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# STUDY ON ANTIBACTERIAL ACTIVITY OF PATOLA NIMBA RASAKRIYA - AN AYURVEDIC PREPARATION FOR TOPICAL APPLICATION ON DUSTHA VRANA

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## ABSTRACT

*Dushta vrana* (chronic non healing ulcers) is a frequently encountered problem in clinical practice. There are many factors which hamper the normal healing process, wound infection being one among them. Early recognition and effective interventions are more important in reducing the health consequences especially in the context of growing resistance to antibiotics. Microorganisms particularly bacteria multiply, invade and damage tissue, delay healing and occasionally cause systemic illness. Hence it becomes necessary to use anti-microbial agents i.e. antiseptics. *Acharya Susrutha* has mentioned 60 *upakramas* for the treatment of *vrana*, *Rasakriya* is one among the topically used methods to treat *dustha vrana.* Rasakriya means a substance which is thick in consistency. Assessment of its antibacterial and antifungal activity may provide scientific evidence for the study. *Patola nimba Rasakriya* was prepared with classical reference and subjected to analysis and invitro antibacterial activity by agar well diffusion method. Different concentrations of the drug were tested against Staphylococcus aureus and streptococcus pyogenes. *Patola nimba Rasakriya* showed significant zone of inhibition againsts. aureus and s. pyogenes.

**Keywords:** *Rasakriya, patola nimba, Staphylococcus aureus, streptococcus pyogenes.*

## INTRODUCTION

*Susrutha acharya's* contribution in the context of *vrana* and its management is unique; he has enlisted 60 different methods for the treatment of *vrana*[1]. Each of these has specific indications. *Rasakriya* means a substance which is in a thick, concentrated, wet form like collyrium[2] is indicated in wounds were *taila upakramas* fail to provide relief and in *sthira mamsa*[3]. *Rasakriyas* are of 2 types. *Shodhana Rasakriya* and *Ropana Rasakriya*[4]. *Patola nimba Rasakriya* is a form *shodhana rasakriya* and is indicated in *shodhana* of *dusta vrana.* As we

know wound infection and its treatment is a continuing challenge even now. Even though it is virtually inevitable that most wounds contain micro-organisms, many heal successfully. However, sometimes micro-organisms (particularly bacteria) multiply, invading and damaging tissues, delaying healing and occasionally causing systemic illness[5].Among the various bacteria Staphylococcus aureus, streptococcus pyogenes are found to be the commonest. Bacteria may produce problems nearby (spreading infection) or cause systemic illness (systemic infection) Localized infection is often characterized by the classical signs and symptoms of inflammation– pain, heat, swelling, redness and loss of function.

Antimicrobial therapy may be required when other methods of reducing wound bacterial load are likely to be insufficient in localized infection, or when the infection is spreading/systemic. Antimicrobial agents – including antiseptics and antibiotics – act directly to reduce numbers of micro-organisms.

**Preparation of *patola nimba Rasakriya***

*Patola* and *nimba twak* were taken 1.25kg each and to it 16 times water was added and heated till it was reduced to1/8$^{th}$. Then the decoction was filtered and the filtrate was further heated till it becomes thick in consistency[6]. To this the following *prakshepa dravyas* were added after *shodhana*-

1. *Haritala*-50g

Purification was done by *swedana* in *churnodaka* for 3 hours[7].

2. *Manashila*-50g

*Bhavana* in *ardraka swarasa* for 7 times[8].

3. *Sphatika*-100g

Frying in *sharava* [9].

4 . *Kasisa*-50g

*Bhavana* in *bhringaraja swarasa* 1 time[10].

The *prakshepa dravyas* were added, mixed well and then the *rasakriya* was stored in air tight containers. Prior to application on *vrana* it was mixed with honey and *matulunga swarasa*.

**Analytical study**

*Patola nimba Rasakriya* was subjected to physico chemical analysis.

**Table 1:** Standardization parameters for *Patola nimba rasakriya*

| Parameters | Results n = 3 %w/w |
|---|---|
| Total Ash | 13.719 |
| Acid Insoluble Ash | 3.286 |
| Water Soluble Ash | 5.714 |

**Antimicrobial study on Staphylococcus aureus**

**Preparation of nutrient broth:**

Beef extract (1 g), yeast extract (2 g), peptone (5 g), Sodium Chloride (5 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.2 and the volume made up to 1000 ml and autoclaved at 121˚C for 20 minutes.

**Preparation of nutrient agar media:**

Beef extract (1 g), yeast extract (2 g), peptone (5 g), Sodium Chloride (5 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.2 and the volume made up to 1000 ml. Finally 15 g agar was added to the media and autoclaved at 121˚C for 20 minutes.

**Agar well diffusion method:**

The work place was cleaned in laminar air flow using 70% ethyl alcohol and the UV was switched on for 20 minutes. One loop of *Staphylococcus aureus* was inoculated from the culture into 10 ml of nutrient broth and mixed well. 15 ml of the agar medium was uniformly poured over the sterile petridish. 1 ml of nutrient broth

containing the organism was uniformly added over petridish, mixed well and the media allowed to solidify. Five equidistant wells on the plate were made. Then different volumes of test sample *Rasakriya* (50 µl, 25 µl & 12.5 µl) were added to different wells. 50 µl of standard (*Ampicillin*) and control (Honey) were taken separately in to different wells. All the petridishes were icubated at 37˚C for 24 hrs. After the incubation period, the zone of inhibition was measured. Experiment was carried out in duplicate.

| Volumes. ( µl ) | Zone of inhibition (mm) *Rasakriya* |
|---|---|
| 50 | 13 |
| | 13 |
| 25 | 12 |
| | 12 |
| 12.5 | 09 |
| | 09 |

**Table 2: *In vitro* antibacterial activity test for sample *Rasakriya* against *S.aureus.***

Zone of inhibition for standard drug *Ampicillin* was 15 mm at 3 mg/ml concentration and there was no zone of inhibition for control.

