ORIGINAL ARTICLE

Antifungal activity of *Karanja taila* on *Indralupt* w.s.r. to alopecia: An *in vitro* study

Brahmdutt Sharma[1], Govind Sahay Shukla[2], Tanuj Bhatia[3], Chinky Goyal[3]

[1]Department of Rasa Shastra and Bhaishjya Kalpana, Shekhawati Ayurved Medical College, Pilani, Rajasthan, India, [2]Department of Rasa Shastra and Bhaishjya Kalpana, Post Graduate Institute of Ayurveda, Jodhpur, Rajasthan, India, [3]Department of Rasa Shastra and Bhaishjya Kalpana, Saraswati Ayurved Hospital and Medical College, Gharuan, Mohali, Punjab, India

Abstract

Introduction: *"Karanja taila"* is an oleaginous medicament indicated in Indralupt, whereby Indralupt disease can be correlated with Alopecia areata of Biomedical Science. There are many reasons of alopecia-like genetic cause, lack of nutrition, tension, and infection. However, in the present study, much emphasis has been laid on the "infective cause," which if persist for a long time can cause hair loss. **Material and Methods:** Antifungal activity of three different samples (S1, S2, and S3) of *Karanja taila* (S1, S2, and S3) prepared as per the reference of Sharangdhara Samhita was conducted on pathogens that causes alopecia, namely, *Trichophyton tonsurans*, *Microsporum canis*, *Trichophyton rubrum*, and *Microsporum audouinii* using well diffusion method. Culture of the above-defined fungi was taken and medicated oil at room temperature was applied on it. After a specific time period, the zone of inhibition was noted to calculate the effectiveness of the drug. **Observation and Results:** The inhibition zone in Samples S1, S2, and S3 and positive control (5% w/w vancomycin) against pathogenic strains *T. tonsurans*, *T. rubrum*, *M. canis*, *M. audouinii* found to be (17, 14, 15, 17 [S1]), (16, 15, 17, 16 [S2]) (15, 14, 16, 17 [S3]) (25, 23, 29, 31 [positive control]) respectively. The activity index in Samples S1, S2, and S3 for pathogenic strains *T. tonsurans*, *T. rubrum*, *M. canis*, *M. audouinii* found to be (0.68, 0.61, 0.52, 0.55 [S1]), (0.64,0.65,0.59,0.52 [S2]) (0.60, 0.61, 0.55, 0.55 [S3]) respectively. **Conclusion:** The activity index of all the samples against the specified fungi is >0.5, which depicts their profound effect on alopecia.

Key words: Alopecia, antifungal, *Ayurveda*, *Indralupt, Karanja taila*

INTRODUCTION

Sneha Kalpana has its peculiar contribution to our system among all other derivative formulations mentioned in Ayurveda. It is a pharmaceutical process to prepare oleaginous medicaments (oil and ghee) from the substances like *Kalka, Kwath,* and Drava *dravyas*, in specific proportions by subjecting to a unique heating pattern and duration to fulfill certain pharmaceutical parameters, according to the need of therapeutics.[1] It ensures the fat-soluble and water-soluble properties of dravya on the basis of their nature (*swabhav),* effect (*prabhav*), and absence (*abhava*). This process leads to the transformation of active therapeutic properties of the ingredients to the solvent. It is a very effective process and has various advantages such as it enhances the absorption of drugs, when used topically in fat media. Usually, Ghrita (ghee) or *Taila* (oil) are used as media of extraction, whereas in rare case, *Vasa* and *Majja* are used. Sneha are of four types: *Ghrita, Taila, Vasa, and Majja*.[2] It also has extra benefits of specific *Taila*/Ghee (nutritive), which is used to preserve the drug for longer time as it increases the bioavailability of drugs due to its *Sukshma, Vyavayee Gunas. Sneha Kalpas* are the only dosage form which can be used both internally and externally. Sneha Kalpana is prepared in three phases. The first phase consists of Sneh Murchana, followed by the second phase of Sneha Paka and finally there is third phase called *Paka Siddhi*.[3] *Acharyas* have different opinions about the use of formulations (ausdha) prepared by *Sneha Kalpana* that is *Pana, Nasya, Basti, Abhyanga,* and *karnpurna*.

