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PREPARATION OF CHANDRAMRUT RAS AND ITS ANTIMICROBIAL ACTIVITY (IN VITRO)

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ABSTRACT

In Ayurveda *Kaas* is a disease as well as a symptom presented in many diseases, including recent situations like the COVID-19 pandemic. Every human being faces *kaas* (cough) in many stages and phases of life. The present study was conducted to analyze *Chandramrut Ras* physico-chemically, as mentioned in *Bhaishajyaratnavali-Kaas rogadhikara*. It is a herbo-mineral combination that acts on *kaas* by normalizing the functions of *vaat* and *kapha*, boosting digestive fire, and helping to increase immunity. Drugs in *Chandramrut Ras* like *pippali*, *shunthi*, and *tankan* show antimicrobial activity. Hence, *Chandramrut Ras* is prepared according to textual reference and then subjected to analytical testings. After that, antimicrobial activity of the drug *Chandramrut Ras* was seen on Gram-positive bacteria (*Staphylococcus aureus*) against the standard drug amoxicillin. According to a current research study, *Chandramrut Ras* showed antimicrobial activity on *Staphylococcus aureus*. It is necessary to develop a drug like *Chandramrut Ras*, which is a safe and potent alternative to antibiotics.

INTRODUCTION

Ayurveda, most renowned traditional system of medicines accepted worldwide, with enormous knowledge of nature-based medicine. Ayurveda explains the relationship of human body constitution and function to nature and the elements of universe that act in coordination and affect living being. In Ayurvedic practice *Rasaushadhi* has been considered as more effective and beneficial due to lesser therapeutic doses, enhancement of action of other

ingredients of formulation, more shelf life, quicker action and palatability as compared to herbal drugs. *Kasa* is the disease or symptom of respiratory tract occurring due to obstruction of *Vata* Dosha and its reverse movement. *Chandramrut Ras* is a herbo-mineral formulation described in *Bhaishajyaratnavali-kaas rogadhikara*. *Chandramrut Ras* contains *Trikatu*, *Triphala*, *Chavya*, *Dhanyak*, *jirak*, *Saindhav*, *Kajjali* and *Tankan*. Respiratory diseases will afflict every human being at some time in their life. Whether it's an associated with the common cold or respiratory distress, allergies and asthma, respiratory challenges are a constant source of irritation and misery for afflicted. Drugs used to treat these cause side effects like Drowsiness, dry throat, upset of stomach, diarrhea, vomiting, stomach pain. Some antihistamines and decongestants make mucus thicker and harder to remove.

After innovations of Antibiotics, these compounds are in danger of losing their efficacy due to the rise in microbial resistance. Currently, it has a significant impact on treatment failures brought on by bacteria that are resistant to many drugs, and it has become a global public health concern. Though "*Chandramrut Ras*" is a textual reference and there is a need of preparation as mention in *Bhaishajyaratnavali*. and to assess its antimicrobial activity against Staphylococcus aureus which is Gram positive bacteria. All the ingredients of this drug are having *aagnivardhan*, *kaphavaatshamak*, *kaphavishleshak*, *yogvahi* properties. Therefore, the goal of the current study is to manufacture "*Chandramrut Ras*," an Ayurvedic compound that is safe, potent, effective, and convenient to use. And evaluate its antimicrobial property.

Aim

To prepare *Chandramrut Ras* and study of its antimicrobial activity. (in vitro)

Objectives

1. To prepare *Chandramrut Ras* as mention in *Bhaishajyaratnavali*.
2. To analyze the study drug physico- chemically.
3. To determine the antimicrobial activity (In-vitro) of the study drug to analyze the results.

MATERIAL AND METHOD

Procurement of ingredient needed - The entire raw material for preparation of *Chandramrut Ras* was collected from APMC Market, Vashi.

Table 1: Raw ingredients used in CR.

