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ANTI DERMATOPHYTIC EFFECT OF KUSHTAGHNA DASHEMANI IN DADRU KUSHTA (DERMATOPHYTOSIS), AN IN-VITRO STUDY

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ABSTRACT

Background:

Shadvirechanashatashritiya adhyaya of *Charaka Samhita* describes fifty group of drugs (*Panchashanmahakashaya*) and *Kushtaghna dashemani* is one among them. *Dadru* is classified under *Kshudra kushta* by Acharya Charaka. On the basis of presenting symptomatology *Dadru kushta* can be interpreted as Dermatophytosis. The prevalence of superficial fungal infection in India is 27.6% of which Dermatophytes are the most common agents (75.6%) though the disease is not a life threatening, the relapsing nature of this disease makes it much annoyance for patient and bothersome for physician too. Hence an attempt was made to evaluate the effect of *Kushtaghna Dashemani* on *Dadru Kushta* through in-vitro study. The In-vitro study on Anti- dermatophytic action of *Kushtaghna dashemani Kashaya* was carried out by Agar Well diffusion method on the dermatophyte species *Trichophyton tonsurans, Microsporum Canis* and *Trichophyton rubrum* with different concentrations of *Kushtaghna dashemani Kashaya.* Anti-dermatophyte effect of different concentrations of *Kushtaghna dashemani kashaya* was seen at the different volume used against the taken dermatophytes species.

Keywords: *Kushtaghna dashemani, Dadru kushta*, Anti-dermatophyte, In-vitro study

INTRODUCTION

The whole universe is the preceptor for the wise. As all the elements in the universe can be used for the purpose of *Chikitsa* on the basis of *Yukti*, A Wise Physician must rightly choose the medicine apt for the particular condition. With the same intent Acharya Charaka explains 50 *Mahakashayas* [1] of 10 drugs each with a specific karma, to treat different ailments and *Kushtaghna Dashemani* is one among them.

Dadru Kushta is one among 18 types of *Kushta*.[2] On the basis of presenting symptomatology *Dadru kushta* can be interpreted as Dermatophytosis.

Dermatophytosis is a term used to describe the superficial infections of the Skin, Nail and Hair caused by a group of fungi called Dermatophytes/Tinea. [3]

The prevalence of superficial fungal infection in India is 27.6% of which Dermatophytes are the most common agents (75.6%). [4] Multiple factors may affect the incidence of this fungal infection within a population. These include geographic area, climate, immuno-competence of the host, pathogenicity of the agent and availability of the treatment. The growing number of immuno-compromised diseases like HIV, Diabetes, COVID 19, patients of carcinoma under chemotherapy, etc... has led to increased incidence of Dermatophytosis and it has significant impact on social, psychological, economical and occupational health compromising the quality of life.

Considering all the facts there is an obvious need for an alternative treatment to treat *Dadru Kushta* (Dermatophytosis). Hence an attempt was made to check the effect of *Kushtaghna dashemani* in *Dadru kushta* (Dermatophytosis) and standardize the different concentration of *Kushtaghna Dashemani* for its Anti-dermatophytic action through an In-vitro study.

Objectives:

- To evaluate the effect of *Kushtaghna dashemani* in *Dadru kushta* (Dermatophytosis) through invitro study.

Methodology:

Materials: Standard MTCC strains of different dermatophyte species (*Trichophyton rubrum, Trichophyton tonsurans, Microsporum Cani*),40% KOH Solution, Saboraud's dextrose, ethanol, spirit

Study drug: The *Kwatha churna* of *Khadira, Abhaya, Amalaki, Haridra, Arushkara, Saptaparna, Aragwadha, Karaveera, Vidanga, Jatipravala* added in equal quantities was procured from SDP Remedies pvt ltd, Puttur, Dakshina Kannada

Name of test sample – *Kushtaghna dashemani Kashaya*

Methods:

Study: Anti-dermatophytic action of *Kushtaghna dashemani Kashaya* on *Trychophyton tonsurans, Microsporum Canis, Trychophyton rubrum* with two different concentrations of *Kashaya* per species

Study design: Microbiological culture and sensitivity test of Dermatophyte species (*Trichophyton tonsurans, Microsporum Canis, Trichophyton rubrum*)

Allotment of species and sample: Lottery method

Preparation of test sample: Ratio of drug and water taken for *Kashaya* preparation - 1 (Drug): 16 (Water)[5]

Reduced to –

Species A (*Trichophyton tonsurans*) - $1/4^{th}$ (Concentration 1), $1/10^{th}$ (Concentration 4)

Species B (*Microsporum Canis*) – $1/6^{th}$ (Concentration 2), $1/12^{th}$ (Concentration 5)

Species C (*Trichophyton rubrum*) – $1/8^{th}$ (Concentration 3), $1/14^{th}$ (Concentration 6)

10 ml of each sample were collected in a sterile container.

