PREPARATION AND ANTIMICROBIAL STUDIES OF UDAYABHASKAR RASA: A HERBOMINERAL DRUG

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ABSTRACT

Five different preparations of Udayabhaskar Rasa were evaluated for their antimicrobial properties against different pathogenic microorganisms on agar plate medium and the zone of inhibition was calculated. Well as disc diffusion methods were tried during the studies of antimicrobial properties of drug. It was observed that Well method of drug sensitivity was more efficient than disc diffusion method. Preparations U₂ and U₅ were observed with highest sensitivity than U₁, U₃ and U₄ respectively. Pseudomonas aeruginosa was found highly sensitive to all different preparations of Udayabhaskar Rasa except sample U₄.

Key words: Udayabhaskar Rasa, Antimicrobial study, Rasa Karpura, Pseudomonas aeruginosa, Disc diffusion method, Well method.

INTRODUCTION

Ayurveda is one of the most ancient medical sciences of the world. This discipline was evolved through intuitive, experimental and perceptual methodology. The main objective of Ayurveda is to promote health thereby preventing the ailments and to relieve the humanity from all categories of miseries i.e. physical, mental, intellectual and spiritual. The approaches are essentially holistic. The Ayurvedic system of medicine is prevalent in India since the Vedic period and as early as the dawn of human civilization. Ayurveda gets flourished in the course of long history. The branch which deals with Rasa (Mercury), metals, minerals, herbals and other herbo-mineral preparation is known as “Rasa Shastra” which has become an integral part of Ayurveda. Since the time of Vedas, Rasasashdhis play an important role in the management of various ailments. The innate qualities like quick action, less duess, tastelessness, prolonged shelf life, better palatability of Rasasashdhis have helped them to conquer the compliance of the patients. As per other sciences; this science too, needs further probes for its total scientific progress according to the demand of present era.

Therefore, to make our treatment scientifically more validated, we can assess the antimicrobial activity of Udayabhaskar Rasa preparations in vitro (i.e. culture and
Total 14 ingredients are used in this formulation but Rasakarpura, Jaypala and Bhavanas of Bijpuraka Rasa are most important ingredients present in this formula.

AIMS AND OBJECTIVES

- Pharmaceutical standardization of Udayabhaskar Rasa.
- Physico chemical standardization of Udayabhaskara Rasa.
- To evaluate the anti-microbial activity of Udayabhaskara Rasa against common pathogenic bacteria.
- A comparative anti microbial study to evaluate the role of Rasakarpura. Rasapushpa and Jaypala seed.

For the above aims and objectives, five different samples of Udayabhaskara Rasa were prepared, as follows with their codes:

- **U₁** - As per the specification of Rasa Kalpa Lata (Rasa Kalpa Lata formulation No.158, Chapt. 7, Pg. No. 22 Verse no. 45-46)
- **U₂** - As per the specification of Rasa Yoga Sagar. (Rasa Yoga Sagar, Part-1, formulation No. 369, Verse No. 1520-1522, Pg. No. 162)
- **U₃** - This was prepared from Rasapushpa in place of Rasakarpura.
- **U₄** - Devoid of Rasakarpura and Rasapushpa.
- **U₅** - Devoid of Jaipala seed (Croton tiglium Linn)

PLAN OF STUDY

A. Pharmaceutical Study

In this study, five different samples were prepared including standard formulation of Udayabhaskara Rasa as per the specification of Rasa KalpaLata in the departmental laboratory of Rasashastra and Bhai-shajya Kalpana, NIA, Jaipur. The main implication of this study was to standardize the processes involved in it.

B. Physico-Chemical Study

In present research work, for physico-chemical standardization, standard sample of Udayabhaskara Rasa as per Rasa KalpaLata specifications prepared (U₁), Sample U₂ as per the specification of Rasa Yoga Sagar and Sample U₃ (prepared from Rasapushpa in place of Rasakarpura) were also taken for the standardization of process involved in it. Materials and methods were followed according to “Pharmacopoeial Standards for Ayurvedic Formulations” – C.C.R.A.S.

