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Evaluation of Antifungal Activity of *Somaraji Taila* Extract & *Taila* in *Dadru Kushta* (Tinea Infections) - An Experimental Study

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ABSTRACT

Dermatophytosis has become a significant health problem affecting children, adolescents, and adults worldwide. In India 5 out of 1000 people suffer from Tinea infections. The present study evaluated the antifungal activity of *Somaraji Taila* in Tinea (Dermatophytosis) in Invitro & Invivo model. Cultures were brought from MTCC a government body and inoculated in SDA media under aseptic condition. Later it was subjected to microscopic and macroscopic examination to identify the organism. A sensitivity test was done using SDA media by well diffusion method, with 4 different concentrations of Hydro-Methanolic extraction of *Somaraji Taila* ingredients. After the incubation period, the zone of inhibition was checked and was measured in mm. As the next phase of the study, animal study was also conducted to check antifungal properties & anti dermal toxicity on albino rat skin.

Key words: *Ayurveda, Dadru Kushta, Tinea Infection, Ringworm Infection, Somaraji Taila, Antifungal Activity*

INTRODUCTION

In *Ayurveda*, references are available regarding the testing of drug and food on animals for evaluating administration to the safety before human beings. *Sushruta Samhita Sutrasthana* has dealt with this by devoting a separate chapter, *Yogya Vidhi*. It is recommended that any procedure performed on human beings should primarily undergo trial on animals or other models with similar characteristics.[1]

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Hence before using *Somaraji* in the form of *Taila*, on humans in dermatophytosis, an experiment to evaluate the efficacy of *Somaraji Taila* on Dermatophytosis *In Vitro* and *In Vivo* Study is essential.

Dermatophyte infections are some of the earliest known fungal infections and are very common throughout the world. Although dermatophytosis does not cause mortality, it causes significant morbidity and posses a major public health problem especially in tropical countries due to the hot and humid climate. No race in any geographical location is entirely free from dermatophytosis. Skin, hair, nail, and subcutaneous tissues in human and animals are all susceptible to infection by several organisms, primarily fungi named dermatophytes and cause ringworm infection (dermatomycoses)[2] A World Health Organization (WHO) review of prevalence studies done on skin diseases among children & adults reported an overall prevalence ranging from 21% to 87%.[3] While several systemic anti-fungal compounds are available for use in humans, these compounds have significant adverse

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effects and their usefulness is limited due to their high rates of toxicity.[4]

Different treatment methods have been in use for the control of dermatophytes but recently the use of some natural plant products has emerged to inhibit the causative organisms. The antimicrobial and antitoxin properties of some plants, herbs, and their components have been documented from the late 19th century. [5] They are safe to human than the chemically produced antifungal compounds and are readily available for use by the rural population who are mostly prone to these infections.[6]

For thousands of years, plants worldwide have been used to treat diseases and are now known to contain chemical constituents that could be of therapeutic importance or as precursors for the synthesis of new drugs. [7] The ingredients of *Somaraji Taila* are reportedly used for treatment of Ringworm, Scabies infections, etc.[8] *Somaraji Taila*[9] comprises of *Bakuchi*[10], *Haridra*[11], *Daruharidra*[12], *Sarshapa*[13], *Kushta*[14], *Karanja*[15], *Chakramarda*[16], *Aragwadha.*[17] The present study aimed to evaluate the efficacy of *Somaraji Taila* in *Dadru Kushta* (Tinea Infections) by In-vitro study and In-vivo study models.

Materials and Methods

Sample Collection

The fungal sample for invitro and *In Vivo* were collected from MTCC Chandigarh in the form of Freezed Dried Samples later they were activated by adding 0.4ml of Sterile Distilled water and transferred into a slant SDA media to grow for 7days once grown then confirmed under microscope (40X).

The ingredients used of *Somaraji Taila* are *Bakuchi* seeds, *Haridra Khanda, Daruharidra Moola, Sarshapa Seeds, Kushta Moola, Karanja Seeds, Chakramarda Seeds, Aragwadha Patra*. All the used parts were authenticated by Dravyaguna Department of Alva's Ayurveda Medical College, Moodubidire.

Preparation of Hydro-Methanolic Extract of *Somaraji Taila* Ingredients

All the ingredient mentioned above were taken and pounded. A total 52gms of pounded drugs taken and rolled in watt man filter paper No 1 and placed in timble of Soxhlet apparatus. 70% hydro-methanal 500ml was used as solvent to extract. At the end of extraction 270ml extract was obtained after 14 cycles. Later gained extract was subjected to water bath for 3days in order to evaporate methanol. At last, a total 8.2gms of extract was obtained.

