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RESEARCH ARTICLE

ANTIFUNGAL ACTIVITY OF MANJISHTADI ADITYAPAKA TAILA AND ITS OINTMENT - AN IN VITRO STUDY

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Abstract

Background : Infectious diseases remain a leading cause of public health concerns, and have the potential to significantly impair the quality of life or can be fatal. *Candida albicans* is the primary species responsible for causing symptomatic skin infections. *Adityapaka* is one of the methods of *sneha* preparation where the *paka* of *sneha* is done using sun rays instead of direct fire. Such *Adityapaka Snehas* are exclusively used for the treatment of skin diseases as they absorb Ultra Violet rays from sunlight. *Manjishtadi Adityapaka Taila* is one such herbo mineral formulation used externally for the treatment of *Pama kushta*, mentioned in *Kushta Adhikara* of *Chakradutta*. Its preparation is explained to keep the *Tilataila* with powdered *Manjishta, Hareetaki, Vibhitaki, Amalaki, Laksha, Nisa,* Purified *Manashila*, Purified *Haratala* and Purified *Gandhaka* under the sunlight. So, this study is aimed to evaluate the antifungal activity of *Manjishtadi Adityapaka Taila* and its modified dosage form, the ointment, against two selected fungi, *Candida albicans* and *Aspergillus niger* by in vitro agar well diffusion method.

Methodology : The antifungal activity was evaluated by in vitro agar well diffusion method. Both the *taila* and ointment samples were divided into duplicates and each duplicate was prepared into three concentrations, 25%, 50% and 80% with the solvent hexane, and their antifungal activity was assessed against *Candida albicans* and *Aspergillus niger* with Fluconazole 500 ppm and Clotrimazole 2000 ppm as the respective standard controls and their zone of inhibition were measured in mm.

Results : From the in vitro study, against *Candida albicans*, the *taila* showed 15 mm, 17 mm and 18 mm of zones of inhibition in concentrations 25%, 50% and 80% respectively, while the ointment showed more activity as it showed 19 mm and 18 mm of zone of inhibitions in 80% and 50% concentrated samples respectively. Both the *taila* and ointment showed antifungal activity by 17 mm of zone of inhibition only in 80% concentration against *Aspergillus niger*. The zone of inhibition of respective standard controls were 20 mm.

Conclusion : The results imply that both the *taila* and ointment possess significant antifungal activity against *Candida albicans* and *Aspergillus*

niger, and the pharmaceutical modification of the *taila* into ointment has contributed in enhancing its antifungal activity.

Introduction:-

Infectious diseases significantly contribute to the major public health concerns globally. Approximately, 20-25% of the total global population suffers from different fungal skin infections, which can significantly impair the quality of life or even be fatal.[1] In people with compromised immunity, the skin's fungal ecosystem is disrupted, allowing different fungal species to flourish[1]. *Candida albicans* is the primary species responsible for causing symptomatic skin infections.[1] Antimicrobial agents are a powerful tool against various microbial infections.

Adityapaka is one of the methods of *sneha* preparation other than *Agnipaka*, where sunlight is used for *sneha paka* rather than direct fire. *Adityapaka Snehas* are exclusively used for the treatment of skin diseases, due to more penetration capacity to the skin, capability to reduce inflammation, enhance the capacity to shed the damaged skin and to grow new healthy skin.[2]

Efforts should be taken to evaluate the antimicrobial activity of such classical Ayurvedic formulations, against human microbial infections.

Manjishtadi Adityapaka Taila is mentioned in the *Kushta Adhikara* of *Chakradutta* - for the treatment of *Pama kushta* as external application.[3] While comparing the clinical features of *Pama*, they were found similar with that of some fungal skin infections.

Aim:-

To evaluate the antifungal activity of *Manjishtadi Adityapaka Taila* and its ointment against *Candida albicans* and *Aspergillus niger* by in vitro agar well diffusion method.

Materials and Methods:-

The *Manjishtadi Adityapaka Taila* and its ointment were prepared at the Department of Rasashastra and Bhaishajya Kalpana, Govt. Ayurveda College Kannur.