C. Antimicrobial Study

The aim of this study was to assess the comparative antimicrobial activity of different samples. For this study, Aqueous and Methanol extracts of the samples were prepared. In vitro studies were undertaken to assess the antimicrobial activity. For both types of extracts, disc diffusion method and for different samples, which were in fine powder form, Well method was employed.

Following common pathogenic strains of bacteria were procured from “Institute of Microbial Technology” (IMTECH), Chandigarh.

Table- Showing the Species of Bacteria and their MTCC NO. -
These stock cultures were maintained on solid media, Blood Agar for Strep-
tococcus pyogenes and Nutrient Agar for other bacteria, of Himedia Lab. Pvt. Ltd.,
Mumbai. This anti-microbial study was car-
ried out at ‘Birla Institute of Scientific Re-
search’, Jaipur. The antimicrobial effects
were assessed on the basis of the scale de-

2. Relevance of the study:
In Ayurvedic literature the word “krimi” has
very much potential. There are abundant
materials available regarding krimi in Vedic
literatures especially in Atharvaveda. Maha-
rishis classified them on the basis of visibili-
ty, colour, size, shape and their place in hu-
man body. Size dependent classification has
two divisions, one is Sukshama (Microor-
organisms) and another is Sthula (Macroor-
organisms). The details of krimi have also
been described very efficiently in Samhita-
Granthis.

The word ‘krimi’ was used in a broad sense
in Ayurvedic literature i.e. it includes all the
pathogenic and non-pathogenic organisms
covering a wide range of infection and infes-
tation caused by a host of agent ranging
from viruses to worms. As per Charaka
Samhita,( Ch.Su., 11/45) ‘Bhutabhishanga’
is also responsible factor to cause diseases1.

One of the three major divisions of disease
i.e. ‘AgantujaVyadhis’ is also caused due to
krimi2. Similarly, Acharya Sushruta has illu-
strated the means of spread of ‘Aaupsargi-
kaRogas’ (Infectious disease)3.

According to the Dalhana Commentary on
Sushruta

“Upasargaja Iti Upasrijyanta Ityupasargah,

Piditajanasamipotpannah Jvaradayah”

( Dalhana Commentary on Su.Su. 24/7 )

That means in the etiology of many
diseases, microbial relation plays a role that
was realized by the modern medicine only a
century ago. But the specific idea of the na-
ture of disease producing germs did not de-
velop till modern microbiology came into
existence. Now, it has become possible to
investigate the action of drugs on pathogenic
microorganisms. Therefore, to make our
treatment scientifically more validated, we
assess the antimicrobial activity of such
preparations in vitro (i.e. culture and sensi-
tivity tests).

Since long, a number of Ayurvedic classical
formulations were being used in cases of
infections, and they were being found to be
effective clinically. But it is a matter of great
concern that such a famous formulation
“Udayabhaskar Rasa” is not studied in de-
tail. The first reference of the formulation
was quoted in Rasa Kalpa Lata by Magni-
ram5. Total fourteen formulations were men-
tioned in Rasa Yoga Sagar6 as Udayab-
haskara Rasa, but Udayabhaskara Rasa (8th)7
is found similar to the Udayabhaskara Rasa
(1st) of Rasa KalpaLata. Two gunja matra

<table>
<thead>
<tr>
<th></th>
<th>Staphylococcus aureus</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td></td>
<td>3160</td>
</tr>
<tr>
<td>II.</td>
<td>Streptococcus pyogenes</td>
<td>1928</td>
</tr>
<tr>
<td>III.</td>
<td>Escherichia coli</td>
<td>901</td>
</tr>
<tr>
<td>IV.</td>
<td>Pseudomonas aeruginosa</td>
<td>424</td>
</tr>
<tr>
<td>V.</td>
<td>Salmonella typhi</td>
<td>733</td>
</tr>
</tbody>
</table>

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with suitable anupana has mentioned in the treatment of Jalodara, Gulma and Aamvata rogas and also in some other diseases. Similar formulation can be found into Bhaarat Bhaishajya Ratnakara as Udayabhaskara Rasa with some modifications. Many of these preparations have not even evaluated for their antibacterial properties.