Preparations of Sabouraud's agar media: [6]

Glucose (40 g) and peptone (10 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 5.5 and the volume was made up to 1000 ml. Finally, 20 g agar was added to the media and autoclaved at 121˚C for 20 minutes.

Preparation of the inoculum:[7]

Trichophyton tonsurans (MTCC 8475), *Microsporum canis* (MTCC-2820), *Trichophyton rubrum* (MTCC 8477) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh.

Loopful of 7 days,10 days and 7 days old culture respectively from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum.

Well diffusion method: [8].

The media was cooled to around 45-55˚C, around 20ml each was poured into sterile Petri plates. One ml of the inoculum was immediately added to the plate, swirled for uniform distribution. Wells were bored using a sterile borer. The samples and the antibiotic were dispensed into the wells. Plates were incubated overnight at 25˚C and observed after 7 days for *Trichophyton tonsurans, Trichophyton rubrum* and 10 days for *Microsporum canis,*

The Agar diffusion study was measured by following zones –

1. Sensitive (S) zones
2. Intermediate (I)zones
3. Resistant (R) zones

The drugs used in *Kushtaghna dashemani Kashaya* with their Latin name, family, used parts and chemical composition and *karma* are as follows.

Table.no -1 Drugs mentioned under *Kushthaghna dashemani* with their latin and family name and parts used

Sl. No	Drug	Latin name	Family	Part used
1.	*Khadira*[9]	*Acacia catechu*	Mimosaceae	*Sara*
2.	*Abhaya*[10]	*Terminalia chebula*	Combretaceae	Fruit
3.	*Amalaki*[11]	*Embilica officinalis*	Euphorbiaceae	Fruit
4.	*Haridra*[12]	*Curcuma longa*	Zingiberaceae	*Kanda*
5.	*Bhallataka*[13]	*Semecarpus anacardium*	Anacardiaceae	Fruit
6.	*Saptaparna*[14]	*Alstonia scholaris*	Apocynaceae	*Twak*
7.	*Aragwadha*[15]	*Cassia fistula*	Caesalpinaceae	*Twak*
8.	*Karaveera*[16]	*Nerium indicum*	Apocynaceae	Root, leaves
9.	*Vidanga*[17]	*Embelia ribes*	Myrsiceae	Fruit
10.	*Jati pravala*[18]	*Jasminum grandiflorum*	Oleaceae	*Panchanga*

Table no.2 - Chemical composition and *karma* of *Kushtaghna dashemani* drugs

Sl no	Name	Chemical Composition	*Karma*
1	***Khadira***	10% of Acacatechin, Quercitin, gum querticin	*Kushtaghna,Dantya,Kandughna,Kasaghna,Ruchya,Medohara,Krimighna, Pramehahara,Jwarahara,Shothahara,Shwitrahara,Pachana*
2	***Abhaya***	Chebulic mycrobalan (Source for Tannin),35% Tannin, Chebulic acid ,Chebulinic acid, Gallic acid, Sorbitold, ethyl galate, punicalagin terflavin A ,Terchebin	*Rasayamna,Medhya,Chakshushya,Brimhana,Anulomana,Shwsahara,Kasahara,Pramehahara,Arshoghna,Kushtaghna,Shothahara, Krimighna,Vibandhahara,Trishnahara,Chardighna,Kandughna, Hridya,Shulahara,Lekhana,Deepana,Pachana,Vayasthapaka,Smrithiprada,Jwarahara,Vrinahara*
3	***Amalaki***	Fruit-Ellagic acid, Amlaic acid, Phyllantine, Phyllantidine, Zeatin, Zeatin nucleotide, Zeatin riboside, Benzenoid, Chebulic acid, Chebulinic	*Rasayana,Pramehaghna, Jwaraghna, Vrishya, Dahahara,Chardighna,Sophaghna,Ruchya,Medohara,Bhagna,Sandhanakara, Keshya,*

