshu Rasa showed remarkable antibacterial activity against *E-coli, pseudomonas aeruginosa,* and *Staphylococcus aureus, Klebsiella pnemoniae.* Compared to gram +ve and gram –ve micro-organisms, Sheetamshu Rasa exhibited more significant antifungal activity against *Candida albicans*.

Keywords: *Sheetamshu Rasa*, Antimicrobial, in-vitro, *E-coli, pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, Klebsiella pnemoniae*

INTRODUCTION

Rasashastra is a branch of science which deals with the metals, minerals, gems, and animal originates. This branch is responsible for the preparation of many formulations to overcome many ailments. Herbo-mineral formulations are considered under kharaliya rasayana. Sheetamshu rasa is one among them. This particular formulation is explained only in the text Basavarajiyam under sannipataja jwara chikitsa[1]. It contains two minerals and three herbs. As sheetamshu rasa is indicated[2] in infectious conditions like kushta, arbuda, shwasa, timira, vidradhi and used externally also as lepana. By keeping its wide range of therapeutic indications, present study has been undertaken to screen its antimicrobial activity by comparing with selected standard drugs like Streptomycin and itraconozole. Hand-made pills were made by triturating fine powders of ingredients with nimbu swarasa, dried and then taken for in-vitro evaluation.

MATERIALS AND METHODS

Raw materials were collected from NIA pharmacy Jaipur. Preparation of Sheetamshu rasa was carried out in Rasashastra lab NIA Jaipur. Antimicrobial study was carried out in B. Lal institute of Biotechnology Jaipur.

Pharmaceutical study

Minerals like Haratala (Orpiment) and Manashila (Realgar) were subjected to shodhana procedure[3,4] as per the reference.

Herbs like Shunti (*Zingiber officinalis*) Maricha (*Piper nigrum*), Pippali (*Piper longum*) were made into fine powder separately[5].

Preparation of Sheetamshu Rasa[6]:

Table No.1. Ingredients with quantity as follows-

S.No.	Ingredients	Eng/Bot. Name	Part	Quantity
01	Haratala	Orpiment	Purified	1 part
02	Manashila	Realgar	Purified	1 part
03	Shunti	*Zingiber officinalis*	Rhizome	2 part
04	Maricha	*Piper nigrum*	Fruit	2 part
05	Pippali	*Piper longum*	Fruit	2 part
06	Nimbu	*Citrus lemon*	Fruit	Q.S.

Method of preparation[6]:

Measured quantity of all ingredients was taken in khalwa yantra triturated in presence of Nimbu swarasa until it becomes homogenous mixture. After attaining siddhi lakshana rolled into pills. Dried and preserved.

Table No.2 Organoleptic characters of Sheetamshu Rasa

Colour	Taste	Odour	Touch	Consistancy
Deep Greenish yellow	Pungent	Irritant	Soft	Solid

Thus prepared pills were subjected to analytical study[7]. Qualitative and quantitative analysis were carried out. Elemental analysis was carried out in IIT Mumbai.

ANTIMICROBIAL STUDY[8, 9]

Microbial strains

Antimicrobial and antifungal activities of the Sheetamshu rasa, were evaluated against 2 Gram-positive and 2 Gram-negative bacteria, 1 fungus by the Kirby-Bauer and Stokes' method. The microorganisms were used *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-27853), *Klebsiella pneunomonia* (NCTC-5046), and *Candida albicans* (ATCC-10231), using Streptomycin [5mg (w/v)] as antibiotic/Itraconozole as antifungal as reference and (DMSO) Di Methyl Sulfoxide for dilution. Bacterial strains were cultured overnight at 37°C in Mueller Hinton Agar.

Method: The working supply of antimicrobial disks should be stored in the refrigerator (4°C). Upon removal of the disks from the refrigerator, the package containing the cartridges should be left unopened at room temperature for approximately 1hour to allow the temperature to equilibrate. This reduces the amount of condensation on the disks. If a disk-dispensing apparatus is used, it should have a tight fitting cover, be stored in the refrigerator, and be allowed to warm to room temperature before using.

Apply the antimicrobial disks to the plates as soon as possible, But no longer than 15minutes after inoculation. Place the disks individually with sterile forceps or with a mechanical dispensing apparatus, and then gently press down onto the agar. In general, place no more than 12 disks on a 150-mm plate and no more than 4 disks on a 100-mm plate. This prevents overlapping of the zones of inhibition and possible error in measurement.

Recording and interpreting results

After the disks are placed on the plate, invert the plate and incubate at 35°C for 16 to 18 hours. After incubation, measure the

diameter of the zones of complete inhibition (including the diameter of the disk) and record it in millimeters. The measurements can be made with a ruler on the undersurface of the plate without opening the lid. The distance from the colony closest to the disk to the outer clear zone should be recorded as well an interpretation recorded for each diameter. The colony inside the zone should be picked, re-isolated, re-identified, and retested in the disk diffusion test to confirm the previous results. The presence of colonies within a zone of inhibition may predict eventual resistance to that agent.

RESULT

Table No.3 Physico-chemical analysis of Sheetamshu Rasa

Parameters	Sheetamshu Rasa
L.O.D	9.356%w/w
Total Ash	6.498%w/w
A.I.A	0.669%w/w
Water soluble extractive	33.169%w/w
Disintegration time	1 h, 15 min to 1h, 45 min
Tablet Hardness	7.2

Table No. 4 Antimicrobial activity of Sheetamshu Rasa

Sl. No	Microorganism	Positive Control Zone of Inhibition(mm)	Sample Zone of inhibition(mm)			
	Antibacterial Sensitivity Streptomycin [5mg(w/v)]					**Mean**
1	*Escherichia coli*	36.5	18.5	21.5	19	19.6
2	*Pseudomonas aeruginosa*	42.5	14.5	16	15.5	15.3
3	*Klebsiella pnemoniae*	47.5	14.5	17	17	16.1
4	*Staphylococcus aureus*	40.5	18.5	19	18	18.5
	Antifungal Sensitivity(Itraconozole 5mg/well)					**Mean**
5	*Candida albicans*	18	15.5	18	17.5	17

Result Interpretation:

If mean value of the zone of inhibition is **<13** then it is considered as **Inactive**

If mean value of the zone of inhibition is **13-18** then it is considered as **Bioactive** If mean value of the zone of inhibition is **>18** then it is considered as **Highly Active**

DISCUSSION AND CONCLUSION

Sheetamshu rasa can be prepared easily, because of easily available ingredients. The formulation has fewer ingredients with more therapeutic usage. As it contains haratala, and manashila as ingredients it must possess antimicrobial effect, because haratala and manashila were already proved drugs against microbial activity.[10] Here trikatu churna acts as antidote for arsenic. Analytical study revealed that, hardness of the sample is more, that might be because of minerals like haratala and manashila and stickiness of the nimbu swarasa. Because of this reason, increase in disintegration time also observed.

Antimicrobial study revealed that, the sheetamshu rasa showed significant sensitivity against tested organisms.

Compared to gram +ve and gram –ve organisms, against fungi test drug showed much sensitivity. Means zone of inhibition of standard drug itraconozole was 18mm where as test drug on *candida albicans* was 17mm. By this it can said as one of the good antifungal formulation.

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