3. Udayabhaskar Rasa:
The use of herbomineral drug in the therapeutics was found since Samhita period. Health is the supreme foundation of virtue, wealth, enjoyment and salvation. In Ayurveda, it is described in one of the quadrupies of the treatment next to the physician. When the multiple groups of symptoms present, single drug formulations might cover only a fraction of the treatment. Hence, the compound preparations or ‘Yogas’ (combinations) came into practice.
The Khalveeya Rasayanas are the combination of herbal, mineral and animal products and effective against a number of diseases in a single formula. These are administered in smaller doses to get faster relief and combating many ailments by proper Anupana and Sahapana. It takes less space for manufacturing and storing. The most important aspect is that it preserves the properties of freshly added churnas, swarasa etc. with the help of Moorhchita Parada i.e. Kajjali, Rasindura, Rasakarpura, Rasapushpa and Hingula etc. Because of which Khalveeya Rasasushadhis occupies greater portion in therapeutics as compared to other kalpanas such as Vati, Gutika, Taila, Ghrita, Avaleha etc.

“Udayabhaskar Rasa” (UBR) is also a compound drug which comes under “Khalveeya Rasayana Kalpana.” Most of the Khalveeya Rasayanas come under “Sagandha and Niragni Moorchhana preparations. Udayabhaskar Rasa is one of such preparation. Some of Khalveeya Rasayanas viz. Kaphaketu Rasa, Bhuvaneshwara Rasa etc. are termed as Rasayoga but are not having any type of Moorhchita Parada preparations. Table 1 shows different ingredients of Udayabhaskar Rasa as described in R.K.L.

**Table 1: Ingredients of Udayabhaskar Rasa (UBR).**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>English/Botanical Name</th>
<th>Part used</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Shu. Parada</td>
<td>Mercury</td>
<td>---</td>
<td>1 Part</td>
</tr>
<tr>
<td>2.</td>
<td>Shu. Gandhaka</td>
<td>Sulphur</td>
<td>---</td>
<td>1 Part</td>
</tr>
<tr>
<td>3.</td>
<td>Shunthi</td>
<td><em>Zingiber officinal Roxb.</em></td>
<td>Rhizome</td>
<td>1 Part</td>
</tr>
<tr>
<td>4.</td>
<td>Maricha</td>
<td><em>Piper nigrum Linn.</em></td>
<td>Fruit</td>
<td>1 Part</td>
</tr>
<tr>
<td>5.</td>
<td>Pippali</td>
<td><em>Piper longum Linn.</em></td>
<td>Fruit</td>
<td>1 Part</td>
</tr>
<tr>
<td>6.</td>
<td>Saindhava Lavana</td>
<td>Rock Salt</td>
<td>---</td>
<td>1 Part</td>
</tr>
<tr>
<td>7.</td>
<td>Sauvarchala Lavana</td>
<td>Black Salt</td>
<td>---</td>
<td>1 Part</td>
</tr>
<tr>
<td>8.</td>
<td>Vida Lavana</td>
<td>Ammonium Chloride</td>
<td>---</td>
<td>1 Part</td>
</tr>
<tr>
<td>9.</td>
<td>Sita</td>
<td>Crystalline Sugar</td>
<td>---</td>
<td>1 Part</td>
</tr>
<tr>
<td>10.</td>
<td>Dhanyaka</td>
<td>Coriandrum sativum Linn.</td>
<td>Fruit</td>
<td>1 Part</td>
</tr>
<tr>
<td>11.</td>
<td>Brihadela</td>
<td><em>Amomum subulatum Roxb.</em></td>
<td>Seed</td>
<td>1 Part</td>
</tr>
<tr>
<td>12.</td>
<td>Rasakarpura</td>
<td>Mercuric Chloride</td>
<td>---</td>
<td>1 Part</td>
</tr>
</tbody>
</table>
Preparation of different samples of Udayabhaskar Rasa:

We have prepared five different combinations of Udayabhaskar Rasa by varying its constituents as shown in Table 2 and evaluated the antimicrobial properties against different pathogenic bacterial strains.