Preparation of Trial Drug Extract

In preview of *In Vitro* and study methanol is considered as toxic, hence to nullify the toxicity other alternative solvent were tested. DMSO (Dimethyl Sulfoxide) was used in various proportion like 5%, 10%, 15%, 20%, 40%, 60%, 80%, 100%. Out of which 80% showed maximum solubility. To prepare mother solution trial extract was taken and dissolved in 80% DMSO and further diluted to required proportion by using 5% DMSO and this solution was used as mother sample for further analysis.

For preparation of 1ml sample, 1mg of sample was dissolved in 100µL of 80% DMSO once sample dissolves completely with the heating on boiling water bath, later it was cooled and 900µL of 5% DMSO was added to make up the volume to 1ml this sample was used all other analytical studies.

Polyphenol estimation was done using FC Reagent and sodium carbonate at the end of analysis total polyphenol was 4.38µg/75µL/10mg.

In Invitro Various concentrations were used like 25µg/l, 100µg/l, 250µg/l, 500µg/l.

Invitro Studies

Susceptibility Test

The antifungal activities of Hydro-Methanolic extract of *Somaraji Taila* ingredients against clinical fungi isolates were evaluated using agar well diffusion method. A total of 4 fungal sample used in the study they are *Trichophyton Rubrum* (MTCC7859), *Trichophyton Mentagrophytes* (MTCC 7687), *Microsporum Gypsum* (MTCC 2819), *Microsporum Canis* (MTCC 2820).

Sabouraud Dextrose Agar (SDA) well diffusion method used in plate was inoculated with 0.1ml of

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standardized inoculum ($1x10^6$ cfu/ml) of all the fungus mentioned above by streaking on surface of agar. Equidistant wells of 4mm size of 4 holes were made with sterile cork borer into agar plate containing the fungi inoculums. A 20µl of 100% concentration and Antifungal drug 1% Fluconazole and 5% DMSO was also introduced in it.

A 20µl of constituted trial extract of different concentrations was carefully introduced into each well. The plates were kept in room temperature for one hour to allow pre-diffusion of the extract into the agar before incubation at 25°C for 3-7days. The plate was observed periodically during this period the presence or absence of suspectable organism was measured at the end of incubation period.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the methanol extract was determined using a EUCAST Guidelines Microbroth dilution method standard agar dilution method. The antifungal activities of the extract were tested at various concentrations The MICs were determined after 3-7 days of incubation at appropriate conditions suitable for fungi growth. The trial drug showed 350 ppm was regarded as the lowest concentration that prevented visible growth.

Determination of Minimum Fungicidal Concentration (MFC).

Minimum Fungicidal concentration of *Somaraji Taila* extract was determined by EUCAST Guidelines Microbroth dilution method used in MIC. To 0.5ml extract at different concentrations as used in MIC assay that showed no visible growth on the agar plate. Samples were streaked onto Extract free SDA to determine the minimum concentration of the extract required to kill the organism indicated by failure of organism to grow. The lowest concentration that prevented the growth of fungal after days on incubation was 450 ppm was recorded as minimum fungicidal concentration (MFC).

In Vivo Study

Before proceeding animal study, the room where animal was kept was bubble wrapped entirely to limit the spread the infection. Wister albino rats aged 14-15 weeks weighing 200-250gms obtained from Alva's Animal House, Alva's Ayurveda Medical College and Hospital, Moodubidire. The rats were individually housed in propylene cage at room temperature. The animals were feed pellet diet and water.

In Vivo Antifungal Assay[18]

The animals were randomly assigned to treatment group as presented in **Table No. 2.** The hairs on the nape of neck of each animal were shaved, cleaned, and the area to be infected was disinfected with cotton swab saturated with 70% ethyl alcohol before infecting. The fungal inoculum was prepared from 14days old culture of *T. Mentagrophytes* suspended in sterile potato dextrose broth. Following filtration through Whatman filter paper No.1 to remove hyphal fragments and residual agar. The final suspension was adjusted to $1x10^6$ Conidia/ml and 0.2ml of inoculum was applied using sterile cotton swabs by streaking over nape of neck and left for 14days. The establishment of an active infection was confirmed on day 7. Isolation of the pathogens from skin scales cultured from infected loci on SDA plate. Infection was confirmed by visual examination of the animals on day 7-14. In animals with confirmed active infection, treatment commenced on day 15 of post infection. The groups were treated topically with various formulations and skin was examined daily. The therapeutic effect of formulations was compared with standard formulation. The treatment was applied twice in a day and infected skin was scored visually from inflammation, patchy lesion and scaling. Clinically assessment of inoculated skin area was performed using lesion score as presented in **Table No. 1**. And the Results are under **Table No.4**

Table 1: Description of lesion score for assessment[19]

Score	Description
0	No Visible Lesion
1	Few Erythematous Lesion
2	Well Defined Lesions

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3	Large areas of marked redness, incrusting, scaling, patchy lesions
4	Mycotic foci well developed.