Address for correspondence:
Dr. Chinky Goyal, Associate Professor,
Department of Rasa Shastra and Bhaishjya Kalpana,
Saraswati Ayurved Hospital and Medical College,
Gharuan, Mohali, Punjab, India.
Mobile: +91-9815144152. E-mail: chinkygoyal52@gmail.com

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"Karanja taila" is also an oleaginous medicament indicated in *Indralupt.*[4] *Indralupt* is described by *Acharya Sushruta* and *Vagbhatt* as a *shiroroga,* in which the vitiation of Pitta and Vatados has cause hair loss. *Pitta* presents in hair follicles is usually associated with *Vata* which makes the hairs to fall off, then afterward *Slesma (Kapha)* along with *Sonita* (blood) blocks the orifices of hair follicles, so as to block the growth of new hairs.[5]

Alopecia described in Biomedical Science can be correlated with *Indralupt* in *Ayurveda.* It usually starts with one or more small, round, smooth bald patches on the scalp and can progress to total scalp hair loss (alopecia totalis) or complete body hair loss (alopecia universalis). There are many reasons of alopecia-like genetic cause, lack of nutrition, tension, and infection. However, in the present study, much emphasis has been laid on the "infective cause," which if persist for a long time can cause hair loss. In ancient time, this was a rare disease, but nowadays, it is common, but challenging and capricious disease affected approximately 1.7% of the world population and can have a profound effect on one's physical and emotional state.[6] To overcome this, patients have to take the medicines for lifelong, which further leads to the side effects and chronicity of the disease. Thus, it is the need to prepare an effective, potent, and standardized drug for the Ayurvedic treatment of alopecia. Topical Ayurvedic herbs and oils like *Karanja* oil remain effective to stimulate hair follicles by subsiding the infection to a great extent. The effectiveness of *Karanja* as a source of biomedicines is very well reported.[7] *Karanja* seed oil contains Karanjain, a bioactive molecule with important biological attributes and it is good in scabies, leprosy with antifungal, antibacterial, antiviral activity, and antifilarial potential.[8] Hence, it can be the best alternate to cope up with alopecia. Hence, the present study was formulated to evaluate *in vitro* antifungal activity of *Karanja tailaw. s.r* to its action on alopecia.

MATERIALS AND METHODS

Three different samples of *Karanja taila* (S1, S2, and S3) as per the reference of *Sharangdhara Samhita* were used for the present study.[9] Antifungal activity was conducted on specific pathogens, namely, *Trichophyton tonsurans, Microsporum canis, Trichophyton rubrum,* and *Microsporum audouinii* that cause alopecia. Culture of the above-defined fungi was taken and medicated oil at room temperature was applied on it. After a time period, the zone of inhibition was seen, which showed the effectiveness of the drug.

The pathogenic strains of different species of fungi were procured from the "Institute of Microbial Technology", Chandigarh, and the antifungal study was conducted at 'Microbiology lab', Institute of Biomedical and Industrial Research, Jaipur, Rajasthan.

- *T. tonsurans*: (MTCC NO: 8475)
- *T. rubrum*: (MTCC NO 296)
- *M. canis:* (MTCC NO: 2820)
- *M. audouinii:* (MTCC No: 8197).

Preparation of Media and Media Plates

- Mueller-Hinton agar medium was taken for all pathogens.
- Agar - 38 g
- Distilled water - 1 L
- pH - 7

Heated the agar with water at 100°C till it became transparent, then kept it in hot air oven for 15 min. The sterilized media was poured in sterile Petri dishes aseptically in a Laminar flow cabinet and agar (solidifying agent) was added in the broth medium.

It is the property of agar (which is used as a solidifying agent) that it use to get hardened after cooling when added in broth medium. After solidifying of agar plates (nearly about 15–20 min), they were kept inverted in incubator at 37°C for overnight for checking any contamination. The ready agar plates were then transferred in zip seal plastic cover and kept in a cold room. The media and media plates were prepared time to time as per requirement and used for streaking purpose and also for antifungal evaluation.

Revival of Microbial Cultures

Like all other living forms, microorganisms need suitable nutrients and favorable environments for growth. A simple way to obtain fungi is to grow them in a flask in broth medium. 100 mL nutrient broth medium was transferred into conical flasks (of quantity 100 mL) 20 mL each. The flasks were capped with cotton plug and autoclaved at 121°C for 20 min at 15 lb pressure per square inch.