Table 2: Ingredients of five different samples of UBR.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Material</th>
<th>U₁</th>
<th>U₂</th>
<th>U₃</th>
<th>U₄</th>
<th>U₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Shudha Parada</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.</td>
<td>Shudha Gandhaka</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Shunthi (Rz.)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Maricha (Fr.)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Pippali (Fr.)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6.</td>
<td>Saindhava Lavana</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Sauvarchala Lavana</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>Vida Lavana</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Sita</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10.</td>
<td>Dhanyaka</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>Brihadela</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12.</td>
<td>Rasakarpura</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13.</td>
<td>Rasapushpa</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14.</td>
<td>Shuddha Jaipala (Sd.)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>15.</td>
<td>Bijapuraka Swarasa (Fr.) for 7 Bhavanas</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Each drug was taken in 10 gm of quantity except Jaipala seed which quantity was the total sum of other ingredients.

3.1.1 Preparation of sample U₁ (R.K.L.)

Shudha Parada was taken into khalva yantra and then Rasakarpura was added in equal quantity and triturated well. Afterwards Trilavana was mixed followed by Shudha Gandhaka and finally other ingredients were added one by one and mixed well. Bhavana was performed with fresh juice of Bijapuraka in 50 ml. quantity and triturated until whole swarasa had dried up and powdered lastly.

3.1.2 Preparation of sample U₂ (R.Y.S.):

Sample U₂ had same ingredients as sample U₁ but procedure for its preparation was somewhat different. In this preparation, firstly Shudha Parada was triturated with equal quantity of sulphur, then Trilavana followed by Rasakarpura. The ingredients were mixed and grinded. After that one by
one other ingredient were added and properly triturated. Seven Bhavanas of Bijapuraka swarasa were given in the quantity of 50 ml. for each Bhavana. After complete drying of the drug, it was collected and stored till further use.

3.1.3 Preparation of sample U₃:

The procedure of preparation of sample UBR₃ was same as sample U₁ but, Rasapushpa was used in the place of Rasakarpura.

3.1.4 Preparation of sample U₄:

The sample U₄ is devoid of both the ingredients i.e. Rasakarpura and Rasapushpa. Other pharmaceutical procedure was same as the formulation of U₂.

3.1.5 Preparation of sample U₅:

It is devoid of Suddha Jaipala seed. Other ingredients and pharmaceutical process for preparation of this sample was same as sample U₁. After preparation of all five different samples of UBR, the total weight of drugs was recorded

Table 3: Weight of different samples of Udayabhaskar Rasa.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Sample Code</th>
<th>Weight of the total Ingredients before Bhavana (In gm)</th>
<th>Weight of the final product (drug) after 7 Bhavanas (In gm)</th>
<th>Total Weight increased (In gm)</th>
<th>Weight gain in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. U₁</td>
<td>240.0</td>
<td>279.0</td>
<td>39.0</td>
<td>16.25 %</td>
<td></td>
</tr>
<tr>
<td>2. U₂</td>
<td>240.0</td>
<td>280.0</td>
<td>40.0</td>
<td>16.67 %</td>
<td></td>
</tr>
<tr>
<td>3. U₃</td>
<td>240.0</td>
<td>260.0</td>
<td>20.0</td>
<td>8.33 %</td>
<td></td>
</tr>
<tr>
<td>4. U₄</td>
<td>220.0</td>
<td>261.0</td>
<td>41.0</td>
<td>18.63 %</td>
<td></td>
</tr>
<tr>
<td>5. U₅</td>
<td>120.0</td>
<td>157.0</td>
<td>37.0</td>
<td>30.83 %</td>
<td></td>
</tr>
</tbody>
</table>

Therapeutic Applications:

In the treatment of Aamvata, Jalodara, Gulma etc. at a single dose of two gunja (250 mg).