Sr. No.	Ingredient	Latin name	Part used	Quantity
1	Pippali	*Piper longum*	Dry fruit	1 Part
2	Shunthi	*Zingibar officinale*	Rhizome	1 Part
3	Marich	*Piper nigrum*	Dry fruit	1 Part
4	Amlaki	*Embilica officinalis*	Dry Fruit Pericarp	1 Part
5	Haritaki	*Terminalia chebula*	Dry Fruit Pericarp	1 Part
6	Bibhitak	*Terminalia belerica*	Dry Fruit Pericarp	1 Part
7	Chavya	*Piper retrofractum*	Stem	1 Part
8	Dhanyak	*Coriandrum sativum*	Dry fruit	1Part
9	Jirak	*Cuminum cyminum*	Dry fruit	1 Part
10	Saindhav	*Rock Salt*		1 Part
11	Gandhak	*Sulphur*		1 Part
12	Parad	Mercury		1 Part
13	Lauha	Iron	Bhasma	1 Part
14	Tankan	Borax		4 Part
15	Marich	*Piper nigrum*	Dry Fruit	2 Part
16	Goat milk			Q.S
17	Rason for shodhan	*Allium sativum*	bulb	Q.S
18	Ghrita for shodhan	Ghee		Q.S
19	Dugdha for shodhan	Cow Milk		Q.S
20	Kadalianda for Shodhan	*Musa paradisiaca*		Q.S
21	Hingul for Marana	HgS		Q.S
22	Triphala kashay for shodhan			Q.S
23	Kumari	*Aloe barbadensis*	Leaf	Q.S
24	Jambeer	Citrus lemon	fruit	Q.S
25	Gomutra			Q.S

A) **Method of preparation of chandramrut ras**[1]

Step 1: Preparation of Kajjali (Ref. R.R.S 8/5)[2]

a. Samanya shodhan of Parad was done w.r.t Rastarangini[3]: 5-27/28.

a. Equal part of Parad and Lime powder was taken and triturated for 3 days and stained with a double folded cloth, then this parad was taken in khalva yantra with an equal part of peeled garlic cloves were added along with half of saindhav lavan until garlic turns into krushna varna. Then it was washed carefully with hot water.

b. Shodhan of Gandhak was done w.r.t Ayurved Prakash[4] 2/21–24.

a. One part of ashudha gandhak was melted with cow's ghee and poured in milk through a cloth so as to allow the filtered to go into liquid, then solidified gandhak in milk was washed with hot water. Same procedure was repeated three times.

c. Shudha Parad and shudha Gandhak in equal quantities were taken in khalva yantra. And ground well to form a soft, fine powder that kajjali was formed.

Step 2: Preparation of Lauha Bhasma (Rasachandanshu 508):[6]

a) Samanya Shodhan of Lauha (R.T. 15/7):[3]

Ashudha Lauha was taken. Lauha bricks were heated until red hot. Quenching was done in Kadalikanda swaras. The same procedure was followed seven times. Every time, fresh swaras would have taken.

b) Vishesh Shodhan of Lauha (R.T. 20/18):[3]

Samanya shodhit Lauha churn was taken in an iron vessel. Tivragni was given till the lauha churn becomes red hot. Triphala Kwath and Gomutra were taken in proportion 1:1. The quenching of the Red Hot Lauha Churn was done in Mixture of triphala kwath and gomutra.

c) Shodhan of Hingul for Maran of Lauha (R.T. 9/16–17):[3]

Ashudha Hingul was taken in Khalva yantra and turned into powder form. Nimbu Swaras was added to the khalva yantra and triturated. Similarly, 7 Bhavna of Nimbu Swaras was given. Shudha Hingul was obtained in dried form.

d) Maran of Lauha (With reference To Rasachandanshu 508):[6]

Vishesh shodhit Lauha Churn and Shudha hingul were taken in the khalva yantra. Bhavna was given by Kumari Swaras. Chakrika was prepared. Dried Chakrika was kept in sharavsamput. Sandhibandhan and the drying of sharava were done. After that, sharava was kept in Gajaputa. Sharava was kept for self-cooling. Then the drug was taken out, and again bhavna of kumari swaras was given. Same The procedure was repeated seven times for maran of Lauha. It was continued till the sidhi lakshana of Bhasma.

Step 3: Preparation of Churn from Herbal Drugs:

Churna Preparation was done according to Sharangdharsamhita Madhyam Khanda 6/1 And API Part 2 Volume 1.

Triphala, Trikatu, Chavya, Dhanyak, Jirak, and Saindhav was collected and dried separately. The powder of the above herbs was made by using Mixer.

Each Powder was sieved by mesh no.85.Collected and store separately.