Table 2: Grouping of Animals

Groups	Name of Group	Treatment
Group 1	Control Group	Normal Saline
Group 2	Induced Group	---
Group 3	Induced and Treated with Standard Formulation	*Chakramarda Taila*
Group 4	Induced and Treated with Trial Formulation	*Somaraji Taila*
Group 5	Induced and Treated with Vehicle Drug	*Sharshapa Taila*

RESULTS

Phytochemicals screening of *Somaraji Taila* extract revealed the presence of Flavonoids, Terpenoids, Phenols, and Sterols. The extraction yield was 8.2gms. The antifungal activity of *Somaraji Taila* extract were determined against *Trichophyton rubrum* (MTCC7859), *Trichophyton mentagrophytes* (MTCC 7687), *Microsporum gypsum* (MTCC 2819), *Microsporum canis* (MTCC 2820). Hydro-Methanolic extract of the *Somaraji Taila* ingredients inhibited the testing fungus as shown in **Table No. 3** and **fig 1 (plate A-D).** The MIC Value is 350ppm and MFC value is 450ppm respectively.

Table 3: In-Vitro Zone of Inhibition.

Organisms	Standard Drug	Trial Drug(µg/l)			
		25	100	250	500
Trichophyton rubrum	34 mm	0	13 mm	18 mm	21 mm
Trichophyton mentagrophytes	17 mm	0	11 mm	16 mm	18 mm
Microsporum gypsum	16 mm	0	0	0	14 mm
Microsporum canis	10 mm	0	0	13 mm	14 mm

