Rasamruta

ISSN 0976-0075 Ayurveda e-Journal Rasamruta

World's First e - journal of Ayurveda

Scientific Revolution in Ayurveda!

Antifungal Activity of Rasakarpoor – An Experimental Study

Dr.Dinesh Gupta*, Dr.Suvarna P.Nidagundi**, Dr J.G.Mitti***and Dr.M.C.Patil****

D.G.M Ayurvedic Medical College and Hospital Gadag, Karnataka, India

Rasamruta, 5:33, October 2013

Abstract:

Rasakarpoor is a Kupipakva Rasayana, which is said to be of *Nirgandha* (*a preparation without sulphur)* type. According Rasatarangini Rasakarpoor is having a property of *krimigna* (antimicrobial). It is also used in *atisar* (diarrhoea), *twachagatarog* (skin disorders,) *raktadosha shaman* (pacify the diseases because of blood), *spota* (swelling), *kandu* (itching), *mandala*(skin disorder), *phiranga*(syphillis), *kushta*(skin disorder) and *vrunanashana*(destroy the wound) and Ayurveda Prakash mentioned it as *sarvarogahara.* (can be used in any disorder because of its properties) Rasakarpoor is mentioned as *krimigna* (antimicrobial). Hypothesis is that, Rasakarpoor has got some antifungal activity and objective is to evaluate the antifungal activity of Rasakarpoora with the standard drug.(Fluconazole). Antifungal activity of Rasakarpoor is assessed by following Cup-plate method. The results were encouraging. Rasakarpoor exhibits good antifungal activity against many microbes. The results have revealed the new dimension of antifungal activity of Rasakarpoor. The details of the experimental study are explained in the paper.

Key words: *Rasakarpoor*, Antifungal, Fluconazole, *Krimighna* (Antimicrobial).

Introduction:

Kupipakva rasayanas (preparations prepared in the glass bottle) are classified into two types viz. *Sagandha* (a preparation with sulphur) and *Nirgandha*(1) (a preparation without sulphur) . Rasakarpoor

is a *Kupipakva rasayana*(2) (preparations prepared in the glass bottle), which is said to be of Nirgandha type i.e. during the preparation of Rasakarpoor, *Gandhaka* (Sulphur) will not be used directly. Few of the authors have recommended utility of *Gandhakamla* (Sulphuric acid) in the process of Rasakarpoor(3). According Rasatarangini Rasakarpoor is having a property of *krimigna* (antimicrobial). It is also used in *atisar* (diarrhoea), *twachagatarog* (skin disorders,) *raktadosha shaman* (pacify the diseases because of blood), *spota* (swelling), *kandu* (itching), *mandala*(skin disorder), *phiranga*(syphillis), *kushta*(skin disorder) and *vrunanashana*(4)(destroy the wound) and Ayurveda Prakash mentioned it as sarvarogahara(5). (Can be used in any disorder because of its properties)

In case of infection because of microorganisms, many antibiotics such as penicillin cephalosporins, fourquinolones, antiprotozoals, antifungals etc are heavily prescribed by modern medical practitioners, which are considered as the weapons of the Allopathic medical Science. But these drugs cause many hazards to the body such as nausea, vomiting, gastric irritations, metallic taste, destruction of gastric flora, anaphylactic reaction causing even death. There are some good medicines in terms of antibiotics in Ayurvedic treasure of therapeutics to treat the infection by killing Krimi (Microorganisms) without or less complication. Therefore Rasakarpoor has been selected to treat the infection against fungus without complications in vitro for the study.

The microbe (fungi) as the source of infection and diseases is not an alien thing to ayurvedic people. Various herbal, herbomineral and mineral formulations are working against all these diseases from centuries prior to the invention of modern microbiology and germ theory of diseases. As mentioned - rasakarpoor is having *krimigna* (antimicrobial) property. So the hypothesis is that - rasakarpoor possesses antifungal property.

