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CULTURE FOR FUNGI AND SENSITIVITY TEST OF KARANJBEEJADI LEPA- A YOGA OF CHAKRADATTA

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ABSTRACT

Karanjabejadi lepa is a *yoga* of *Chakradatta* indicated in the disease *alas* (T.pedis). Experiments of classical medicines on modern parameters are essential today. Cultures of skin scrapings were done for fungi and then sensitivity test of this drug was done. This drug was found not effective experimentally but was found effective clinically. Further studies are required for its sensitivity test in other solvents.

Keywords: *Karanjbeejadi lepa*, culture for fungi, sensitivity test, DTM, SDA

INTRODUCTION

The experimental study of classical drugs mentioned in the *Ayurvedic* texts is the need of today. Experiment is the base of development of science. Any branch of science cannot grow without experimentation. *Karanjbeejadi Lepa* has been mentioned in *Chakradatta* in the management of *Alas*[1]. To see the characteristics of alas[2,3], it can be correlated with tinea pedis[4,5]. This *yoga* contains *Karanja (Pongamia glabra)*[6], *Haridra (Curcuma longa)*[7], *Mulethi (Glycerrhiza glabra)*[8], *Honey and Kasis.Gorochan* and *hartal* have not been included in the yoga due to problem in availability. *Neem (Azadirachta indica)*[9] has been included in the yoga due to its antimicrobial activities. The experimental study of *"Karanjbeejadi churna"* was done in the department of microbiology Dr. G. C. Negi College of veterinary sciences, C.S.K.H.P.K.V. Palampur, Himachal Pradesh, India.

MATERIAL AND METHODS

Collection of samples –

Skin scrapings: Infected areas were cleaned with 70% alcohol and allowed to dry. Then skin scrapings were collected from the edge or the active border area of lesions with sterile scalpel blade. The scraping was kept in sterile vials and brought to the laboratory.

Processing of samples for fungal organisms –

1. *Direct microscopic examination* – Samples were taken on a clear glass slide with 2-3 drops of 10% KOH. The slide was gently heated to clear off debris and thereafter cover slip was placed over it. The preparation was examined under both low and high power of microscope for the fungal structures (conidia, hyphae, spores)

2. *Cultural examination (isolation of fungi)* – 6 samples of skin scraping were cultured in petriplate having DTM. The preparation was kept in fungal incubator at 25^0C (Fig. 1). The growth of fungal organisms along with change in the color of the DTM from yellow to red within 14 days indicated the presence of dermatophytes (Fig. 2 (a) & (b)). In those samples of skin scraping, Trichophyton spp. were detected in 2 samples.

In one sample mucor was detected and in another sample rhizopus was found. There was no any growth found in the rest of 2 samples. The details of culture were as follows:

Sr. No.	Date of inoculation	Result seen on	Finding
1.	06 Oct 09	20 Oct 09	Trichophyton spp.
2.	06 Oct 09	20 Oct 09	Mucor
3.	06 Oct 09	07 Nov 09	No growth
4.	28 Oct 09	11 Nov 09	Trichophyton spp.
5.	28 Oct 09	29 Nov 09	No growth
6.	11 Nov 09	18 Nov 09	Rhizopus

The pertriplates showing fungal growth were kept for further identification of fungi and those showing no fungal growth at 25^0C upto 4 weeks were discarded.

Macroscopic structure of dermatophytes

Sr. No.	Organisms	Growth				
rate	Texture	Topo-graphy	Surface pig-ment	Reverse pigment		
1.	Microsporum canis	Rapid	Cottony	Flat	White to yellow`	Translucent yel-low or absent
2.	M. gypseum	Rapid	Powdery to granular	Flat	Tan to cin-namon brown	Yellow to tan
3.	M. ferrugineum	Slow	Waxy to slight velvety	Flat	Yellow rusty or pale	Yellow rusty or pale
4.	M. audouinii	Moderate	Velvety	Flat	With	Pale salmon to pale brownish

* Fungi were seen in the laboratory.

Microscopic structure of dermatophytes

Sr. No.	Organisms	Macroconidia	Microconidia
1.	Microsporum canis	Abundant spindle shaped, thick echinulated	Present
2.	M. gypseum	Abundant, elliptical thin, echinulated	Presented
3.	M. ferrugineum	Very few	Rare
4.	M. audouinii	Rare, deformed	Drop shaped microconidia pecti-nate branching
5.	Trichophyton men-tagrophytes*	Clavate or variable thin, smooth	Spherical

6.	T. verrucosum	Rare, irregular, induced in thiamine medium	Rare, inducedon thi-amine medium

* Fungi were seen in the laboratory.