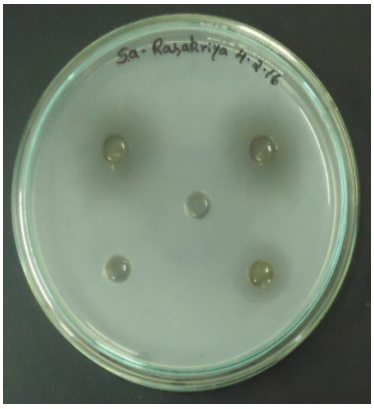


**Figure 1**

**Table 2 and Figure 1:** The antibacterial activity test for sample *Rasakriya* against *S.aureus.*

**Conclusion**: It was observed that antibacterial activity of the test drug increased with increased volume.

**Antimicrobial study on streptococcus pyogenes**

**Preparation of trypticase soya broth:**

Glucose (5 g), casein peptone (15 g), soya peptone (5 g), Sodium Chloride (5 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.3±0.2 and the volume made up to 1000 ml and the media autoclaved at 121˚C for 20 minutes.

**Preparation of trypticase soya agar:**

Glucose (5 g), casein peptone (15 g), soya peptone (5 g), Sodium Chloride (5 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.3±0.2 and the volume made up to 1000 ml. Finally 15 g agar was added to the media and autoclaved at 121˚C for 20 minutes.

**Agar well diffusion method:**

The work place was cleaned in laminar air flow using 70% ethyl alcohol and the UV was switched on for 20 minutes. One loop of *Streptococcus pyogenes* was inoculated from the culture into 10 ml of trypticase soya broth and mixed well. 15 ml of the agar medium was uniformly added over the sterile petridish. 1 ml of trypticase soya broth containing the organism was uniformly added over the petridish, mixed well and the media allowed to solidify. Five equidistant wells were made on the plate. Different volumes of test sample *Rasakriya* (50 µl, 25 µl & 12.5 µl) were added to different wells. 50 µl of standard (*Ampicillin*) and control (Honey) were taken separately in to different wells. All the petridishes were incubated at 37˚C for 24 hrs. After the incubation period, the zone of inhibition was measured. Experiment was carried out in duplicate.

**Table 3: *In vitro* antibacterial activity test for sample *Rasakriya* against *S. pyogenes.***

| Volumes. ( µl ) | Zone of inhibition (mm) *Rasakriya* |
|---|---|
| 50 | 10 |
| | 10 |
| 25 | 09 |
| | 09 |
| 12.5 | 00 |
| | 00 |

Zone of inhibition for standard drug *Ampicillin* was 12 mm at 3 mg/ml concentration and there was no zone of inhibition for control.

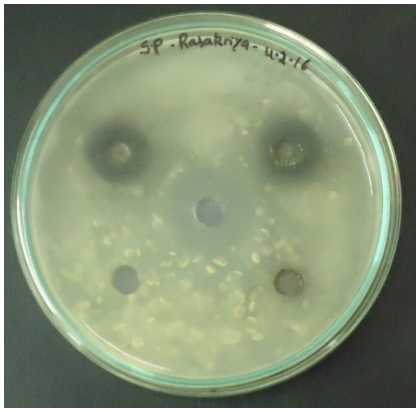


**Figure 2**

Table 3 and Figure 2: **The antibacterial activity test for sample *Rasakriya* against *S. pyogenes.***

**Conclusion**: It is observed that 25 µl and 50 µl volumes of *Rasakriya* showed antibacterial activity against *S.pyogenes.*

## DISCUSSION

*Patola nimba rasakriya* was undertaken for antibacterial study and tested against *Staphylococcus aureus* and *Streptococcus pyogenes*. It was found that with different concentrations of *patola nimba rasakriya*, the zones of inhibition were found to be significant. Higher the concentration of *patola nimba Rasakriya* more was the zone of inhibition. The standard drug used in both study was ampicillin where the zone of in-

hibition was 15mm at 3mg/ml concentration against *Staphylococcus aureus* and 12mm at 3mg/ml concentration against *Streptococcus pyogenes*. However there was no zone of inhibition by the control drug honey against both the bacteria. *Nimba*[11] and *honey*[12] are having *krmighna* property similarly *Haritala* is also attributed to have *bhutahara* property[13] where *bhuta* can be considered as microorganisms, researches on citrus medica have proved its antimicrobial action[14]. From this it is clear that the antibacterial activity of *patola nimba Rasakriya* is due to the combined effect of the drugs present in it.

## CONCLUSION

*Patola nimba Rasakriya* has demonstrated antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes*. The above study has contributed for the evidence base to rationality of using *patola nimba Rasakriya* as topical application in chronic non healing infected ulcers. It also gives further scope for experimental and clinical study on various microorganisms.

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