Figures - I

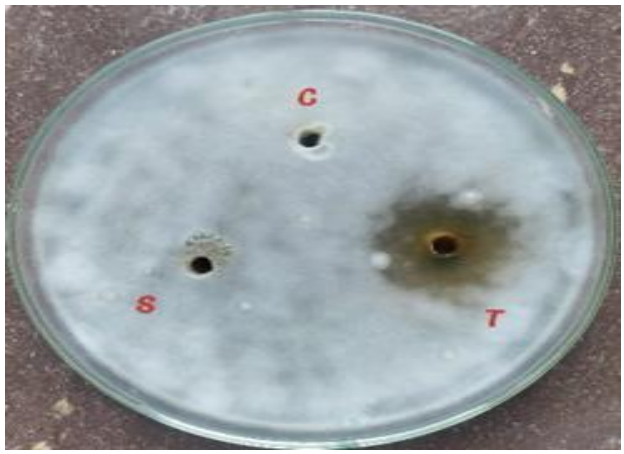

Fig. A: Trycophyton Mentagryphates

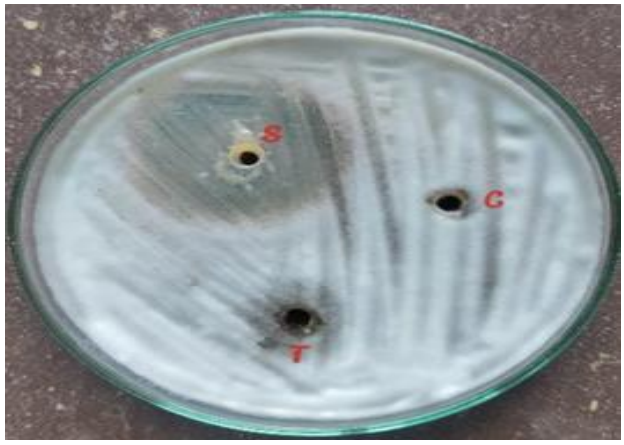

Fig. B: Trycophyton Rubrum

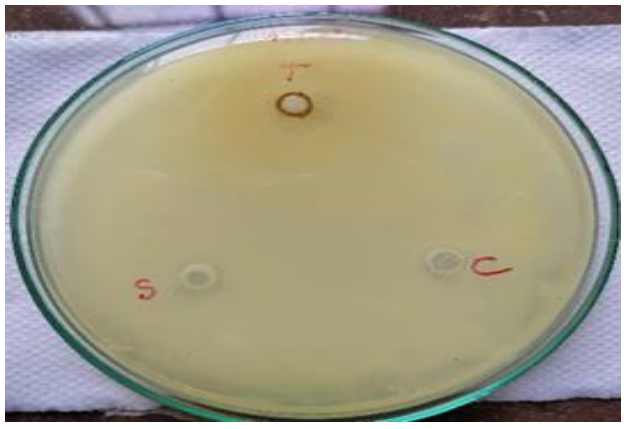

Fig. C: Microsporum Canis

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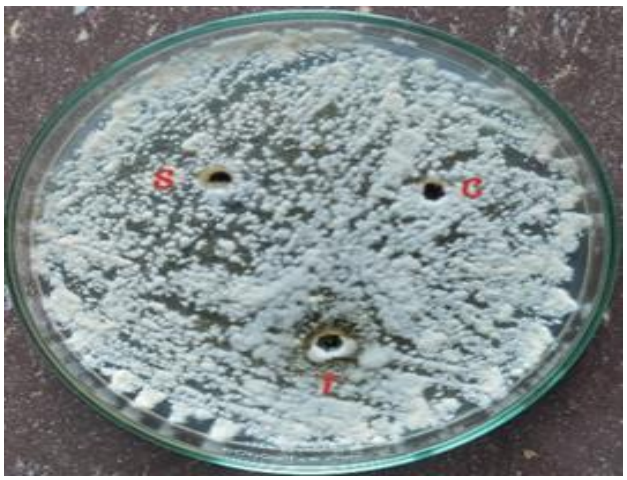

