



Research Article

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STUDY OF ANTIFUNGAL ACTIVITY OF HINGULIYA MANIKYARASA IN *CANDIDA ALBICANS* BY ITS VARIOUS CONCENTRATIONS

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ABSTRACT

By the use of microbiological studies an Ayurvedic physician can choose accurate drug for particular disease. Keeping this view in mind present study was conducted to screen antifungal activity of Hinguliya manikya rasa on *Candida albicans*. For this, Hinguliya Manikyarasa was prepared by Kupipakwa rasayana method as given in Rasatarangini. The culture and sensitivity method used was tube dilution method (Agar slant method) so in the present study the drug was serially diluted from 0.5mg/ml concentration to 8mg/ml concentrations. All 90 samples were used and it was found that *Candida albicans* shows significant sensitivity to Hinguliya manikya rasa at 8mg/ml concentration level as compare to Placebo.

Keywords: *Candida albicans*, Hinguliya Manikyarasa, Culture and sensitivity by Tube dilution (Agar slant Method).

INTRODUCTION

Diagnosis of the diseases can be done by Biochemistry, Microbiology and Pathology in medical science. They help the clinicians for the proper diagnosis of diseases because improper or late diagnosis and failure in the treatment leads to death. Acharya Charaka in Indian Medical Science has given three sutras or basic pillars as Hetu (Causes), Linga (Symptoms) and Aushadha (Medicine), in which Aushadha have unique importance¹. In this modern era Aushadh i.e. Ayurvedic drugs are to be tested in the light of modern scientific techniques. Rasaushadhis are mainly prepared with the help of mineral or metallic compounds. These Rasaushadhis requires lesser dose, these are tasteless and having immediate action in body. Due to these properties Rasaushadhis are better than herbal drugs². An arsenical compound comes under Rasaushadhi category. There are several Ayurvedic Rasaushadhis in practice to cure skin diseases since long time. But the need of the hour is to provide clear cut evidences to show the effectiveness of the Ayurvedic drug in a disease by laboratory findings. By employing microbiological analytical techniques for assessing the severity and type of the disease an Ayurvedic physician can easily choose the drug and try to evaluate its effectiveness specifically to the particular disease, by which specificity of the drug can be attained. A microbiologist can analyze the Indian medicinal plant based drug for its antifungal effects, on pathogens and at different dose levels. For example, if a skin diseased patient comes, the skin scrapping can be examined for the type of organism and proved antifungal Ayurvedic drugs for the particular organism can be prescribed to cure the patient quickly and effectively. Organic Arsenicals are useful in the treatment of protozoal infections caused by trypanosomes. Very little information is available about the usage of inorganic Arsenical Ayurvedic drugs as antimicrobial agents so it is important to study antimicrobial activity of Arsenicals compounds which are used in Ayurved. In 1990, Dr. U K Pillai did Ph.D. thesis

on "Standard Preparation of Harital Bhasma and its clinical efficacy in certain skin diseases at I.P.G.T. and R.A. Gujarat Ayurveda University, Jamnagar and found that the Harital Bhasma produced significant growth inhibition of *Staphylococcus aureus* and *Candida albicans* while it did not produce any growth inhibition to *Pseudomonas aeruginosa* and *Escherichia coli*. As Harital is one of the ingredients of Hinguliya Manikyarasa and according to the above mentioned study Harital bhasma shows antifungal activity, this study was planned to assess the effect of Hinguliya Manikyarasa in serial dilution on the common fungus i.e. *Candida albicans*.

Aim

Observe the antimicrobial activity of Hinguliya Manikyarasa against *Candida albicans* by culture and sensitivity method.

Objectives

Identify the organism by its morphological and cultural characteristics. To observe the sensitivity or resistance of Hinguliya Manikyarasa, *in vitro*.

Hypothesis

Does *Candida albicans* are sensitive to Hinguliya Manikyarasa?

MATERIALS AND METHODS

Materials

Literature review was done from classical Ayurvedic texts, Modern literature and Web references regarding Hinguliya Manikyarasa and culture sensitivity of microorganisms (i.e. fungus).

Test fungus

Candida albicans - The cultures of the fungus were obtained from the Department of Microbiology M.P. Shah Medical College, Jamnagar, India.