Preparation of *Taila* :

The preparation of *Manjishtadi Adityapaka Taila* was done according to the reference in *Kushta Adhikara* of *Chakradutta* :[3]

मञ्जिष्ठा त्रिफला लाक्षा निशा शिलाऽऽलगन्धकैः।

चूर्णितैस्तैलमादित्यपाकं पामाहरं परम्।। (*Chakradutta*. 50/157)[3]

Table 1:- Ingredients of *Manjishtadi Adityapaka Taila* :

Sl No :	Drug	Botanical name	Quantity as per reference	Quantity taken
1	*Manjishta*	*Rubia cordifolia*	1 part	30 g each
2	*Haritaki*	*Terminalia chebula*		
3	*Vibhitaki*	*Terminalia bellerica*		
4	*Amalaki*	*Emblica officinalis*		
5	*Laksha*	*Kerria lacca*		
6	*Nisa*	*Curcuma longa*		
7	*Manahsila*	*Arsenic disulphide*		
8	*Haratala*	*Arsenic trisulphide*		
9	*Gandhaka*	Sulphur		
10	*Manjishta*	*Rubia cordifolia*	4 parts	1080 ml

After *shodhana*, all the mineral ingredients were powdered. All the herbal ingredients were also powdered and both of them were sieved through sieve no. 85 and were mixed together to form the *kalka*. To this, *tila taila* was added, stirred well and exposed to adequate sunlight for 7 hours per day (from 10 am – 5 pm) for 7 consecutive days, after which the *taila* was filtered and stored.

Preparation of Ointment :
For the preparation of ointment, beeswax was selected as the base and the ointment was prepared by the ratio of *Siktha taila* as per *Rasa Tarangini*,[4]ie., 1:6 (for 1 part beeswax, 6 parts of *Manjishtadi Adityapaka Taila* was taken). The beeswax was melted in the *taila* by double boiling method for preparing the ointment and was stored in a glass container after cooling.

In vitro Agar Well Diffusion Method :
The antifungal activity of *Manjishtadi Adityapaka Taila* and its ointment was carried out by in vitro agar well diffusion method[5,6] at CARe KERALAM Ltd, Thrissur.

The two samples, ie., *Manjishtadi Adityapaka Taila* and Ointment were made into 3 concentrations each – 25%, 50% and 80% concentrated samples in duplicates.

The solvent used was Hexane.
Standard controls used were :
For *Candida albicans* – Fluconazole 500 ppm.
For *Aspergillus niger* – Clotrimazole 2000 ppm.
Then, the zone of inhibitions of all the samples, corresponding standards and the solvent hexane were measured using a Hi Antibiotic Zone Scale.

Observations:-

Table 2:- Results of Antifungal activity against *Candida albicans* :

Sl No :	Test Sample	Zone of Inhibition in mm			Standard Drug	Solvent Control (Hexane)
		Sample 80%	Sample 50%	Sample 25%		
1	*Taila*	18, 18	17, 17	15, 15	Fluconazole 500 ppm – 20, 20 mm	NZ, NZ
2	Ointment	19, 19	18, 18	15, 15		

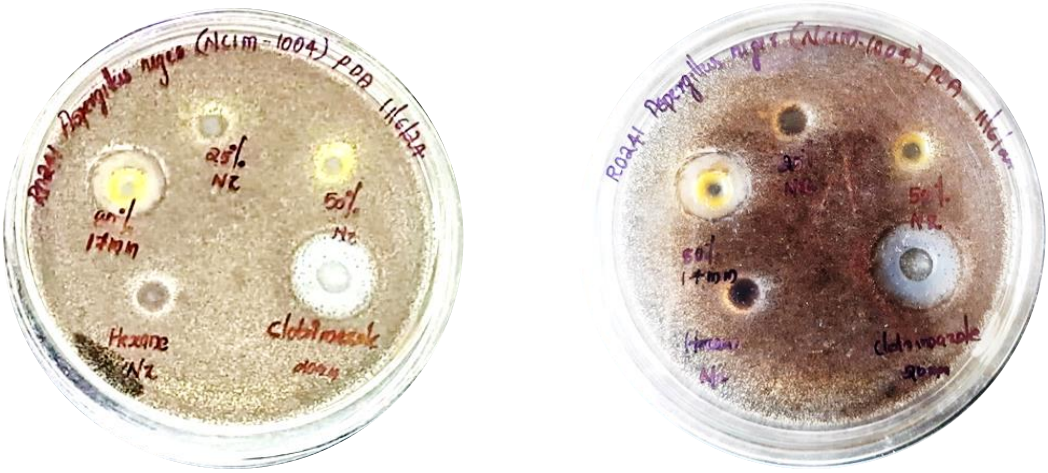
Table 3:- Results of Antifungal activity against *Aspergillus niger* :

Sl No :	Test Sample	Zone of Inhibition in mm			Standard Drug	Solvent Control (Hexane)
		Sample 80%	Sample 50%	Sample 25%		
1	*Taila*	17, 17	NZ, NZ	NZ, NZ	Fluconazole 500 ppm – 20, 20 mm	NZ, NZ
2	Ointment	17, 17	NZ, NZ	NZ, NZ		