Fig. D: Microsporum Gypsum

Figures - II

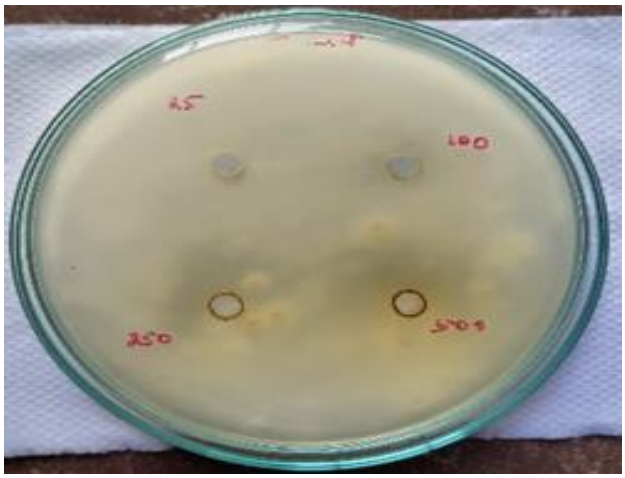

Fig. A: Trycophyton Mentagryphates

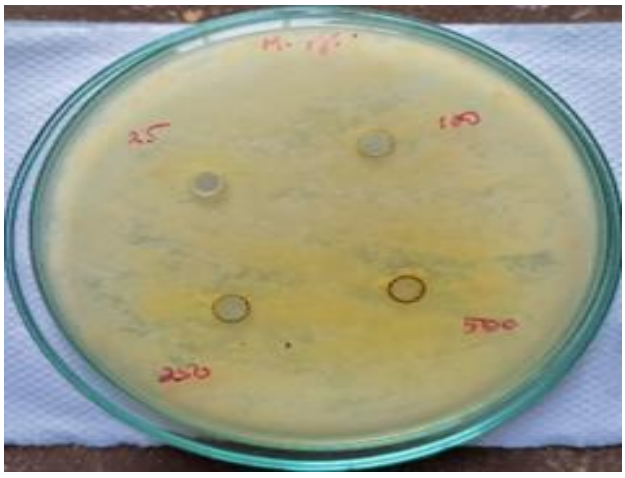

Fig. B: Trycophyton Rubrum

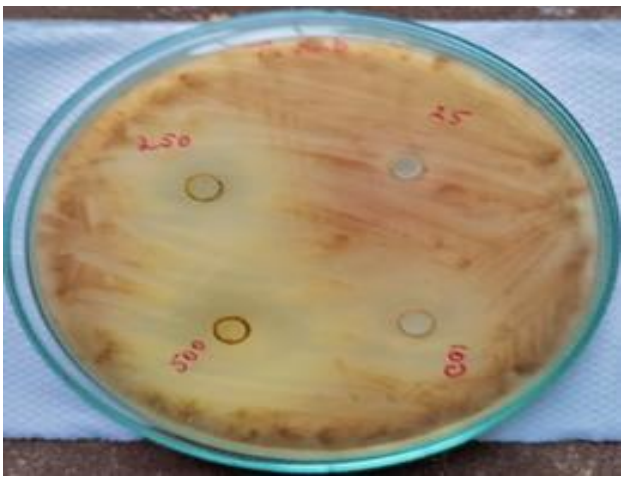

Fig. C: Microsporum Canis

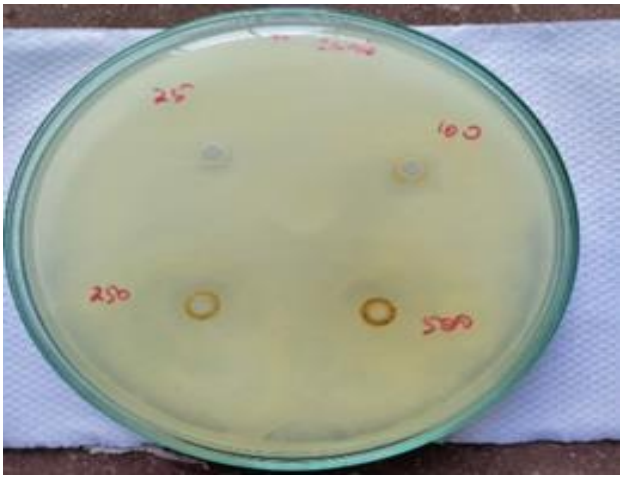

Fig. D: Microsporum Gypsum

Figures – III: Invivo Study

Fig. A: Bubble wrapped room

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Fig. B: Bubble Wrapped Rack

Fig. C: Bubble Wrapped Cage