Methods

Organisms were grown on artificial media in laboratory and then isolated. The fungus *Candida albicans* was isolated. The tubes containing the fungus were exposed to Hinguliya Manikyarasa and these tubes were kept in incubator at 37°C for 24 hours. Then the results were observed. This study includes collection of appropriate sample, fungal culture, isolation and identification, preparation of Hinguliya Manikyarasa and sensitivity or resistance of fungus to Hinguliya Manikyarasa.

Collection of specimen

The Collections of appropriate specimen was taken into a sterilized container and was sent for culture and sensitivity to the laboratory. 15 samples were used for each dilution as well as for placebo i.e. total 90 samples.

Fungal culture, isolation and identification

Fungus from specimen was grown on an artificial medium in laboratory. Isolation and identification was made after growth of fungus, by cultural, morphological and biochemical characteristics.

Material for fungal culture

Sabouraud's agar, Petri dish, Nichrome wire loop, Spirit lamp, Incubator, Hot air oven, Normal saline, Slides, Autoclaved test tubes, Gram stain kit, appropriate specimen, Inoculation chamber, Gloves, Microscope and Sterile container.

Procedure for fungal culture and sensitivity

The antimicrobial parameters were determined by serial dilution with agar slant method³.

Dilution tests

Employ medicine in serially diluted concentrations in solid agar or broth media containing a culture of the test microorganism. The Minimal Inhibitory Concentration (MIC) was determined by the agar slant method. The lowest concentration of the tubes which did not show any visible growth after 18-24hours of incubation in macroscopic evaluation was considered as the MIC. Many anaerobes can only be tested by a dilution method and this is also true for many fungi. The dilutions can be made in broth or in agar⁴.

Observation of colony character

Candida albicans differ in their shapes, size, elevation, margin, surface texture, color and consistency.

Test micro-organisms (*Candida albicans*)

Candida albicans is yeast like fungus. It is an ovoid or spherical budding cell, which produces pseudomycelia both in cultures and in tissues. *Candida* species are normal inhabitants of the skin and mucosa. Cultures can be obtained readily on Sabouraud's and on ordinary bacteriological culture media⁵.

Isolating characteristics

Colonies are creamy white, smooth and are with a yeasty odor.

Pathogenicity

Candida albicans causes infections of skin, mucosa and rarely of internal organs known as Candidiasis⁶.

Test Drug

Ingredients of Hinguliya Manikyarasa

- Purified Hingula (Cinnabar) - 90g
- Purified Gandhak (Sulphur) - 90g
- Purified Harital (Yellow Orpiment) - 90g
- Juice of Flower of Palash (*Butea frondosa*) - Q.S.

Hinguliya Manikyarasa prepared by textual method as given in Rasa Tarangini by Kupi-pakwa Rasayan method before that Hingul, Harital and Gandhaka were purified as per the references given in the texts and the purified minerals were triturated with the juice of flowers of *Butea frondosa*⁷⁻⁸. The drug prepared by this method was used in the form of suspension in 3% Tween 80 solution.

Media

Subourauds agar (Himedia India Limited)

Antifungal activities of drug were screened by serial tube dilution method as described by Cruickshank and Co-workers (1975). This method consisted of incorporating Hinguliya Manikyarasa in various concentrations in growth media suitable to the test organism and monitoring the growth. Initially pilot study was carried out on 7 samples of *Candida albicans* by using 0.5mg/ml concentrated and 8mg/ml solutions of Hinguliya Manikyarasa.

Composition of medium - Sabouraud's Agar

- Dextrose - 20g
- Peptone - 5g
- Agar - 10g
- Distilled Water - 500ml

All the three ingredients dissolved in 500ml of distilled water and pH was adjusted to 7.6

