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# Antimicrobial Study of *Nishothamadi Kashayam* in Microbes Causing Dermatophytosis W.S.R to *Dadru Kushtha*

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**Abstract**
**Introduction:** *Dadru* is one of the most common but miserable variety of *Kushtha* affects the population of all the age groups and stands as a challenge to different medical systems. Dermatophytosis can be closely correlated with the classical disease *Dadru kushtha.* Dermatophytosis is the most important cutaneous infection, generally confined to the layer of the skin and its appendages. There are about 40 species of fungus that can cause Dermatophytosis in humans, but the most common belong to three genera- Trichophyton, Microsporum, and Epidermophyton. Superficial fungal infections are one of the commonest infections affecting humans. The recent prevalence of dermatophytosis in India ranges from 36.6-78.4%. It is presumed that the antimicrobial property present in most of the ingredients of *Nishothamadi kashayam* are useful in deciding the probable action of this formulation on *Dadru*.
**Aim:** Antimicrobial study of *Nishothamadi kashaya* is against Dermatophytes causing *Dadru*.
**Materials and Methods:** *Nishothamadi kashaya* made from Haridra, Haritaki, Amalaki, Vibhitaki, Patola, Katuki, Vacha, Manjishta, Nimba and an Anti-bacterial study was done by using Well Diffusion Method and Sabouraud's agar was used to evaluate anti-bacterial activity against 3 selected Dermatophytes.
**Results:** The studied formulation shows very enthusiastic result to treat the fungal disease of the skin.

**Keywords:** Dermatophytosis, *Nishothamadi Kashaya*, *Dadru*, Dermatophytes

## Introduction

Dermatophytosis or tinea is a predominance in about 20%-25% of all total world populations [1]. Dermatophytosis is the disease that mainly caused by different species of dermatophytes within the cutaneous layer of the skin. This disease considers a common fungal infection in different parts of the human body which enrichment with keratin, especially hair, skin, and nails [2]. It is also called ringworm when the lesion appears as a ring shape with a clear centre and inflammatory edge. Tinea is another term of dermatophytosis which could take a different name based on the infected site of the human body. This Infection usually occurs in both genders at different ages. Scaling, pustules, itching, inflammation, and hair and nail loss are the characters of most dermatophytosis infection [3].

The fungal diseases of the skin can be divided into superficial mycoses and the deep mycoses. Superficial infection involving keratinized tissue is called as dermatophytosis [4].The infection is commonly designated as ringworm or tinea. The term literally means insect's larva. The dermatophytosis are classified as tinea cruris, corporis and so on depending on the part affected. These infections are restricted to invasion of horny structures like stratum concern, the nails and the hair [5]. They do not involve deeper

structures. Ringworm is caused by twenty species of dermatophytes fungi which are grouped into three genera. Most species of dermatophytes produce two types of asexual conidia. Microconidia are small and unicellular whereas macroconidia are large and septate and may have thick or thin walls. The latter spore, provide differentiation of the dermatophytes into three genera [6] Epidermophyton, Microsporum, Trichophyton are mainly considered to be the most common disease-causing dermatophytes.

*Nishothamadi kashayam* is a concentrated decoction prepared out of herbal ingredients. Ingredients of this formulation are safe, easily available and economical and are also having pitta kapha hara, vrana ropana and kushtaghna properties. Nishothamadi kashayam is explained in Ashtanga hridaya chikitsa sthana [7]. It is also mentioned in Yoga Ratnakara, Ashtanga sangraha, Charaka Samhita and Vaidhya sara sangraha [8]. While describing the treatment of pitta- kaphaja kushta.

Numerous antifungal medicines are mentioned in various textbooks of Ayurveda which are used to cure various skin infections and other diseases. Despite their slow action, their therapeutic use is becoming popular because of lower side effects. Unlike synthetic drugs, Ayurvedic medicines have the ability to control resistant microorganisms. These Ayurvedic drugs have been used from centuries to cure various cutaneous fungal infections (*Kustha Roga*), but there is no scientific explanation for their actions. In this research work, an initiative was taken to prove the antimicrobial activity of one such common Ayurvedic formulation on an experimental basis. For this, an Ayurvedic formulation named *Nishothamadi Kashaya* was taken for study to assess the antimicrobial effect on dermatophytes.