Before Treatment and Infection Day

Fig. D: On the Day of Neck Shaving

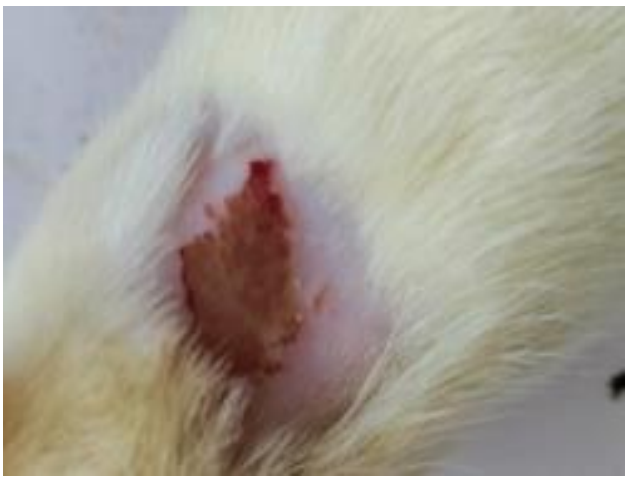

Fig. E: Before Treatment

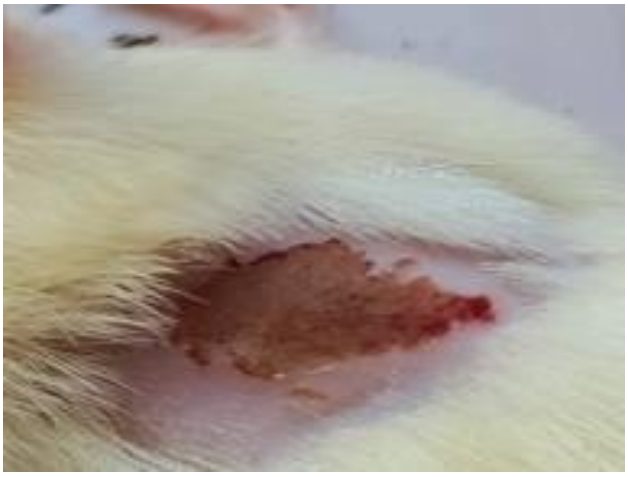

Fig. F: Day 14 Infection

After Treatment

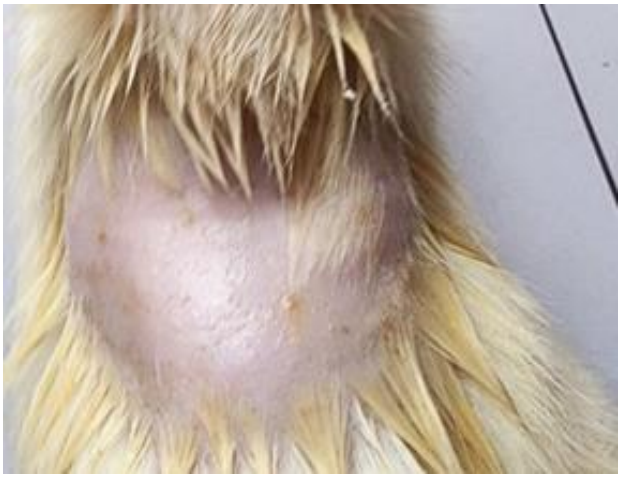

Fig. G: After Treatment Day 10

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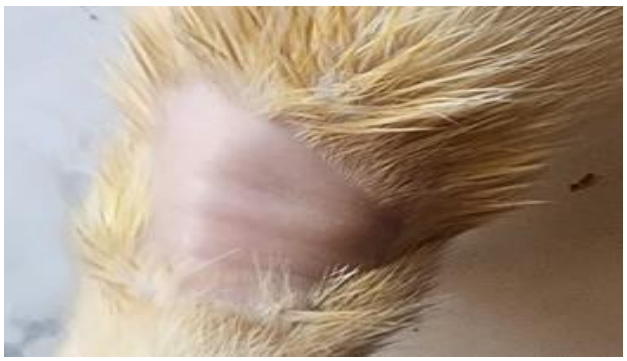
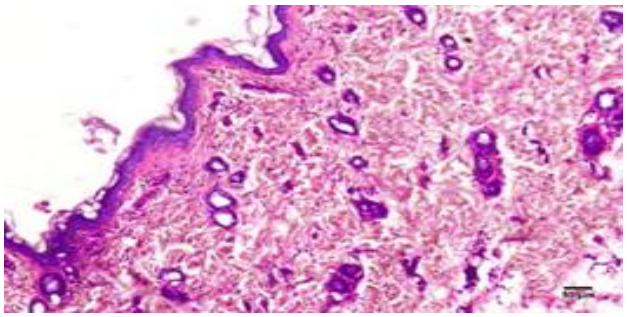
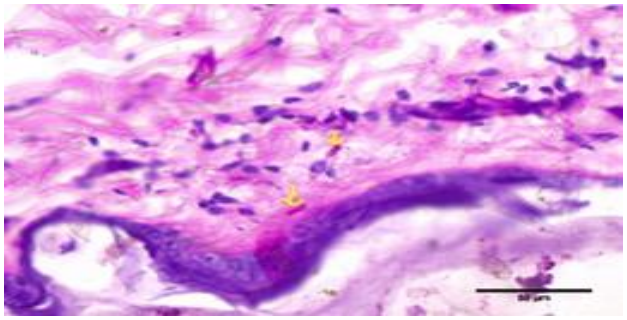