Objective:

Various pharmaceutical procedures (like preparation of rasakarpoora by *kupipakwa* i.e preparations prepared in the glass bottle) are employed to reduce the toxicity, increasing the potency, increasing bioavailability of the rasakarpoora. Objective of the study is to evaluate antifungal activity of rasakarpoora as compared to Fluconazole and to suggest the better medicine for fungal with minimum side effects.

Materials and methods:

Materials:

1) Drugs:

a) Rasakarpoor
b) Fluconazole

2) Micro organism:
a) *Candida albicans*
b) *Aspergillus flavus*

3) Media Ingredients:

a) Potato dextrose agar
b) Nutrient agar
c) Nutrient broth
d) Alcohol

4) Equipments:a) Autoclave
b) Incubator
c) Test tubes
d) Petriplates
e) Conical flasks
f) Cork borer
g) Cotton
h) Vernier caliper
i) Mask and Glows

Test and Standard drugs were prepared in distilled water.

Table no.1: showing the pathogenic activity of each individual.

Sr.no	Pathogen	MTCC No.	Pathogenic activity
1	*Candida albicans*	35	Causes oral and genital infections in humans. It causes systemic fungal infections(fungemias) in immunocompromised patients.(7)
2	Aspergillus flavus	62	Second most common agent of aspergillosis. May invade arteries of the lung or brain and causes infarction. May cause corneal,otomycotic,and naso-orbital infections.(8)

Method:

Anti fungal activity was carried out by Cupplate method and the following procedure were conducted during Antifungal study at S.C.S.Collage of Pharmacy Dept of Microbiology, Harapanhalli**,** Karnataka

Cup-Plate Method: (9)

In this technique the test solution is placed in contact with agar, which is already inoculated with test organism. After incubation, zone of inhibition are observed. The cups were made aseptically with cork borer having 8mm diameter and 3/4th part test solution was poured.

Procedure:

The complete procedure was carried out in 3 stages for Anti Fungal activity of standard and tested drugs. All the chemicals used were of HiMedia company U.S.A and the glassware's used for study are sterilized by the following standard procedure.

Stage I

Preparation of inoculum

Preparation of solutions of different drug concentration (Standard and Tested)

Stage II

Preparation of agar media

Inoculation of test organisms

Application of solutions (Standard and Tested)

Incubation

Stage III

Reading of zone of Inhibition

Interpretation of Results

Stage I

Preparation of Inoculum for Fungi:

Fungi are used in antifungal activity:

1. *Candida albicans* MTCC 35

2. *Aspergillus flavus* MTCC 62

Table No 2: Potato Dextrose Agar composition [HIMEDIA MO96]:

Sr No	Ingredients	Quantity
1	Peptone	200 gm.
2	Dextrose	20 gm
3	Agar	15 gm
4	Distilled water	1000 ml
5	pH	5.6±0.2

Required quantity of PDA was prepared as per the standard ratio.

The solution was sterilized in an autoclave for 15 min at 15lbs/sq.inch.

It was tested for pH and was found 5.6.

After cooling individual fungal cultures are inoculated into separate conical flask by adding 1 ml of stock culture and are shaken well to assure proper mixing.

They are incubated at $27^0C \pm 2^0C$ for 48 hrs.

Preparation of solutions of Tested and Standard drug concentration:

Preparation of test solutions

The Rasakarpoor solutions were prepared with 50 µgm/ml and 100 µgm/ml concentrations and are labeled as T_1 and T_2 respectively. The samples were prepared in distilled water.

Preparation of standard Anti fungal solutions

The "fluconazole" was selected for antifungal activity as standard drug. The solutions were prepared with 50 µgm/ml and 100µgm/ml concentrations and are labeled as S_1 and S_2 respectively

Stage II

Preparation of agar media:

Potato dextrose Agar media was used for testing antifungal activity. The required quantity of Agar media was prepared as per the standard composition of HIMEDIA REF MOO1.

The pH was maintained at 7.4

It was essential for solidification and media has to be sterilized by autoclaving 15 lbs / sq inch for 15 min.

