

IN-VITRO ANTIFUNGAL ACTIVITY OF GANDHAKA RASAYANA

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Abstract

Many herbo-mineral remedies individually or in combination have been recommended in various medical treatises for the cure of different diseases. *Gandhaka Rasayana* is a compound preparation, being used by Ayurvedic physicians in clinical practice for thousands of years, in the treatment of skin diseases of bacterial and fungal origin. So the drug is selected to study its anti-fungal effect on *Candida albicans*. Candidiasis is the most common mycotic disorder of the skin affecting the immune-compromised patients and those on the anti-biotic therapy. Hence the anti-candidal activity was examined in the study. For the study Broth Dilution method was adopted to study the Minimum Inhibitory Concentration (MIC) and Minimum Contact Time (MCT) of the Gandhaka Rasayana, following NCCLS guidelines. **Results:** *Gandhaka rasayana* showed antifungal effect against *Candida albicans* with Minimum inhibitory concentration (MIC) of 5.00mg/ml of Brain Heart Infusion (BHI) Broth and Minimum Contact Time (MCT) above one hour.

Key words: Candidiasis, Minimum inhibitory concentration, Minimum contact time, *Gandhaka rasayana*,

Introduction:

Fungal diseases of the skin can be divided into superficial mycoses and deep mycoses. Superficial mycoses are infections restricted to invasion of horny structures like the stratum corneum while the deep mycoses infect deeper layers. Deep mycoses are much more common in immuno-compromised patients including these with AIDS, transplanted patients on corticosteroid or immuno-suppressive therapy. *Candidiasis* is one of the most common fungal infections affecting both the superficial as well as deeper layers of the skin and mucous membranes. Though candidiasis can be caused by various species of *Candida* (like *C. albicans*, *C.stellatoidea*

tropicalis, *C. krusei*, *C. viswanathii* etc), *Candida albicans* is a causative species in about 80-90% of cases. *Candida albicans* is a normal inhabitant of gastro-intestinal tract, oral and vaginal cavities. Various manifestation of candidiasis include mucocutaneous lesions (like oral thrush, vulvovaginitis, conjunctivitis etc), various skin and nail infections (commonly found in areas macerated by perspiration like axillae, groin, perineum, diapered area of infants, etc). Deeply invasive candidiasis is often preceded by increased superficial colonization of mouth, vagina, GIT, skin etc., mostly following broad spectrum antibiotic therapy. Factors which decrease the immune-competence of the person (e.g.: diabetes mellitus, HIV infections, corticosteroid therapy etc.) favour its growth. *Candida* can pass from colonized surface into deep tissue when integrity of skin or mucosa is violated. Local factors like

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third degree burns, trauma and wounds, surgery, intravenous drug abuse may prove favourable for deep invasion.

To find an affective *Ayurvedic* remedy for the treatment of the candidiasis *Gandhaka rasayana* is selected for the study. *Gandhaka Rasayana* is one of the important formulations used in the treatment all types of *kusthas* (skin diseases), *nadivrana* (sinuses) and *kostha gata roga* (Gastro-intestinal disorders). Thus based on its use on certain skin diseases, since ancient times, the present study “In-Vitro Anti-Fungal Activity of *Gandhaka Rasayana*” was undertaken to prove its efficacy as an Anti-fungal agent especially on *Candida albicans*.

Aims and objectives:

- To carry out Purification of the raw drug – *Gandhaka* (sulphur) and.
- To prepare *Gandhaka Rasayana*
- To find the Anti-fungal action of the prepared *Gandhaka Rasayana* and
- To determine the Minimum inhibitory concentration (MIC) & Minimum Fungal dosage (MFD) of prepared sample against *Candida albicans*.

Materials and methods:

Raw materials were collected from genuine sources and subjected initially for the purification process.

Gandhaka Shodhana (Purification of Sulphur):(1) (*Rasa ratna samuchaya 3/21-23*)

Materials: *Gandhaka* – 500 gm (Figure 1), *Godugdha* – 2 lts, Mud pot, *Sthali yantra*, Cloth, lace or wire.

Method: *Bhudhara method* (Figure 2)

Procedure: A mud pot was taken and filled with 2 lts of *Godugdha* (Cow milk) and a cloth is tied to the mouth of the pot with a lace or wire. On the cloth 500 gm of *Gandhaka* was placed and is covered with

Sthali yantra (Lid). Then the pot is placed in a pit made in the ground such that the pot along with the lid exactly fits into it. Then it is covered with sufficient number of *Vanyopalas* (Cow dung cakes). The cow dung cakes are then burnt till they become ash. The *Gandhaka* melts and drops into the milk leaving the impurities in the cloth. After the ashes get self cooled, the mud pot is taken from the pit and the *Gandhaka* is collected from the milk and washed in hot water. Thus the pure *Gandhaka* (Figure 3), devoid of impurities is obtained. This is used for the preparation of the compound *Gandhaka Rasayana*.