## Material & Methods

To evaluate the antimicrobial activity of *Nishothamadi Kashaya* a study was carried out by Agar Well Diffusion method on 3 selected Dermatophytes.

### Materials

- **Drugs**
  Test drug: *Nishothamadi Kashaya*
  Standard drug: Amphotericin B
- **Micro-organisms**
  Trichophyton Tonsurans
  Microsporum Canis
  Trichophyton Rubrum
- **Equipments**
  Loops and loop holder, Petri dish, Conical flask, Hot air oven, Test tubes, Inoculation hood, Beakers, Autoclave, Funnel, Incubator, Stirrer, Spirit lamp, round bottom flask.

### Methodology

#### a) Preparation of Sample

Haridra, Haritaki, Amalaki, Vibhitaki, Patola, Katuki, Vacha, Manjishta and Nimba were taken in fresh form and converted into small pieces and crushed. All the drugs(90gms) were mixed together thoroughly to prepare a homogenous blend, which was shifted to a stainless-steel vessel, added with 16 parts of water (1440ml) and subjected to mild heat. When the volume was reduced to one fourth (180ml), the contents were filtered through a clean cloth into a stainless-steel vessel to obtain kwatha.

#### b) Preparation of Sabouraud's Agar Media

Glucose (40 g) and peptone (10 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 5.5 and the volume was made up to 1000 ml. Finally, 20 g agar was added to the media and autoclaved at 121˚C for 20 minutes.

#### c) Preparation of the Inoculum

The test fungal species was procured from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh. Loopful of 10 days old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum.

#### d) Identification of Fungal Colony Morphology

Rate of growth, pigmentations, texture and colony surface was assessed for macroscopic appearance of the colony.

#### e) Microscopic Examination of Fungi

To study the microscopic appearance of the fungal isolates grown in culture by lactophenol cotton blue.

**Requirements**

- Lactophenol cotton blue
- Glass slides
- Teasing needle
- Microscope

A bit of fungal colony was taken out from the culture tube and lactophenol cotton blue mount was made on a slide and viewed under microscope. Septate or aseptate, hyaline, narrow or wide and conidia were observed.

#### f) Antifungal Sensitivity Test

**Requirements**

- SDA media plates
- Micro pipette
- Four different concentrations of sample
- Incubator

Well diffusion method was selected for the present study. This method includes incorporation of wells. Sensitivity test was done using SDA (Sabouraud's dextrose agar) media by well diffusion method, with four different concentrations (25 ml, 50 ml, 75ml, 100 ml) of the sample.

#### g) Well Diffusion Method

The media was cooled to around 45-55˚C, around 20ml each was poured into sterile Petri plates. One ml of the inoculum was immediately added to the plate, swirled for uniform distribution. Wells were bored using a sterile borer. The samples and the antibiotic were dispensed into the wells. Plates were incubated overnight at 25˚C and observed after 10 days. After the incubation period, the zone of inhibition was measured.

## Results

**Table 1:** In vitro antifungal activity of *Nishothamadi Kashaya* against Microsporum canis.

| Sample | Volume | Zone of inhibition-(Radius in mm) | |
|---|---|---|---|
| Nishothamadi Kashaya | 25 µl | 9 | 9 |
| | 50 µl | 12 | 12 |
| | 75 µl | 15 | 15 |
| | 100 µl | 16 | 16 |
| Control (DD) | 50 µl | 0 | 0 |
| Standard (Amphotericin B) 10 mg/0.5 ml | 100 µl | 10 | 10 |