Fig. H: After Treatment Day 15

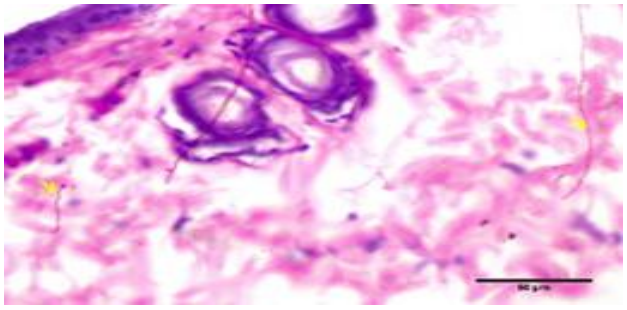
Histopathology Imaging



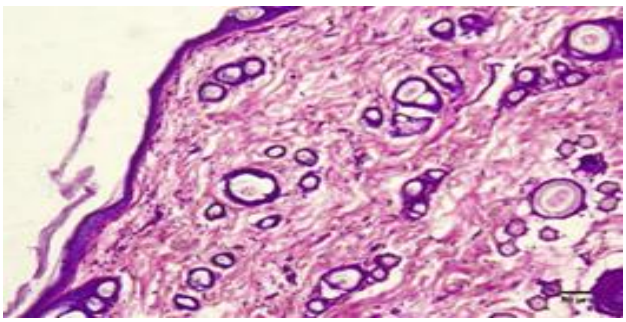
I- Control Group



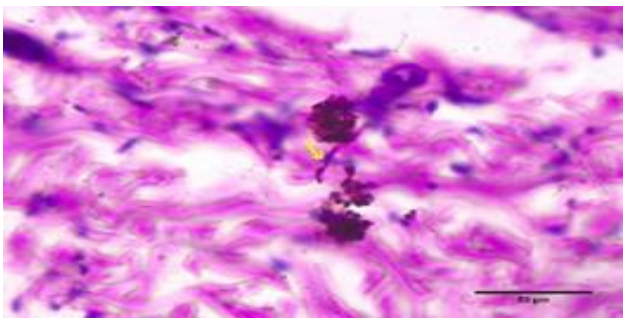
II- Induced Group Showing Fungal Hyphae



III- Standard Group Showing Fungal Hyphae after Taila Application



IV- Trial Group Showing no Fungal Hyphae after Taila Application



V- Vehicle Group Showing Fungal Hyphae After *Taila* Application

Table 4: Showing Scoring details of In-Vivo Study

Group	Day 0	Day 8	Day 15	Day 21
Group 1	0	0	0	0
	0	0	0	0
	0	0	0	0
Group 2	3	3	3	3
	3	3	3	3
	3	3	3	3
Group 3	3	2	1	1
	2	2	2	1
	3	2	1	0
Group 4	3	3	2	0
	3	2	2	0

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	3	2	1	0
Group 5	3	2	2	1
	3	2	2	1
	3	3	2	1

DISCUSSION

Discussion on In-Vitro

In search of new Anti-fungal agent with lower toxicity, the antifungal activity of hydro-methanol extract of *Somaraji Taila* ingredients were investigated in some common dermatophyte. Phytochemical screening of the extracts revealed Flavonoids, Terpenoids, Phenols, and Sterols

Bakuchi (*Psoralea corilifolia*) belonging to Fabaceae family having *Katu, Tikta Rasa, Laghu, Rooksha Guna, Ushna Veerya, Vata-Kapha Hara* which help in reducing *Kledata* used part is *Beejas* and contains Psoralen, Isopsoralen, Bavachromene, Bakuchiol. Psoralen a compound has the ability to disrupts fungal cell wall and inhibits fungal DNA synthesis, Bakuchiol exhibits broad spectrum antimicrobial activities.

Haridra (*Curcuma longa*) belonging to Zingiberaceae Family having *Tikta, Katu Rasa, Laghu Rooksha Guna, Ushna Veerya, and Tridosha Hara* helps in reducing *Daha, Kledata, Kandu* used part is *Moola* and contains Curcumin, Curcuminoids, Volatile Oils. Curcumin has strong antifungal properties which exerts by inducing oxidative stress within fungal sells, disrupting membrane integrity and interfering with fungal cell signaling pathway.

Daruharidra (*Berberis aristata* DC) belonging to Berberidaceae Family having *Tikta, Kashaya Rasa, Laghu Rooksha Guna, Ushna Veerya* and *Kapha Hara* which help in reducing *Kleda, Daha*. Used part is *Moola* contains Berberin, Berbamine, Oxycanthine, Berberrubine. Berberine an alkaloid with significant antifungal effect acting by inhibition ergosterol synthesis which is vital to fungal cell wall membrane.

Sharshapa (*Brassica nigra* L. Koch) belonging to Cruciferae Family having *Katu Rasa, Teekshana Guna, Ushna Veerya, Vata-Kapha Hara* Property which help in *Kleda, Daha Hara*. Used part is Seeds and Contains Steraric acid, Oleic Acid, Linolenic Acid, Esosenic Acid, Indole. Seeds contain allyl-isothiocynate, this compound disrupts the cell membrane integrity of fungi leading to leakage of cellular contents & leads to cell death. Glucosinolate a compound when hydrolyzed it produces various compound including isothiocyanate which have potent antifungal effect.

Kushta (*Saussurea lappa* CB. Clarke) belonging to Asteraceae family having *Tikta, Katu, Madhura Rasa, Laghu, Rooksha, Teekshna Guna, Ushna Veeyra,* having *Vata-Kapha Hara* which helps in *Kleda Hara* and stops spreading of infection. Used part is *Moola* and Contains Kusthin, Saussureal, Taraxasterol. Sesquiterpene lactone, dehydrocosten lactone, costunolide known to their strong antifungal & antimicrobial activities. Sesquiterpene exhibits antifungal property by inhibiting the synthesis of fungal cell wall & interfering with fungal enzyme activity. Castanosides help in apoptosis in fungal cells by generating reactive oxygen species (ROS) & disrupting mitochondrial function.