Step 4: Shodhan of Tankan (Ayurved Prakash 2/224)[4]:

Ashudha Tankan was powdered. This powder was kept over the pan, which was heated with mandagni, then tivragni was given. The water content evaporated. Atlast, a lighter substance, was obtained.

Step 5: Preparation of Chandramrut Ras:

1 Bhavna of Ajadugdha was given to Churn of Herbs that are *trikatu, triphala, chavya, jeerak, dhanyak, saindhav* (1 tola each), *Kajjali* of *parad* and *gandhak* in equal quantity (1 *karsha*), *Lauha Bhasma* (12gm), *Shudha Tankan* (48gm) and *Marich* (24gm) in the *khalva yantra*. And tablet of weight 1125mg (9 Gunja) was formed.

Table 2: Quantity of each drug in CR preparation.

Churn of Herbs	*Kajjali*	*Lauha Bhasma*	*Shudha Tankan*	*Marich*
1 *tola*	1 *karsha*	1 *karsha*	1 *pal*	½ *pal*
12 gm each	12 gm each	12 gm	48 gm	24 gm
1 part	1 part	1 part	1 part	1 part

A) In-Vitro study of chandramrut ras

The experimental study (In vitro) was conducted at Jeevanrekha Analytical Services, Aurangabad.

The following methods was carried out during the study. Plan of Antimicrobial (In Vitro) Study

A. Steps

1. Procurement of cultures of microorganisms from culture collection centre.
2. Preparation of antimicrobial study materials (CR test samples) as mentioned above.
3. Evaluation of antimicrobial activity (in vitro).

B. Microbial assay:

Antimicrobial activity test was performed by the commonly used Agar diffusion method which is designed to determine the smallest amount of the antibiotic needed to inhibit the growth of microorganism.

Requirements

- 18 hrs Nutrient broth culture of Test organism
- Standard Amoxicillin
- Mueller –Hinton agar plates
- Cork borer
- Sterile std bioassay filter paper disc.
- Sterile cotton swabs
- Alcohol
- Ruler
- Laminar flow chamber
- Test samples of which the activity has to be measured.

C. Method:

1. A sterile cotton swab was taken and dipped it into a culture of Test organism suspension.
2. The entire agar surface of each plate first was incubated in horizontal and then in vertical direction to ensure the even distribution of the organism over the agar surface using the swab.
3. Agar surface was allowed to dry for 5 min.
4. A cork borer was sterilized by autoclaving it by rinsing in alcohol followed by sterile water. A Mueller –Hinton agar plates were obtained and aseptically punched (4-5mm) holes in the agar using a cork borer. Using a wax pencil, underside of the Petri was marked to label the wells, with the help of micropipette, test solution of *Chandramrut Ras* was added in the well. The procedure was repeated for all wells.
5. Incubated all plates at 37-degree c for 48 hrs. in an incubator.

Observation

Physico-Chemical analysis of *chandramrut ras*

A. *Panchabhautika parikshana*:

Shabda- No specific sound

Sparsha- Round Rough

Roop- Greyish Black

Rasa- Katu, Kashay

Gandha- Characteristic smell

B. Modern parameters

Table 3: Chemical analysis of CR.

Tests	Results
pH value	7.3
Loss on Drying	9.29%
Total Ash	26.73%
Acid Insoluble Ash	14.58%
Alcohol Soluble Extractive	15.33%
Water Soluble Extractive	27.98%
Friablility	0.11%
Hardness	9.75 kg/cm^2
Disintegration Time	85 min.

Observation of in vivo study of chandramrut ras

Chandramrut Ras showed antimicrobial activity on *Staphylococcus aureus* with a zone of inhibition of 14.25mm. Zone of inhibition was measured using ruler. Amoxicillin was the standard drug used to compare the results. But Amoxicillin didn't show any activity against *Staphylococcus aureus.* It shows that Amoxicillin is resistant to bacteria *Staphylococcus aureus.*

Table 4: Microbial information used in this study.

Sr. No.	Genus	Species	Kingdom	Atcc No.
1	*Staphylococcus*	*Staphylococcus aureus* (Gram +ve)	Eubacteria	ATCC 6538

Table 5

Test sample	Zone of Inhibition in mms Staphylococcus aureus
Chandramrut Ras	14.25 mm
Amoxicillin	nil

RESULT AND DISCUSSION

A) Result of *chandramrut ras*

Result of Physico-chemical analysis of *Chandramrut Ras* are given in above table.