Inoculation

To start a fungal culture, a number of cells (the inoculums) are to be transferred (inoculated) into a sterilized broth media. The loop full of freeze-dried cultures were transferred. In this inoculation procedure, the loop that was used to transfer microorganisms has been heated to redness by flaming immediately before and after the transfer. Flaming use to destroy living forms f fungi on the surface of the loop. During transfer, the flask was held in the left hand and the cotton plug between the fingers of the right hand. The mouth of the flasks into which the cultures were transferred, were also passed through the burner flame immediately before and after the loop was introduced and then removed. In addition to destroying organisms on the mouths of the flasks, flaming creates outward convection currents, which decreases the chance of contamination. This inoculation procedure was done in a laminar flow chamber. After inoculation, the fungal cultures were incubated at room temperature overnight in

a shaker for their growth. Growth, in this case, means the development of a population of cells from one or few cells. Next day, the mass of daughter cells became visible to the naked eye as cloudiness (turbidity) in all flasks.

Streaking

Fungal grow very well in fluid media, i.e., nutrient broth. Hence, they are used as enriched media before plating on solid media. Solid media is essential for the isolation of organism in pure form. Hence, for isolation of microorganisms in pure form without contamination, streaking was done on solid media plates. A microbial culture was applied to the surface in a Petri plate and was spread with cotton swab sticks. The prepared plates were then incubated in inverted position at 37°C for 24 h. After incubation, we got the pure cultures. This procedure is termed as 'subculturing'. In this way, frequent subculturing was done whenever required during antifungal study.

Applying a microbial culture to the surface of agar in a Petri plate and spread them with a loop or a bent needle or cotton swab. This process is called 'streaking' and the plate so prepared is called a 'Streak plate'.

Preparation of Positive Control

The concentrations of 5% w/w vancomycin was prepared in sterile Eppendorf tubes.

Preparation of Discs

For fungicidal assay *in vitro*, well diffusion method was adopted (Gould and Bowie, 1952), because of reproducibility and precision. The different test organisms were introduced separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition was measured around sterilized wells (5 mm in diameter).

Well Diffusion Method

Wells (of about 5 mm diameter) were made on the plates with the help of sterile stainless steel borer. About 20–30 µL of different concentrations of plant solvent extracts were added using sterile syringe into the wells and allowed to diffuse at room temperature for 2 h (Sen *et al*. 2012). Control groups comprising inoculums without plant extract was also set up. The plates were incubated at 37°C for 48 h for fungal pathogens.

Groups Design

Positive control

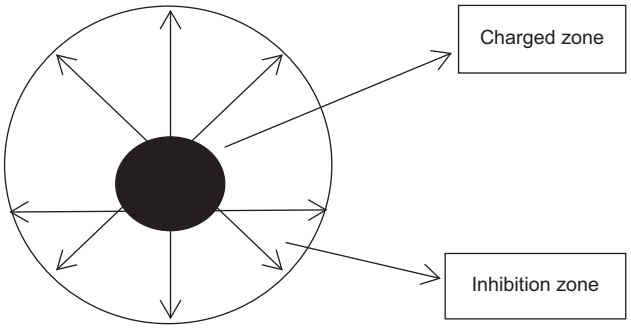
5%w/w vancomycin.

Test groups

S1, S2, and S3.

Recording and Interpretation of Results

After the discs were placed on the plate, plate was inverted and incubated at 35°C for 48 h. After incubation, diameter of the zones of complete inhibition was measured (including the diameter of the disk) and recorded in millimetres. The measurements were made with a ruler on the under surface of the plate without opening the lid. In the instance, slight growth (80% inhibition) should be ignored and the zone diameter should be measured to the margin of heavy growth. The zones of growth inhibition were compared and recorded. Colonial growth within the clear zone of inhibition represents resistant variants or mixed inoculums. The distance from the colonies closest to the disk to that of the disk centre was measured and then was doubled to obtain the diameter. The diameter of the outer clear zone should be recorded as well and an interpretation recorded for each diameter. The presence of colonies within a zone of inhibition may predict eventual resistance to that agent. Experiment was carried out in triplicate and the averages diameter discs were measured in mm with the help of a scale. The zone of inhibition of fungal growth around the well and activity index was noted. The readings were taken at four different planes as shown in figure below.



