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Research Article

"EVALUATION OF IN VITRO ANTIFUNGAL ACTIVITY OF DADRUNASHAK LEPA"

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ABSTRACT

In recent years, there is leap in incidences of skin disorders in tropical developing countries like India. Due to various environmental factors, unhygienic conditions, poor food quality, poverty etc. Dadru is one such Kushtha prakaar. It is a Kapha Pitta pradhan twakvikaar affecting all age groups. It can be broadly correlated with Tinea infection in modern science.

"**Dadrunashak Lepa**" is one such exclusive lepa from Ravankruta Arka Prakash which is useful in Dadruchikitsa. Here is a study of Antifungal activity of this Dadrunashak lepa on two fungi namely Trichophyton Rubrum and Microsporum Canis which are the causative agents of Tinea infection (Dadru Kushtha).

Antifungal activity was done using well diffusion method and it was observed that this lepa has an excellent Antifungal activity due to synergistic action of its ingredients such as Kushtha, Laksha, Chakramarda, Haridra, Sarshapa, Saindhava and Amrasthi on the fungi.

Keywords: Dadru**,** Tinea infection, Antifungal activity (Well diffusion method).

INTRODUCTION

Since India is fast developing nation with newer techniques coming up each day, there is a leap in pollution, health ignorance, increased hot and humid climate, lowering the standard of living and unhygienic conditions.

This broadly comprises the cause of skin diseases mainly fungal infections, bacterial infections and allergies. Tinea fungal infection is one of the most common fungal infection growing in such conditions.

In Ayurveda the causes, symptoms, signs, and treatment of skin diseases is comprised in Kushtha Chikitsa. Tinea infection is highly comparable with Kshudra Kushtha prakaar DADRU. Dadru is an original Sanskrit word which means daridra "durgato". Due to this disease, man lost his wealth and consequently lost his good health. Due to Dadru, the varna (the contour) of the body gets ceased. So it causes daridrya of health. It is one type of skin ailment (Kushtha in ayurvedic term),where it's Nidaan and Chikitsa is well explained in Ayurvedic texts.

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Since this disease which is highly increasing in today's world it should be accounted for its treatment. Thus looking forward for a chance to prove the world the veracity of Ayurveda. Arka Prakash is a classical treaty written by Lankapati Ravana, it is a book in which he has given importance to both Bhaishajya and Rasashastra.

This book deals with the special dosage form Arka (Distillation) which has more shelf life and better palatability. Apart from classical reference it has mentioned five main preparations as panchavidha kashaya Kalpana like Kalka, Choorna, Swaras, Taila, Arka. In the fifth chapter of Arka Prakash book, there is a refrence of Dadrunashak Arka which contains seven major ingredients namely Kushtha, Laksha, Chakramard, Haridra, Saindhav, Sarshap and Amrasthi. This formulation can be used in Dadruchikitsa by preparing Arka out of it or can be used for local application by preparing choorna of the above drugs as explained in the shloka. This lepa selected in this research study is supposed to be efficacious in treatment of Dadru (Tinea infection) on the basis of their properties according to several classical references.

This study has been taken to evolve new treatment for Dadru kushtha with respect to Ayurvedic formulations.

कुष्ठं कृमिजददृघ्ननिशासैन्धवसर्षपाः।

आम्रास्थिश्चैतदर्कौ वा लेपाद्दद्रूंविनाशयेत् ॥ (अ. प्र. ५/१०१)

AIMS AND OBJECTIVES

Aim:

To study the Antifungal activity of Dadrunashak Lepa.

Objectives:

Study the literature on Dadru & Tinea Infection from Modern texts.

Perform Antifungal Activity of Dadrunashak lepa.

MATERIAL AND METHOD

Under this heading antimicrobial study of three sample of Dadrunashak Arka was studied against Trichophyton Rubrum and Microsporun Canis.

Materials –

A) Drugs: Sample of Dadrunashak Arka

B)Microorganisms: –

a) Trichophyton Rubrum b) Microsporum Canis.

C) Equipments:–

1) Digital balance

2) Incubator

3) Autoclave

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4) Inoculation hood
5) Hot air oven
6) Heating mantle

D) Glasswares:-
1)Petri dish
2)Conical flask
3)Test tube
4)Beaker
5)Funnel
6)Stirrer

E)Chemicals:-
1)Nutrient broth
2)Nutrient agar
3)Disttiled water
4)Surgical spirit

Method:-
Antifungal activity of Dadrunashak lepa was carried out by Agar well Diffusion method.

Principle:-
The microbial assay is based on the comparison at the inhibition of growth of micro organisms by measured conc.of the drug to be examined.
The antifungal activity was carried out by Agar well diffusion method.

The method depends on the diffusion of the drug from a cavity or well through the solidified agar layer of a petridish to an extent, such that growth of the added microorganisms is prevented entirely in a circular area or zone around the cavity containing a solution of the drug.
The rate and degree of diffusion may be affected by concentration and type of salt, viscosity of solution, solubility, temperature, etc. It is the improper to compare the therapeutic value of the antimicrobial agent on the basis of size of the zone of inhibition as same excellent therapeutic agents diffuse poorly in agar vice versa.

Procedure:-
Preparation:-All aspects of the Agar well diffusion method procedure are standardized to adhere to these standards. The media used in this testing was Sabouraud dextrose agar containing Chloramphenicol and cycloheximide at only 4mm deep was poured into either 100mm or 150mm petridishes. The pH level of agar must be between 7.2 and 7.4.