Procedure

The Sabouraud's medium was reconstituted and pH was adjusted to the required level and sterilized by autoclaving for 1hour under 20pound pressures per square inch. Simultaneously a series of drug concentration were prepared by serial dilution. Initially in one test tube drug solution of 16mg/ml was prepared. 1ml drug solution was taken from above test tube in which 1ml distilled water was added to it, thus drug solution of 8mg/ml was prepared. Again 1ml drug solution from this test tube was taken and by adding 1ml distilled water a drug solution of 4mg/ml was prepared. By the same process drug solution of 2mg/ml, 1mg/ml and 0.5mg/ml concentration were prepared. For each time 6 autoclaved test tubes were used in which one was for control and 5 were for test drug solutions. 5 test tubes were taken and 9.5ml Sabouraud's agar was added in each test tube and 0.2ml drug solution of each concentration was added in each test tube except control in which 0.2ml of 3% Tween 80 was added and mixed well. Again the test tubes were autoclaved. After autoclaving Sabouraud's medium (with drug solution) slant were prepared and kept for incubation for 24hours to rule out contamination. The cultured organisms were

taken and inoculated over slants. The slants were incubated and fungus observation was made after 8days. The same procedure was carried out for 15 samples of each dilution.

RESULTS AND DISCUSSION

Initially a pilot study was carried out in which 0.5mg/ml and 8mg/ml drug concentrations of Hinguliya Manikyarasa were selected. In each group 7 samples along with Placebo group were observed. The results showed that growth inhibitions were not observed i.e. complete turbidity was seen in all the 7 samples of 0.5mg/ml concentration and Placebo group which indicates that the *Candida albicans* was not sensitive at this drug concentration level. The growth inhibition was seen. It means that the tubes were completely clear in all the 7 samples of 8mg/ml concentration indicating that the

Candida albicans was sensitive at this drug concentration level. So the study was conducted by using serial dilution from 0.5mg/ml to 8mg/ml concentrations (Table 1). The drug Hinguliya Manikyarasa was serially diluted and concentrations were maintained as 0.5, 1, 2, 4 and 8mg/ml. In case of 0.5mg/ml and 1mg/ml drug concentrations of Hinguliya Manikyarasa, the results shown that growth inhibitions were not detected i.e. turbidity was seen in all the 30 samples which mean that the *Candida albicans* was resistant at these drug concentration levels (Table 2). In case of 2mg/ml and 4mg/ml drug concentrations of Hinguliya Manikyarasa, the results showed that partial growth inhibitions i.e. intermediate turbidity were seen in all the 30 samples indicating that *Candida albicans* were partially sensitive at these drug concentration levels (Table 3).

Table 1: Table showing Effect of 0.5mg/ml and 8mg/ml concentrations of Hinguliya Manikyarasa and Placebo on *Candida albicans* during Pilot study

Concentration	Turbidity	Intermediate Turbidity	Clear
Placebo	07	00	00
0.5mg/ml	07	00	00
8 mg/ml	00	00	07

Growth inhibitions were not observed in all the 7 samples of 0.5mg/ml concentration and there was growth inhibition seen in all the 7 samples of 8mg/ml concentration. So the study was conducted by using serial dilution from 0.5mg/ml to 8mg/ml concentrations.

Table 2: Table showing Effect of 0.5mg/ml and 1mg/ml concentrations of Hinguliya Manikyarasa and Placebo on *Candida albicans*

Concentration of Hinguliya Manikyarasa	Turbidity	Intermediate Turbidity	Clear
0.5mg/ml	15	00	00
1mg/ml	15	00	00
Placebo	15	00	00

Growth inhibitions were not observed in all the 15 samples of 0.5mg/ml and 1mg/ml concentrations and in Placebo group as well.

Table 3: Table showing Effect of 2mg/ml and 4mg/ml concentrations of Hinguliya Manikyarasa and Placebo on *Candida albicans*

Concentration of Hinguliya Manikyarasa	Turbidity	Intermediate Turbidity	Clear
2mg/ml	00	15	00
4mg/ml	00	15	00
Placebo	15	00	00

The intermediate turbidity was observed in all the 15 samples of 2mg/ml and 4mg/ml concentrations and complete turbidity was observed in Placebo group.

Table 4: Table showing Effect of 8mg/ml concentration of Hinguliya Manikyarasa and Placebo on *Candida albicans*

Concentration of Hinguliya Manikyarasa	Turbidity	Intermediate Turbidity	Clear
8mg/ml	00	00	15
Placebo	15	00	00

The tubes were observed completely clear in all the 15 samples of 8mg/ml concentration and complete turbidity was observed in Placebo group.