Preparation of Inoculum:

For Fungal culture:

Sterile Nutrient agar medium was cooled to 45^0C and mixed with 20% of respective fungal culture individually (That is 80 ml media and 20 ml culture).

Applications of solutions:

The inoculation prepared has to be transferred to petriplates immediately of its preparation. After solidification of the media, 4 holes are made at equal distance with the help of sterile cork borer (8 mm diameter). These wells are used to inoculating different antifungal drug Fluconazole [S_1 – 50 µgm/ml, S_2 – 100 µgm/ml] and Tested drug Rasakarpoor [T_1 – 50 µgm/ml, T_2 – 100 µgm/ml]

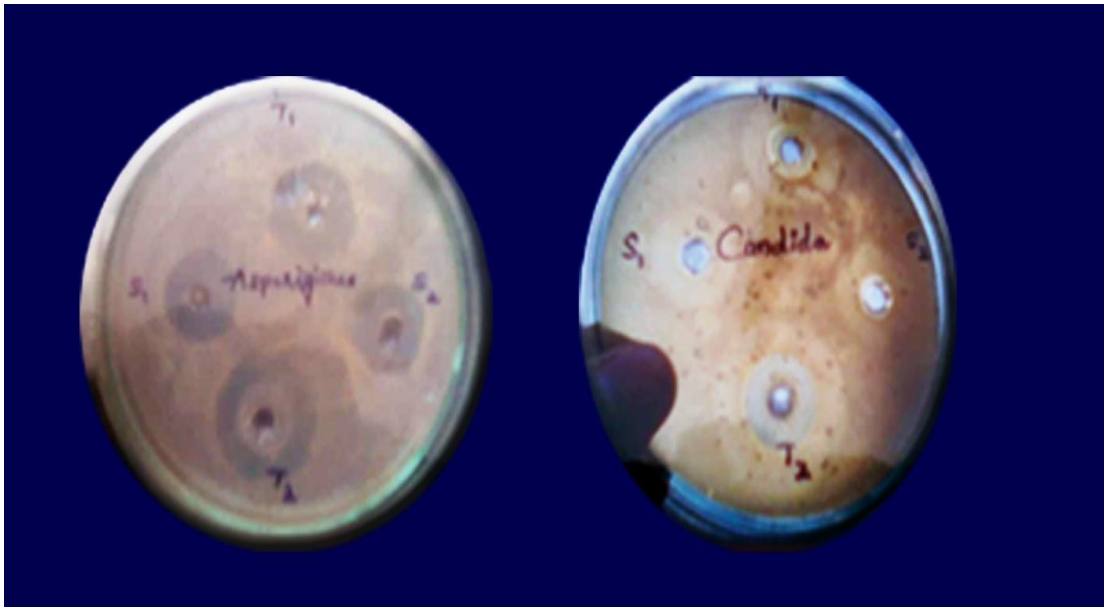
Inoculated media is poured in to the petriplates to a depth of 3 to 4 mm. Ensure that the layer of media is uniform in thickness, by placing the plate on a level surface. All the procedure was carried out under aseptic conditions by using Laminar airflow.

Incubation:

After introduction of Test and Standard drugs, the plates were placed in a refrigerator at 8^0C - 10^0C for diffusion of drugs into the media. After two hours of cold incubation, fungal petriplates were incubated at 27^0C ± 2^0C and maintained at 37^0C ± 2^0C for 48 hrs

After the incubation period, the petriplates were observed for zone of inhibition and measured using the Vernier caliper.

IMAGES : ZONE OF INHIBITION



Results

Antifungal activity:

Table No.4: Efficacy of standard and tested drug against Fungal organism:

Sr No	Organisms	Zone of Inhibition (in mm)			
		50μgm/ml		100μgm/ml	
		Fluconazole S_1	RasakarpoorT_1	Fluconazole S_2	RasakarpoorT_2
1	*Candida albicans*	21.66	29.00	23.50	32.00
2	*Aspergillus flavus*	19.33	28.66	20.66	31.50

The fungal organisms have shown significant zone of inhibition in 50μgm/ml, 100 μgm/ml concentrations when compared with the Standard "Fluconazole" at 50 μgm/ml, 100 μgm/ml concentrations respectively.