Incubation Procedure:
1) Using cork borer a well of 8mm diameter was punched in the medium.

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2) Using an aseptic technique, the fungal spores were harvested and standardized to approx. 10^7 CFU/ ml. It was individually spread by a sterile swab evenly over the face of agar plate and then gently remove the excess liquid by gently pressing or rotating the swab against the tube.
3) Test material - Dadrunashak lepa choorna in 100 ul quantity was then applied to each well. The plates were incubated at 28˚C for 5 – 7 days.
Zone of inhibition were measured by calibrated ruler.
4) Allow the plate to dry for approximately 5 minutes.
5) The plates were incubated at temperature of 28°C.

A) Preparation for inoculums:-
A loop of organisms was emulsified in 100 ml sterile growth media under proper sterile condition and incubated for72 hrs at 37^0C in incubator.

E)Preparation of Agar Plates:-
-5ml of inoculums prepared was added to 45ml of flask containing nutrient agar at 37^0C.
-This was immediately powered into dry sterile petri dish to the depth of 5mm.
-The Petri dish were placed on a leveled surface to ensure that layers of medium are of uniform thickness.
-Allow the plates to solidify at room temperature for 12hrs.
-Incubate the agar plates at 35^0C to check sterility.
-The surface of the agar layer was kept dry before use.
-With the help of sterile borer (diameter 8 mm) cylinder were made in agar plates.
-Uniform volume (i.e.0.5ml) of test solutions were added to each cavity.
-After 30 mins agar plates were incubated at 37^0C for 72 hrs.
-Zone of inhibition was measured after 72 hrs.
-The diameter of the circular zone is the measurement of the zone of inhibition.

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similar to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or tip, and a volume (20–100 mL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. The agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

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RESULT

Observations:

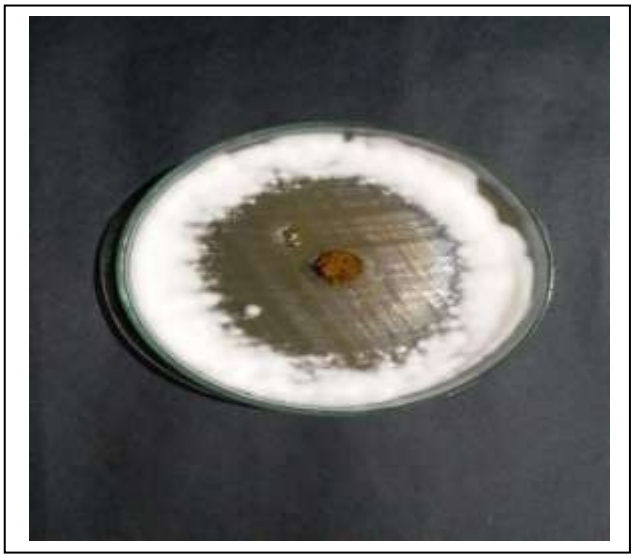
3. Observations and Result of Antifungal study.

In vitro Antifungal Test of Dadrunashak lepa choorna was carried out on following Microorganisms:

a) Trichophyton Rubrum
b) Microsporum Canis

Following Table shows Zone of Inhibition in Agar Media in mm of the Dadrunashak Arka:

Sr.No.	Test fungus	Sample of Dadrunashak Arka Zone of inhibition in mm
1.	Microrosporum Canis	30 mm
2.	Triichophyton Rubrum	29 mm



According to zone of Inhibition and criteria of assessment the results are as follows-

1. The zone of Inhibition was found to be 30 mm against Microsporum Canis Fungus, while it was found that the zone of inhibition to be 29 mm against Trichophyton Rubrum.
2. Hence we can say that this Kalpa, Dadrunashak Arka is definitely beneficial in Microsporum genus than in Trichophyton genera.

Thus we can state that, in Invitro Antifungal Activity of Dadrunashaka Arka for above organisms shows good fungicidal activity against both Microsporum Canis and Trichophyton Rubrum

CONCLUSION

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- Invitro antifungal activity of Dadrunashaka Arka clearly shows that this kalpa has an excellent inhibitory action on Microsporum Canis fungi as compare to Trichophyton Rubrum.
- Agar well Diffusion method is convenient, cost effective method for evaluation of Antifungal Activity of this kalpa.

DISCUSSION

Trichophyton Rubrum and Microsporum Canis were selected for the study. Since these fungi are most commonly found in natural environment. These are the major causative factors for many fungal infections.

In present study nutrient medium is the Agar medium which is chosen as culture media for the fungus to grow.

Results were expressed by determining the zone of Inhibition measuring in mm by using vernier caliper. Microsporum Canis and Trichophyton Rubrumboth has shown remarkable results in Antifungal activity against Dadrunashak Arka. Since the zone of Inhibition is around 30mm and 29mm respectively, it is said to be very effective against both the fungi.

All the ingredients of Dadrunashka Lepa such as Kushtha, Chakramarda, Haridra, Sarshapa, Laksha, Saindhava and Amrasthi are krimighna and kushthagna.

All the drugs are Katu-tikta-kashaya rasatmaka, Ushna veerya, and katu vipaakatmak. Also they are kaphahara and kleda shoshaka. Since Dadru vyaadhi is kaphaj vyadhi the combination of these drugs explained in Ravankruta Arka Prakash aids to eliminate the disease completely. Which hence proves that Ayurvedic formulations prepared and interpretated by our acharyas and explained in our granthas are potent and highy recommended in todays era too.

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