**Result:** *Nishothamadi Kashaya* shows marked antifungal activity against Microsporum canis.

**Table 2:** In vitro antifungal activity of *Nishothamadi Kashaya* against Trichophyton rubrum

| Sample | Volume | Zone of inhibition- (Radius in mm) | |
|---|---|---|---|
| Nishothamadi Kashaya | 25 µl | 7 | 7 |
| | 50µl | 8 | 8 |
| | 75µl | 10 | 10 |
| | 100 µl | 10 | 10 |
| Control (DD) | 50 µl | 0 | 0 |
| Standard (Amphotericin B) 10 mg/0.5 ml | 100 µl | 11 | 11 |

**Result:** Nishothamadi Kashaya shows marked antifungal activity against Trichophyton rubrum.

**Table 3:** In vitro antifungal activity of *Nishothamadi Kashaya* against Trichophyton tonsurans.

| Sample | Volume | Zone of inhibition- (Radius in mm) | |
|---|---|---|---|
| Nishothamadi Kashaya | 25 µl | 6 | 6 |
| | 50 µl | 8 | 8 |
| | 75 µl | 10 | 10 |
| | 100 µl | 10 | 10 |
| Control (DD) | 50 µl | 0 | 0 |
| Standard (Amphotericin B) 10 mg/0.5 ml | 100µl | 12 | 12 |

**Result:** *Nishothamadi Kashaya* shows marked antifungal activity against Trichophyton tonsurans.

## Discussion

Antimicrobial activity is a technique in which response of an organism to a particular anti-microbial agent can be established. Many methods are employed for evaluation of anti-microbial activity of a drug. In the present study well diffusion method was selected. The antifungal activity of the *nishothamadi kashaya* was assayed against three selected Dermatophytes namely Trichophyton Tonsurans, Microsporum Canis, Trichophyton Rubrum. The assay was done at different concentrations of the sample using Amphotericin B as standard to understand the most effective anti-microbial activity.

The study revealed that *Nishothamadi Kashaya* has significant antifungal activity in the concentrations like 25µl, 50µl, 75µl and 100µl as shown by agar well diffusion method. 25µl volume of *nishothamadi kashaya* has shown sensitivity towards all the 3 selected dermatophytes, but high sensitivity is towards Microsporum Canis with 9mm of zone of inhibition. 50µl volume of *nishothamadi kashaya* has shown sensitivity towards all the 3 selected dermatophytes, but high sensitivity is towards Microsporum Canis with 12 mm of zone of inhibition. 75µl volume of *nishothamadi kashaya* has shown sensitivity towards all the 3 selected dermatophytes, but high sensitivity is towards Microsporum Canis with 15mm of zone of inhibition. 100µl volume of *nishothamadi kashaya* has shown sensitivity towards all the 3 selected dermatophytes, but high sensitivity is towards Microsporum Canis with 16 mm of zone of inhibition. The standard drug Amphotericin B was sensitive to all 3 fungal organisms and highly sensitive against Trichophyton Tonsurans with 12mm of zone of inhibition. Control group (DD) did not show any sensitivity towards any organisms.

*Nishothamadi kashaya* has shown sensitive against all organisms. This may be due to the presence of *Haridra,* Haritaki, Amalaki, Vibhitaki, Patola, Katuki, Vacha, Manjishta, Nimba which are said to be *krimighna* and have proven anti-microbial properties. Amphotericin B act only as an antifungal agent and may produce adverse effects on human beings but *nishothamadi kashaya* not only act as anti-microbial agent but have additional properties like rejuvenation and promotes positive health by increasing the immunity, thus making the body resistant against disease causing factors.

## Conclusion

*Nishothamadi kashaya* exhibited a broad-spectrum antimicrobial activity against dermatophytes it inhibited the growth of Trichophyton Tonsurans, Microsporum Canis and Trichophyton Rubrum When compared in between the groups, Microsporum Canis has displayed a significant antifungal activity (16mm ZOI) at higher concentration (100µl). In vitro experimental study has shown that selected dermatophytes have significant antifungal activity at higher concentrations (75µl and 100 µl). However, the results are

significant too when compared with standard drug Amphotericin B (11-12mm ZOI). In vitro study of *nishothamadi kashaya* indicates that the formulation was stable and has effective antimicrobial activity.

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