Observations:

<i>Gandhaka</i>	<i>Before shodhana</i>	<i>After shodhana(b)</i>
Weight	500g	496g
Appearance	Yellow, crystalline	Small globular and lustrous

Preparation of Gandhaka Rasayana:(5)

Materials:

- *Suddha gandhaka* (purified sulphur),
- *Godugdha* (Cow Milk),
- *Chaturjata Kwatha* (Decoction prepared by the combination of *Tvak* (*Cinnamomum zeylanicum* Lume.), *Patraka* (*Cinnamum tamala* Nees.), *Ela* (*Elettaria cardamomum* Maton.) and *Nagakesara* (*Mesua ferrea*)),
- *Guduci swarasa* (Juice of the leaves of *Tinospora Cordifolia*),
- *Triphala kwatha* (Decoction prepared by the combination of the fruits of *Hareetaki* (*Terminalia chebula*), *Vibhitaki* (*Terminalia belerica*) and *Amalaki* (*Phyllanthus embelica*)),
- *Sunthi kwatha* (Decoction of dried ginger (*Zingiber officinalis*),

- *Bhringaraja swarasa* (Juice of the whole plant of *Eclipta alba*) and
- *Ardraka swarasa* (Juice of the wet rhizomes of *Zingiber officinalis*)
- Kharala yantra

Method: *Bhavana (Trituration)*

Procedure: *Suddha gandhaka* (purified sulphur) was subjected to *bhavana* (trituration) first with *Godugdha* (Cow milk) followed by the trituration with *Chaturjata Kwatha*, *Guduci swarasa*, *Triphala kwatha*, *Sunthi kwatha*, *Bhringaraja swarasa* and *Ardraka swarasa*, for 8 times each. Then the Gandhaka is collected and mixed with equal quantity of Sugar and preserved in an airtight bottle. This compound is called as Gandhaka Rasayana.

Anti Fungal Study:

After the drug is prepared, the antifungal study was conducted in MICROBIOLOGY DEPARTMENT OF JSS MEDICAL COLLEGE, MYSORE. The study is as follows.

Procedure to know the Minimum Inhibitory Concentration (MIC):

Preparatory procedures:

A) To make *Gandhaka Rasayana* suitable for the study, it was made into fine powder and stock solution was prepared by adding 10 ml of BHI broth (Figure 5) to 100 mg of *Gandhaka rasayana*. The stock solution was then diluted to prepare three different samples of different concentration of the drug and were named as follows.

Sample I – Containing *Gandhaka Rasayana* in a concentration of 10 mg/ml of the BHI Broth.

Sample II – Containing *Gandhaka Rasayana* in a concentration of 5 mg/ml of the BHI Broth.

Sample III – Containing *Gandhaka Rasayana* in a concentration of 2.5 mg/ml of the BHI Broth.

B) Control groups: Two solutions named as positive control group and negative control group were taken as follows:

Positive control group: In which fungal suspension was maintained to check the viability of organisms.

Negative control group: Was only BHI broth for validation of the test was used.

C) A suspension was prepared with standard strain of *Candida albicans* matching to 0.5 Mc Farland turbidity standards.

Method:

To study the antifungal activity, In-vitro study by Broth Dilution Method was adopted. In this five test tubes were taken and filled with 1 ml of the samples of Sample I, sample II, Sample III, Positive control group and Negative control group respectively. To the test tubes named as Sample I, Sample II and Sample III, 1 loop full of *Candida albicans* matching to 0.5 Mc Farland turbidity standards was added. Then all the five test tubes (sample group and control group) were incubated at 37°C for 24 hrs.

After 24 hrs of incubation, the test tubes were taken out on wet mount separately for each sample and were examined microscopically for viability of the *Candida*. Then one loop full of material was taken out from each test tube aseptically and inoculated on five different Sabouraud's dextrose agar (SDA) plates separately for the sample groups and the control groups respectively. All the plates were then incubated at 37°C for 24 hours to promote the fungal growth.

On the third day the plates were examined for presence or absence of fungal growth (colony formation).

Procedure adopted to know the Minimum Contact Time (MCT):

5.0mg of the prepared sample (*Gandhaka Rasayana*) was added to 5ml of

BHI broth containing *Candida albicans*. Subcultures were prepared from the solution at different times i.e., immediate, after half-an-hour, one hour, one and half hour, two hours, two and half hours, three hours and four hours, on the SDA (Sabouraud's dextrose agar) and were incubated at 37° C for 24 hours for the viability of the *Candida*. After 24 hours, the SDA media was examined for the growth of the fungi.