Karanja (*Pongamia pinnata*) belonging to Fabaceae Family having *Tikta, Katu, Kashaya Rasa, Laghu Teekshna, Ushna Guna, Vata-Kapha Hara* which help in *Kleda, Daha Hara*. Used part is *Beeja* and contains Glabarin, Karanjin, Pongapin, Pongamol. Flavanoids, Karanjin & Pongamol having antimicrobial properties. Karanjin & pongamol interfere with fungal cell wall synthesis and disrupts membrane integrity leading to cell lysis and cell death. Flavonoids is having broad spectrum antimicrobial activity & enhance the overall antifungal efficacy by inhibiting fungal enzyme & DNA synthesis.

Chakramarda (*Cassia tora* Linn.) belonging to Caesalpiniaceae family having *Katu Rasa, Laghu Rooksha Guna, Ushna Veerya, Vata Kapha Hara* which help in reducing spread of infection. Used part is *Beeja* and Contains Oleic Acid, Lenolic Acid, Palmitic Acid, Sitosterol's. Seeds are rich ion flavonoids, anthraquinones and phenolics compound which contributes to its antifungal activity. Anthraquinones exhibits antifungal effect by interfering with the

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mitochondrial electron transport chain by fungi leading to energy depletion and leads to cell death. Flavonoids enhances membrane permeability, causing leakage of cellular content and inhibit fungal spore germination.

Aragwadha (*Cassia fistula*) belonging to Caesalpiniaceae family having *Madhura Rasa, Mrudhu, Guru Snigdha Guna, Sheeta Veerya, Kapha-Vata Hara* helps in *Kandu, Daha Hara*. Used part is *Patras* which contains Anthraquinone, Lenoceric Acid, ß-Sitosterol's. Anthraquinone, flavonoids, and tannins which has antimicrobial properties. Anthraquinones these help in disrupt mitochondrial function and energy production in fungal cells. Tannins precipitate proteins and inhibit fungal enzymes, hence preventing fungal growth and reproduction. Flavonoids act by inhibiting fungal cell wall synthesis & inducing oxidative stress within fungal cell wall.

These all herbs work in synergetic effect to balance the impaired *Doshas*, purify the blood and direct combating fungal infection, their unique bioactive compound disrupt fungal cell wall and membrane inhibiting essential enzymes, generating oxidative stress and interfere with fungal DNA and energy synthesis. This synergetic effect enhances the efficacy of *Somaraji Taila* making it a potent natural *Taila* preparation for *Dadru Kushta.*

Discussion on In-Vivio Study

The significant finding of in- vivo study was noted down they are as follows

a) Reduction in lesion

b) Healing of lesion

c) Reducing of symptom

d) Histopathological analysis

a) Reduction in Lesion

- Animals treated with *Somaraji Taila* exhibited a notable reduction in lesion size compared to the control group & Standard group.
- The reduction in lesion size in the *Somaraji Taila* - treated groups was comparable to that observed in the standard treatment group, suggesting that *Somaraji Taila* has a better efficacy then Standard Group.

b) Healing of Lesion

- The trial treated groups showed accelerated healing of the infected skin areas. *Somaraji Taila* not only reduced the fungal burden but also promoted skin regeneration and restoration of normal skin texture.
- This healing effect can be attributed to the combined antimicrobial and anti-inflammatory properties of the formulation's ingredients, such as psoralen from *Bakuchi*, curcumin from *Haridra*, and berberine from *Daruharidra*.

c) Reducing of Symptom

- Treated animals exhibited significant relief from symptoms such as itching and erythema. This symptom relief is aligned with the traditional Ayurvedic properties of the ingredients, such as *Kushta* (*Saussurea lappa* CB.Clarke), *Bakuchi* (*Psorelea corylifolia*), which include anti-itching (*Kandughna*) and anti-inflammatory actions.

d) Histopathological Analysis

- Histological examinations of skin samples from treated animals revealed a reduction in fungal elements within the skin layers. There was also evidence of reduced inflammation and a return to normal skin architecture.
- The histopathological findings corroborated the clinical observations, confirming the antifungal and healing properties of *Somaraji Taila*.

CONCLUSION

This experiment investigated the antifungal properties and anti-dermal toxicity of *Somaraji Taila* ingredients and *Taila*, both *In Vitro* and In Vivo, to validate their traditional use in treating *Dadru Kushta* (Tinea, Ringworm). The *In Vitro* results showed that Fluconazole exhibited 100% antifungal activity, while the test sample (*Somaraji Taila* extract) demonstrated 78% antifungal activity. The *In Vivo* study confirmed the *Taila's* ability to eliminate fungal hyphae from skin

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tissue in rats infected with *Trichophyton mentagrophytes*, indicating its potential as an effective antifungal agent in *Dadru Kushta*

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