Then, the mean was calculated of the four readings taken.

OBSERVATION AND RESULTS

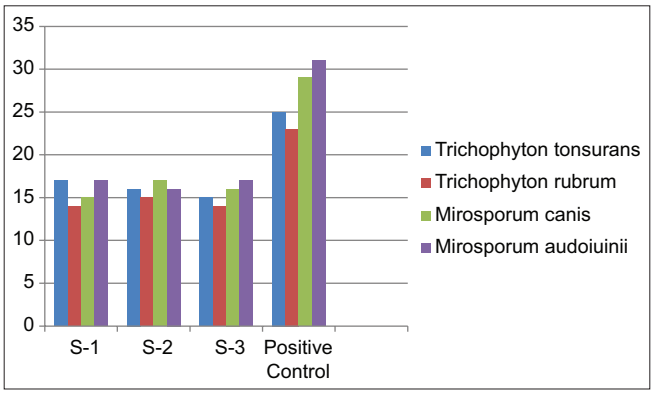


Figure 1: Zone of inhibition in (mm)

Table 1: Zone of inhibition

Pathogenic strains	Zone of inhibition in (mm)			
	S1	S2	S3	Positive control
Trichophyton tonsurans	17	16	15	25
Trichophyton rubrum	14	15	14	23
Microsporum canis	15	17	16	29
Microsporum audouinii	17	16	17	31

Table 2: Activity index

Pathogenic strains	S1	S2	S3
Trichophyton tonsurans	0.68	0.64	0.60
Trichophyton rubrum	0.61	0.65	0.61
Microsporum canis	0.52	0.59	0.55
Microsporum audouinii	0.55	0.52	0.55

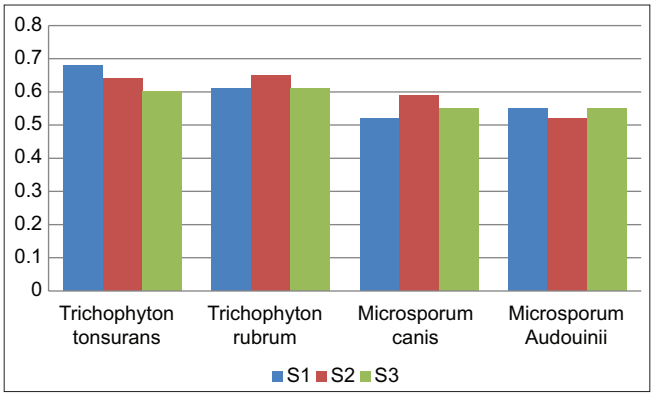


Figure 2: Activity index

DISCUSSION

The zone of inhibition of fungal growth and the activity index are the standard tool parameters to evaluate the action of Karanja taila against the specified fungi. The inhibition zone is directly proportional to antifungal activity. The inhibition zone of samples S1, S2, and S3 and Positive Control (5% w/w vancomycin) against pathogenic strains T. tonsurans, T. rubrum, M. canis, M. audouinii as depicted in table 1 respectively was found to be (17, 14, 15, 17 [S1]), (16, 15, 17, 16 [S2]) (15, 14, 16, 17 [S3]) (25, 23, 29, 31 [positive control]). The same is depicted graphically in Figure 1.

The activity index >0.50 implies the significant activity of *Karanja tail* toward the fungi. It is obtained by dividing the inhibition zone of positive control by inhibition zone of pathogenic strain. The activity index of samples S1, S2, and S3 for pathogenic strains *T. tonsurans*, *T. rubrum*, *M. canis*, *M. audouinii* as depicted in Table 2 was found out to be (0.68, 0.61, 0.52, 0.55 [S1]), (0.64, 0.65, 0.59, 0.52 [S2]) (0.60, 0.61, 0.55, 0.55 [S3]), respectively. The same is depicted graphically in Figure 2.

It is a well-known that the efficacy of a particular drug mostly depends upon the quality of the raw material and method of preparation used that can be used to set the standard parameters for standardisation of a particular type of drug. Similarly, in the present study, we have tried our level best to set or follow the ideal standardization parameters to make more potent *Karanja taila.*

CONCLUSION

The activity index of all the samples against the specified fungi is >0.5, which depicts their profound effect on Alopecia. Hence, it can be concluded that *Karanja taila* can be used in patients with alopecia with no adverse effects.

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