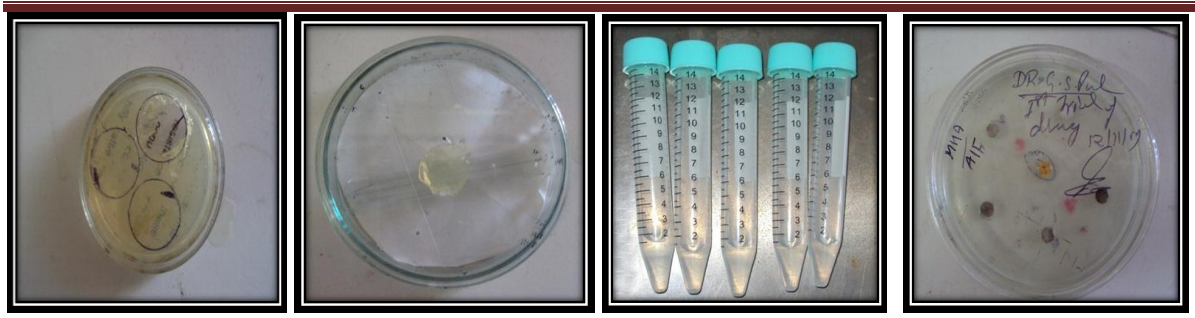
Identification of fungi:-

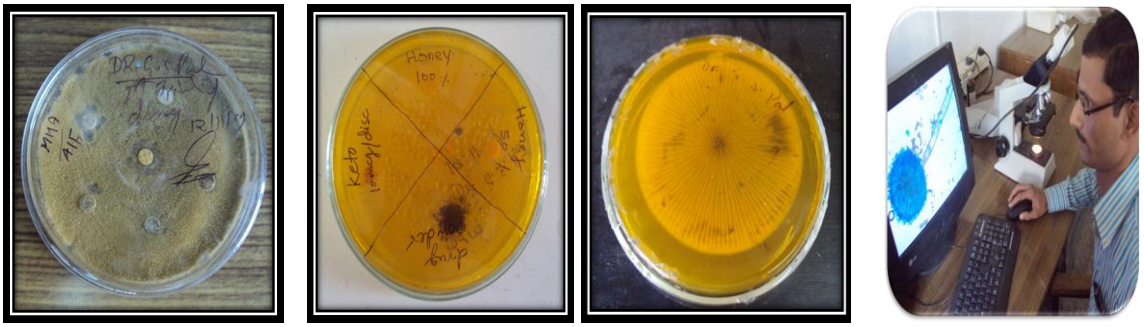
1. *Wet mount technique* –A drop of lacto phenol cotton blue stain was placed on a clean glass slide. With the help of sterile needle, some material of fungal colony was added to the stain and material was teased properly by a sterile needle. A cover slip was properly placed on it. The preparation was examined under microscope and spores and hyphae were identified. (Fig. 3)
2. *Slide culture* – A block of 5x5x2 mm of sterile SDA was cut from the petriplate with the help of sterile needle. The block was placed on a clean sterilized glass slide. With the help of sterile needle, some fungal colony was inoculated at four sites of the periphery of the block. Then sterilized coverslip was placed on the agar block with the help of sterilized forceps. Then, the slide was kept on V shaped glass tube placed at the bottom of the petriplate containing the blotting paper soaked in sterile water. The preparation was incubated at 25^0C. When the growth was complete the cover slip with adhering mycelia was removed and put on a drop of lacto phenol cotton blue stain on another slide and examined under the microscope. (Fig. 4 & 5) Drug sensitivity test-

1%, 2%, 3%, 4% and 5% concentrations of drug were prepared in sterilized distilled water (Fig. 6). Preparation were centrifuged and kept in water bath for sometimes for dissolving. It was found that the drug was not dissolved completely in the distilled water. Some colony of fungi was taken by sterilized swab and swab was dipped in sterile NSS. Then the swab was touched properly on the base of petriplate containing MHA. Then 5 sterilized microdiscs were kept on the periphery of the base of the petriplate. A drop of different concentrations of drug was dropped on these microdiscs with the help of pipette containing sterilized tips. Then a disc of Amphotericin B was kept in the centre of the petriplate (Fig.7). Preparations were kept in incubator. After 4 days the petriplate was examined. No growth was found at the site of Amphotericin B. Fungal growth was found in the petriplate and also at the site of drug. (Fig.8) Thus, this drug is not effective against fungi at these concentrations. Same procedure was repeated with 100% honey, 50% honey, dry drug and ketoconazole, these were applied on the petriplate and kept in incubator at 25^0C (Fig. 9). The petriplate was examined after 4 days. No growth was found at the site of ketoconazole. Fungal growth was found in the petriplate and also at the site of drug.

PROCEDURES





RESULTS

The drugs were found not effective against fungi in the laboratory. Further studies are required to optimize the proper dissolution of drug in other solvents which should then be tried at various concentrations.

CONCLUSION

Although this *yoga* is found not effective in *alas* (T. pedis) experimentally, this is effective clinically. This means the drugs have antifungal property but could not be detected through this method in the laboratory. Other methods or other solvents should be tried for further studies.

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