Images of Zone of Inhibitions :

1. Images of Antifungal activity of *Manjishtadi Adityapaka Taila* against *Candida albicans* :

2. Images of Antifungal activity of *Manjishtadi Adityapaka Taila* against *Aspergillus niger* :



3. Images of Antifungal activity of *Manjishtadi Adityapaka Taila* Ointment against *Candida albicans* :

4. Images of Antifungal activity of *Manjishtadi Adityapaka Taila* Ointment against *Aspergillus niger* :

Statistical Analysis

1. Antifungal activity of *Manjishtadi Adityapaka Taila* and its Ointment against *Candida albicans* :

Graph 1:- Antifungal activity of *Manjishtadi Adityapaka Taila* and its Ointment against *Candida albicans*.

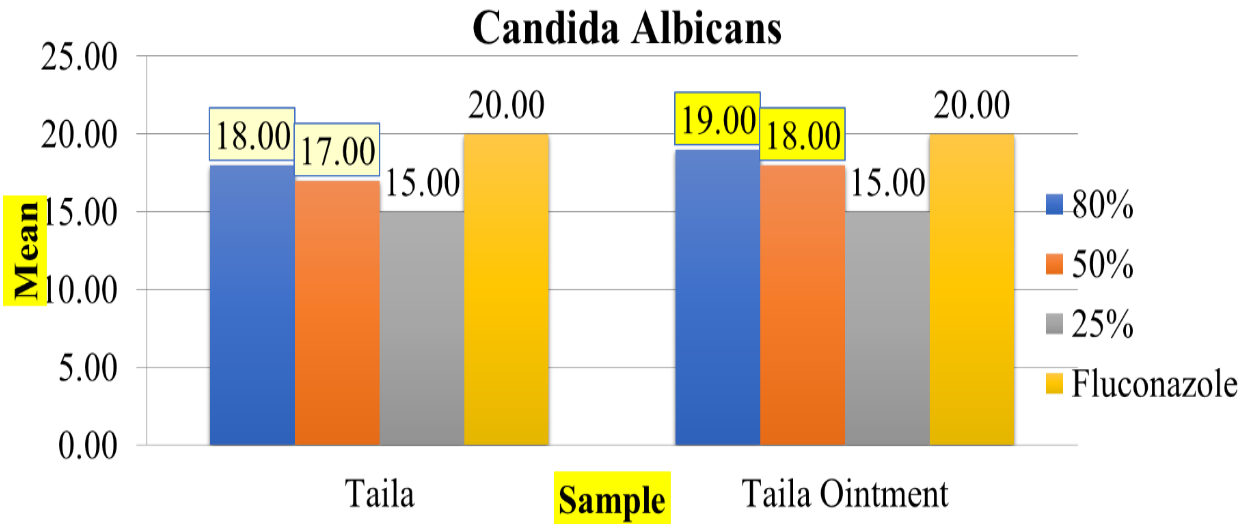


Table 4:- Statistical analysis of Antifungal activity of *Manjishtadi Adityapaka Taila* and its Ointment against *Candida albicans*.

Sample	Candida albicans	Mean	Std. Deviation	Mean Rank	Kruskal-Wallis H	p value
Taila	80%	18.000	0.000	5.500	7.000	0.072
	50%	17.000	0.000	3.500		
	25%	15.000	0.000	1.500		
	Fluconazole	20.000	0.000	7.500		
***Taila* Ointment**	80%	19.000	0.000	5.500	7.000	0.072
	50%	18.000	0.000	3.500		
	25%	15.000	0.000	1.500		
	Fluconazole	20.000	0.000	7.500		
Kruskal Wallis Test, Significant at 0.05						

Since p value > 0.05, there is no significant difference between the antifungal activities of the samples and the standard.

2. Antifungal activity of *Manjishtadi Adityapaka Taila* and its Ointment against *Aspergillus niger* :

Graph 2:- Antifungal activity of *Manjishtadi Adityapaka Taila* and its Ointment against *Aspergillus niger*.