Table 5: Table showing effect of all concentrations of Hinguliya Manikyarasa and Placebo on *Candida albicans*

Concentration of Hinguliya Manikyarasa	Turbidity	Intermediate Turbidity	Clear
Placebo	15	00	00
0.5mg/ml	15	00	00
1mg/ml	15	00	00
2mg/ml	00	15	00
4mg/ml	00	15	00
8mg/ml	00	00	15

Table 6: Table showing statistical analysis of Hinguliya Manikyarasa and Placebo on *Candida albicans*

Concentration of Hinguliya Manikyarasa	Significant / Not Significant
0.5mg/ml	Not Significant
1mg/ml	Not Significant
2mg/ml	Significant
4mg/ml	Significant
8mg/ml	Highly Significant
Placebo	Not Significant

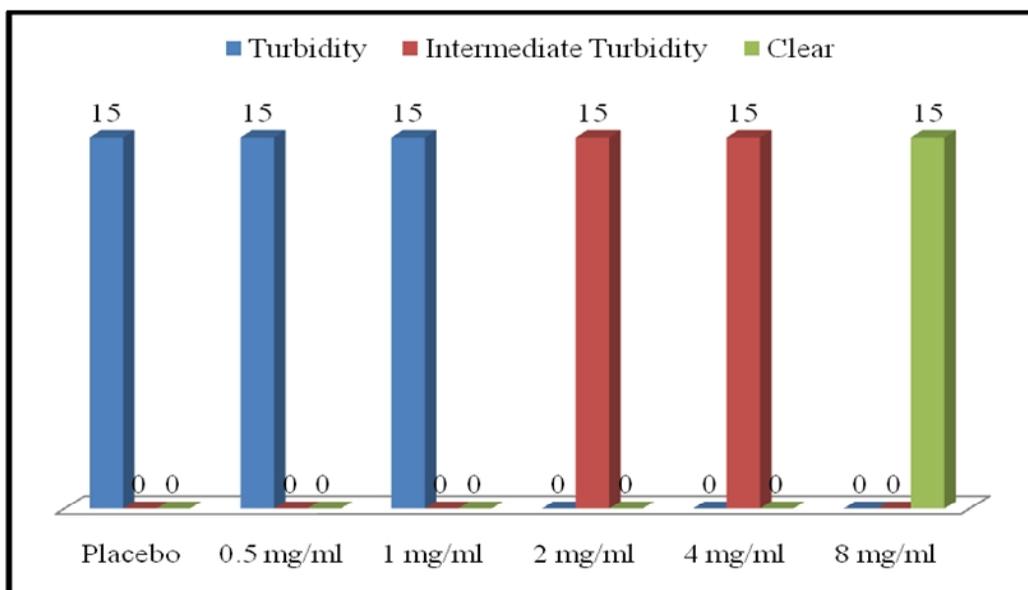


Figure 1: Effect of all concentrations of Hinguliya Manikyarasa and Placebo on *Candida albicans*

When the study was carried out at 8mg/ml drug concentrations of Hinguliya Manikyarasa, the results were observed that complete growth inhibitions i.e. the samples tubes were seen clear indicating that *Candida albicans* were sensitive at this 8mg/ml drug concentration level.

As this was the lowest concentration level where complete growth inhibition was observed which should be considering as minimum inhibition concentration (MIC) (Table 4). In all the Placebo group and samples having 0.5mg/ml, 1.0mg/ml concentrations turbidity was observed in all 15 samples whereas the samples having 2.0mg/ml, 4mg/ml concentrations showed intermediate turbidity (i.e. significant) and the sample having 8mg/ml concentration showed clarity (i.e. Highly significant) (Table 5-6 and Graph 1).

CONCLUSION

In the present study of antifungal activity of various concentrations of Hinguliya Manikyarasa, the drug did not show antifungal activity at 0.5mg/ml and 1mg/ml concentration, but the test drug had shown partial antifungal activity at 2mg/ml and 4mg/ml concentrations whereas complete antifungal activity was observed at 8mg/ml drug concentration (highly significant) for *Candida albicans*.

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