B) Result of in vitro study

The present study deals with the preliminary screening & comparison of antimicrobial activity of *Chandramrut Ras* & Amoxicillin. The study drugs have been tested against *Staphylococcus aureus* for antimicrobial activity. The antimicrobial activity of Chandramrut

Ras &Amoxicillin was equal in 1% concentration against both the organisms by Minimum Bactericidal Concentration (MIC) by Plate Method – ASM: Manual of Microbiology methods. Zone of inhibition was also calculated in mms for *Chandramrut Ras* & Amoxicillin by antimicrobial Property of test by Disc Diffusion Method as per NCCLS guidelines 2005. Zone of inhibition of *Chandramrut Ras* &Amoxicillin was seen 14.25mm and nil respectively.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 12

Fig. 13

Fig. 14

Fig. 15

Fig. 16

Fig. 17

Fig. 18

Fig. 19

Fig. 20

Fig. 21

Fig. 22

Fig. 23

Fig. 24

Fig. 25

Fig. 26

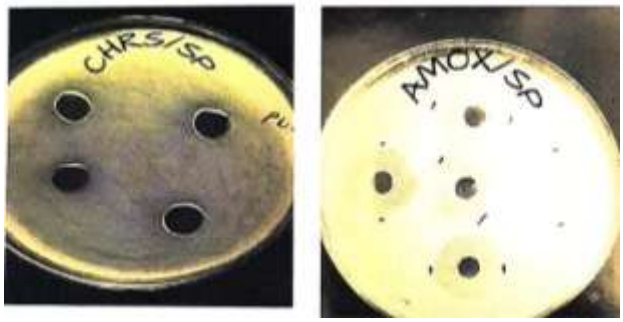

Fig. 27

1. Parad shodhan in lime powder
2. Gandhak shodhan in goghrita and godugdha
3. Shudha gandhak
4. Sgudha parad and shudha gandhak
5. Preparation of kajjali
6. Ashudha tankan
7. Shudha tankan
8. Ashudha loha
9. Heating ashudha loha on flame
10. Loha nirvap in kadalikanda swaras
11. Heating of loha for vishesh shodhan
12. Preparation of Triphala kwath and Gomutra
13. Ashudha Hingul
14. Hingul Shodhan
15. Bhavna of kumari swaras to shudha loha and shudha hingul
16. Chakrika Nirman
17. Sharavsamput
18. Gajaputa
19. Lohabhasma
20. Varitaratva of Loha Bhasma
21. Rekhapurnatva of loha bhasma
22. Churna preparation of herbs
23. Goatmilk
24. Chandramrut Ras Preparation
25. Vati preparation
26. Weighing of vati.
27. Antimicrobial study- CHRS(*Chandramrut Ras*), AMOX(Amoxicillin)

CONCLUSION

From the present study, we can conclude that *Chandramrut Ras* was formulated successfully as per the reference given in the *Bhaishjyaratnavali Kasa rogadhikara* 56-64 & showed significant antimicrobial activity against *Staphylococcus aureus* (Gram +ve). As *Chandramrut Ras* is indicated in *Kaas chikitsa* (Cough), hence we have selected *Staphylococcus aureus*[10] Gram +ve bacteria for in vitro study which is more resistant to

allopathic antibiotics. *Chandramrut Ras* is effective in *Staphylococcus aureus* (gram +ve bacteria) against standard drug amoxicillin with zone of inhibition 14.25mm.

Kasa[8] is the disease or symptom of respiratory tract occurring due to obstruction of *Vata* Dosha and its reverse movement. *Chandramrut Ras* contains *Triphal, Trikatu, Dhanya, Jiraka, Saindhav* acts on *Agni*. It enhances digestive power. These drugs remove the obstruction and hence cures forceful movement of the *Vata* Dosha, gives effect of *vatanuloman*. *Shunthi,*[11] *Pippali*[9] shows antibacterial activity. *Shudha Tankan*[7] helps in expectoration of cough. Aja dugdha is mention in rajyakshma so it acts in Pranvaha strotas vyadhi i.e kasa. Mercurial products show synergistic action when combined with other plants, metalas and minerals as kajjali prepared from parad and gandhak also work as yogvahi drug. Therefore, Chandramrut Ras is Herbo-mineral formulations have gained importance and rising global attention.

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