Observations and results:

Minimum Inhibitory Concentration (MIC): (Table 1)

After 24 hours of incubation of SDA plates, positive control group and the Sample III were showing the growth of the *Candida* (Figure 6), indicating that the *Candida* were viable through out the experiment and the Concentration of *Gandhaka Rasayana* at 2.5 mg/ml of BHI Broth was not sufficient to inhibit the growth of the *Candida*. Thus it can be said that the Minimum Inhibitory Concentration (MIC) of *Gandhaka Rasayana* for *Candida* is 5 mg/ml of BHI Broth.

SDA plates	<i>Candida albicans</i>
Positive control group	Growth present
Negative control group	Growth absent
Sample I	Growth absent
Sample II	Growth absent
Sample III	Growth present

Minimum Contact Time (MCT): (Table 2)

It was observed after 24 hours of incubation that the growth of *Candida* was seen till half an hour of the adding of the *Gandhaka Rasayana* and was absent later. This indicates that the minimum contact

time (MCT) of *Gandhaka Rasayana* to act anti-fungally should be above one hour.

Table 2: Showing the Effect of Gandhaka rasayana on the Candida cultures inoculated at different times

Time of subculture after inoculation	Growth
Immediately after adding sample	Present
Half an hour	Present
One hour	Absent
One & Half hour	Absent
Two hours	Absent
Two & Half hour	Absent
Three hours	Absent
Four hours	Absent

Discussion and conclusion:

Ayurvedic System of medicine has its long history of therapeutic potential. Many drugs used by the Ayurvedic physicians have good therapeutic efficacy, but they lack scientific validity regarding the standard dosage patterns and the duration of the treatment to be adopted. Hence an experimental proof in this will help the mankind to use the medicine much efficaciously in the therapeutic practice.

In the present study, *Gandhaka Rasayana*, a well known Ayurvedic compound was taken for the in-vitro anti-fungal study especially on the *Candida albicans*. *Gandhaka Rasayana* has a wide range of therapeutic actions as mentioned in the Ayurvedic classics. It is used in diseases like Kushta, Kandu etc., which show a clear involvement of bacterial and fungal activity. Thus the drug is selected for the study to know its anti-fungal activity.

Candidiasis is the most common mycotic disorder affecting the immune-compromised patients like Diabetes, AIDS,

post-transplant cases etc. So for this study, *Candida albicans* was selected from the fungal organisms as it is the most common cause of the candidiasis.

Standard sample of the *Gandhaka Rasayana* is prepared and is used. Broth dilution method was adopted to know the Minimum inhibitory concentration (MIC) and the Minimum Contact Time (MCT).

From the above study it was observed that the Minimum inhibitory Concentration of the of *Gandhaka Rasayana* on the *Candida* cultures is above 5 mg/ml of the BHI Broth and the minimum Inhibitory Concentration is above one hour.

Thus it can be concluded that *Gandhaka Rasayana* has the anti-fungal activity on *Candida albicans* organisms when studied in-vitro.

Further research has to be carried out to find out the effect of *Gandhaka Rasayana* on *Candida albicans* and to know its effects in-vivo and complications of using the drug.

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Figure 1: Impure *Gandhaka* (Sulphur)



Figure 2: *Bhudara puta*



Figure 3: Purified *Gandhaka*



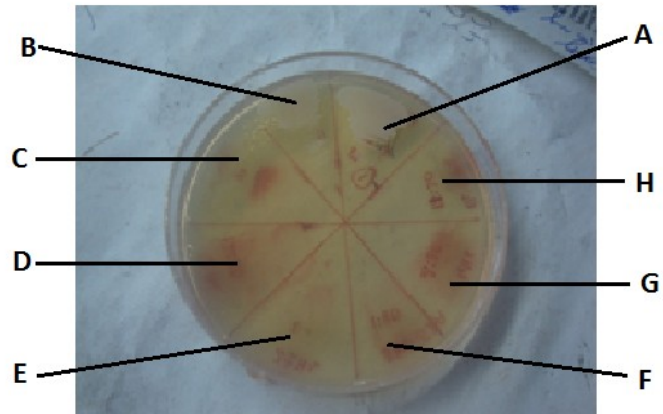
Figure 4: *Gandhaka Rasayana*



Figure 5: SAMPLE WITH BHI BROTH



Figure 6: *CANDIDA ALBICANS*

Figure 7: Subculture of Candida on SDA at different intervals of time

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|------------------------------------|--------------------------|
| A. Immediately after adding sample | B. After half an hour |
| C. After One hour | D. After One & Half hour |
| E. After Two hours | F. After Two & Half hour |
| G. After Three hours | H. After Four hours |

AYURVEDA SAHITI PRABHA'S