4 Antimicrobial Studies:

Five different pathogenic bacteria were procured from Microbial Type of Culture Collection, Institute of Microbial Technology, Chandigarh (Table 4) for evaluating the antibacterial properties of different Udayabhaskar Rasa. The cultures were maintained on solid agar plate media and stored at 4°C till further use. Well and disc diffusion methods were followed for the testing the antimicrobial properties of different samples. 100 µl of well grown bacterial cell were spreaded on the agar plate and a hole of 6 mm diameter was made aseptically. 25 mg of drug was placed in the well (well method) and incubated at 37°C for two days and zone of inhibition was recorded. In case of disc diffusion assay, different concentration of herbal drug was loaded on sterile disc of whatman paper no 1 (diameter 6 mm) and placed on the agar plate to observe the zone of inhibition at optimal conditions.

Table 4: Pathogenic bacterial strains.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of Bacteria</th>
<th>MTCC No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Staphylococcus aureus</td>
<td>3160</td>
</tr>
<tr>
<td>2</td>
<td>Streptococcus pyogenes</td>
<td>1928</td>
</tr>
</tbody>
</table>
3. Escherichia coli
4. Pseudomonas aeruginosa
5. Salmonella typhi

Results and Discussion:
The antibacterial activity of different formulations of Udayabhaskar Rasa were evaluated against all pathogenic bacterial strains and zone of inhibition was calculated based upon the scales mentioned in Table 5.
<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U₁</td>
<td>20,21,19,20</td>
<td>35,36,35,36</td>
<td>22,23,22,23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean -20</td>
<td>Mean -35.5</td>
<td>Mean -22.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U₂</td>
<td>22,23,21,22</td>
<td>35,36,35,36</td>
<td>31,32,31,32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean -22</td>
<td>Mean -35.5</td>
<td>Mean -31.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U₃</td>
<td>19,20,18,19</td>
<td>28,29,28,29</td>
<td>26,27,27,26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean -19</td>
<td>Mean -28.5</td>
<td>Mean -26.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U₄</td>
<td>13,14,13,14</td>
<td>25,26,26,25</td>
<td>21,22,21,22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean -13.5</td>
<td>Mean -25.5</td>
<td>Mean -21.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>U₅</td>
<td>21,22,20,21</td>
<td>34,35,34,33</td>
<td>25,26,26,25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean -21</td>
<td>Mean -34</td>
<td>Mean -25.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Sterile</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Charcoal Powder)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

The antibacterial properties of different formulations of Udayabhaskar rasa (UBR) proved the importance of compound drugs in the treatment of a number of diseases caused by a number of “krimi’s”. In the treatment of infectious diseases due importance should also be given to restoration of Tridosaha-equilibrium i.e. immunity and bala. Comparing the well and disc diffusion method for antimicrobial assay, the former gave maximum inhibition against all pathogens. In case of disc diffusion method, the formulation U₅ was least sensitive against *S. pyogenes* at all concentrations. *S. aureus* was highly resistant against all formulations of Udayabhaskar rasa.

**REFERENCES**

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6. Rasa Yoga Sagar, Part-I, Pg. No. 160-166
8. Ibid, Verse No. 1522.
Fig. 1: Effect of drugs U1 to U5 on *Streptococcus pyogenes*

Fig. 3: Effect of drugs U1 to U5 on *Escherichia coli*

Fig. 4: Effect of drugs U1 to U5 on *Pseudomonas aeruginosa*
Fig. 5: Effect of drugs U1 to U5 on *Salmonella typhi*

Fig. 6: Effect of different concentrations of drugs on *Streptococcus pyogenes* (Disc diffusion method)
Fig. 7: Effect of different concentrations of drugs on *Staphylococcus aureus* (Disc diffusion method)

Fig. 8: Effect of different concentrations of drugs of *Escherichia coli* (Disc diffusion method)
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