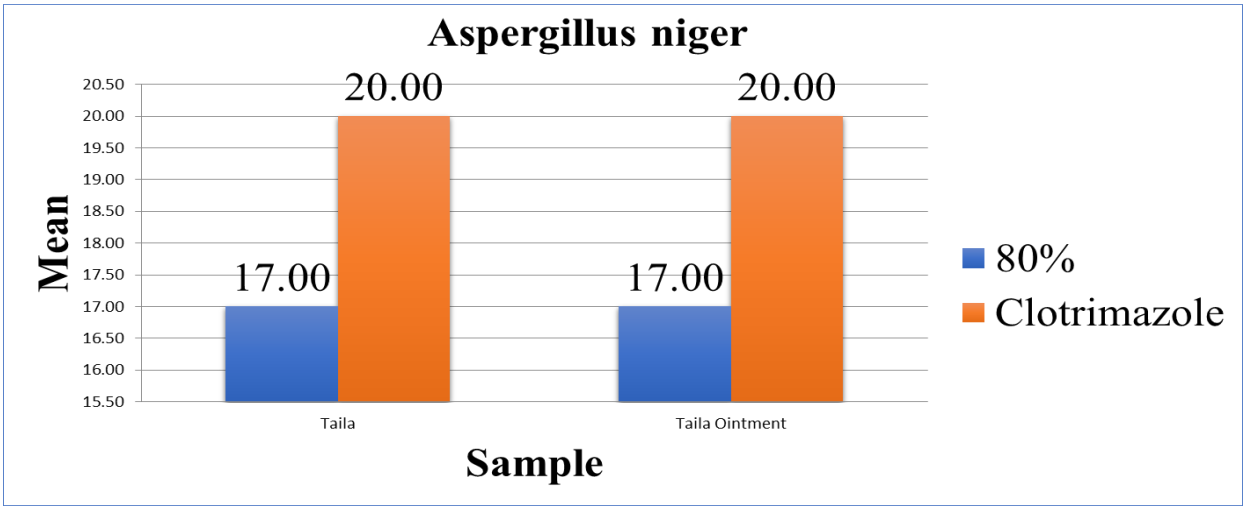


Table 5:- Statistical analysis of Antifungal activity of *Manjishtadi Adityapaka Taila* and its Ointment against *Aspergillus niger*.

Sample	Aspergillus niger	Mean	Std. Deviation	Mean Rank	Z value	p value
Taila	80	17.000	0.000	1.50	-1.732	0.083
	Clotrimazole	20.000	0.000	3.50		
***Taila* Ointment**	80	17.000	0.000	1.50	-1.732	0.083
	Clotrimazole	20.000	0.000	3.50		
Mann-Whitney U Test, Significant at 0.05						

So, there is no significant difference between 80% sample and Clotrimazole in *Taila* & Ointment against *A. niger*. Here, the *taila* and ointment shows significant antifungal activity against *Aspergillus niger* in only one concentration, ie., 80%.

Discussion:-

The *Adityapaka sneha kalpanas* are described to be used as external applications, especially for skin diseases as they absorb UV rays when exposed to sunlight. *Manjishtadi Adityapaka Taila* is one such *Adityapaka* formulation mentioned in Ayurveda classics for the treatment of *Pama kushta*.

So, considering the convenience of an ointment dosage form over the *taila*, it was modified into an ointment form, and the antifungal activities of both dosage forms were evaluated by in vitro agar well diffusion method against two selected fungi, ie., *Candida albicans* and *Aspergillus niger*.

From the results, there was no significant difference between the antifungal activities of samples and their respective standards, ie., both *taila* and ointment showed significant antifungal activity against *Candida albicans* and *Aspergillus niger*. The antimicrobial activity of each ingredient has already been proven by previous researches.

The ointment showed a 1 mm higher zone of inhibition in 80% and 50% concentrated samples than the same concentrated samples of *taila* against *Candida albicans*. The enhanced antifungal activity of the ointment may be due to the antimicrobial property of the beeswax, which is the additional ingredient.

Thus, all the ingredients possess antimicrobial activity.

The probable mode of action may be disrupting fungal cell membrane, generating Reactive Oxygen Species, inhibiting fungal enzymes, inhibiting fungal spore germination and releasing Arsenic ions – which are toxic to fungal cells, thus leading to the disruption of fungal cell membrane and cell death.
Beeswax, used as the ointment base, acts as a physical barrier thus preventing fungal growth by its lipophilic nature and antifungal activity.

Conclusion:-

Statistically significant antifungal activity was obtained for both *taila* and ointment, against both the fungi, in all the three concentrations against *Candida albicans*, and in only one concentration against *Aspergillus niger*.

The ointment showed enhanced antifungal activity than the *taila* (by 1 mm ZOI) against *Candida albicans* in two concentrations.

Pharmaceutical modification of *taila* into ointment has contributed to its antifungal activity.

From the probable mode of action, it may be concluded that both the *taila* and ointment are able to fight fungal skin infections having the presence of *Candida albicans* or *Aspergillus niger*.

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