Discussion:

Rasakarpoor is Nirghandha Kupipakva Rasayana. It is neglected in Pharmaceutical science, if processed properly and administered in minimal dosage, it is highly effective against Krimi. Many methods are employed for evaluation of antimicrobial activity of a drug like Disc diffusion method, Serial dilution method, Solid dilution method, Ditch plate technique, Gradient plate technique, Cup plate technique. In the present study cup plate method was selected. It is simple and relatively inexpensive which makes it still the method of choice for the average laboratory. (10)Fungi culture was incubated at 27^0C ± 2^0C.Inoculated media poured into the petriplates to a depth of 3 to 4mm and place and platform to get uniform spreading of the media. The growth of the fungal cultures were indicated by the turbidity of the media. Zone of inhibition was measured by Vernier caliper.

Rasakarpoor has much better antifungal activity than the proven drug Fluconazole. It seems Rasakarpoor can be a better alternative to Fluconazole and should be tried in clinical trial also So that we can have a showcase of antifungal Ayurvedic drugs**.**

Conclusion:

The selected microorganisms like *Candida albicans and Aspergillus flavus* are causative agents for many common infections manifested in day-to-day life. An antifungal activity of Rasakarpoor has shown significant activity against fungal organisms compared to standard drug in both the concentrations.

Overall it can be concluded that Rasakarpoor has a significant antifungal activity.

* P.G Scholar of Rasashastra Dept., D.G.M Ayurvedic College and Hospital Gadag

** lecturar of Rasashastra Dept., D.G.M Ayurvedic College and Hospital Gadag

*** Astt. Prof of Rasashastra Dept., D.G.M Ayurvedic College and Hospital Gadag

**** H.O.D of Rasashastra Dept., D.G.M Ayurvedic College and Hospital Gadag

Refrences:

1. Dr.Chandrabhushan Jha. Ayurvedeeya Rasashastra, 1st Edition, Varanasi, Chaukhambha Surabharati Prakashan, 1994, Page No 173-174.

2.Sri Sadanand Sharma, Rasatarangini, Kashinath Shastri, 11th Edition, Varanasi, Motilal Banarasi Dass, 2004, Chapter No 6,shloka no.68-71 Page No 115-116.

3. Sri Sadanand Sharma, Rasatarangini, Kashinath Shastri, 11th Edition, Varanasi, Motilal Banarasi Dass, 2004, Chapter No 5, Page No 72.

4. Sri Sadanand Sharma, Rasatarangini, Editor Kashinath Shastri, 11th Edition, Varanasi, Motilal Banarasi Dass, 2004, Chapter No 6, Sloka No 72-75, Page No 117.

5. Acharya Madhava, Ayurveda Prakasha, Editor Sri Gulrajsharma Mishra, 2nd Edition, Varanasi, Chaukhambha Bharati Academy, Reprint 1999, Chapter No 1,Sloka No 416-417, Page No 200-201.

6. R.S.Satoskar, Pharmacology and Pharmacotherapeutics, 16th Edition, Mumbai, Popular Prakashana, 1999, Section XII, Chapter No 45, Page No 682.

7. Warren E.Levinson, Medical Microbiology and Immunology, 3rd Edition, London, Prentice-Hall International Inc. 1994, Part V, Chapter No 50, Page No 245-246.

8. Warren E.Levinson, Medical Microbiology and Immunology, 3rd Edition, London, Prentice-Hall International Inc. 1994, Part V, Chapter No 50, Page No 247.

9. R.S.Satoskar, Pharmacology and Pharmacotherapeutics, 16th Edition, Mumbai, Popular Prakashana, 1999, Section XII, Chapter No 45, Page No 682.

10. W.B.Hugo, Pharmaceutical Microbiology, 2nd Edition, Oxford, Blackwell Scientific Publications, 1980, Part I, Chapter No 10, Page No 189-191.