		acid, Chebulagic acid, Gallic acid, Ethyl galate, B-glucogatin, Digallic acid, Emblicol, Phyllemblic acid, Ascor bic acid, Triterpene, Lupiol, Phyllemblin, Flavonoid, Kaempheral, Rutin, B-Sitosterol, Tannin (28%), Carbohydrates, Protein, Fat, Calcium. Bark-Tannin (10-20%) Leaves- Tannin - 22%	*Chakṣhushya, Kusthaghna.*
4	***Haridra***	Rhizome of Curcuma longa contains 5% volatile oil, Resin, abundant zingeberaceous starch grains and yellow colouring substances known as curcuminoids. Chief component of curcuminoids is known as curcumin (50-60%). Volatile oil contains mono and sesquiterpenes such as a and B-pinene, a-phellandrene, camphor, camphene, zingiberene and a,ß curcumenes.	*Varnya, Twacya, Pramehahara, Sothahara, Vranahara, Kandughna, Vishaghna, Kuṣṭhaghna, Krmighna, Ruchikara, Shitapittahara.*
5	***Bhallataka***	Nuts contain- Bhilawanol, Bioflavanoids A, B and C., Anacardiac acid. Seeds contain- Bhilawanol and Anacordoside, Anacardic acid, cardol, catechol, Anacardol, Semecarpol. Nutshell contains- Bioflavaone ß, Bioflavonoids, Bhilawanol, Tetrahydroa-mentoflavone, Tetrahydroro-bustaflavone.	*Bhallataka phala- Pachana, Chedana,Bhedana, Medhya, Deepana, Vṛaṇahara, Kushtahara, Arsoghna, Jwarahara, Krmighna, Sukrala, Grahi and Balya.* *Bhallataka majja- Vrishya, Brimhaṇi, Dahashamaka, Tarpaka, Dipana.* *Bhallatakasthi- Pachaka, Medhya, Deepaka, Kṛmighna.*
6	***Saptaparna***	Ditamine, echitenine and echitamine are used as alternative to famous medicine- quinine. Akuammidine (rhazine); picrinine; strictamine, tetrahydroalstonine; angustilobine B, 6,7-seco-6-norangustilobine B (losbanine, alschomine, isolschomine, alstonamine: echitamine, tubotaiwine, alstonamine scholaricine etc.	*Hridya ,Deepana Sara, Shulanut Gulmanut, Vranahara, Asrahara, Vishamajwara Kruminut, Kushtam hanti Shwasaghna*
7	***Aragwadha***	Root bark→ Anthraquinone derivates, sennoside A&B, Fistucacidin, B-sitosterol, Volatile oil. mogyors Stem bark and Leaves→ Rhein glycoside.	*Phala (Fruit)→ Sramsana, Shulahara, Ruchya, Kostha shuddhikara, Kṛmighna, Pramehaghna, Jwaraghna, Kustaghna* *Patra (Leaf)→ Meda vishoṣhaka, Virechaka.* *Puhspa (*Flower*)→ Grahi* *Aragwadha phala majja* is.

			best *Sramsaka*
8	***Karaveera***	Karabin, Neriodorein, Neriodorin, B-Sitosterol, Neriodin, Neriodorin, Nerium D, Nerioum E (Anhydrooleandrin), B-D Digitaloside, Nerioside, obandrin, Digitoxigenin, oleandroside, rutin, plumericin etc.	*Jwarapaha* *Chakshushya* *Kushtahrut* *Kandughna* *Akshikampa-Vranapaha*
9	***Vidanga***	Fruits yielded Embelin, Quercitol, Taninn, an alkolied Christembine, Iodoembolin, Bromoembelin, Embelic acid, Quercitol, Fatty ingredients, Volatile oil, Vilangin.	*Krimighna, Dipana,* *Shulahara, Adhmanahara, Vibandhahara, Vishaghna, Ruchya, Medohara,* *Mehahara, Garbhanirodhaka,* *Varnya, Rasayana* and *Kuṣṭhaghna.*
10	***Jatipravala***	The leaf of the plant contains ascorbic acid, anthranilic acid and its glucoside, indole oxygenase, alkaloid jasminine and salicylic acid. The flowers contain pyridine and nicotinate derivatives. The oil extracted from the plant yield benzyl acetate, benzyl benzoate, phytol, methyl jasmonate, linalool, geranyl linalool and isophytol.	*Kushtanut, Varnya ,Mukharogahara, Shirorogahara ,Chakshushya*

Observations and results: The incubated plates of *Trichophyton tonsurans, Trichophyton rubrum* was kept overnight at 25˚C and observed after 7 days and plates of *Microsporum cani* was observed after 10 days.

Table no.3- Invitro antifungal activity of *Kushtaghna dashemani Kashaya* against *T. tonsurans.*

Sample	Volume	Zone of inhibition – (Radius in mm)	
Kushtaghnadashemani Kashaya- 1A	25 ml	0	0
	50 ml	5	5
	100 ml	5	6
Control (DD)	50 ml	0	0
Kushtaghnadashemani Kashaya- 4A	25 ml	0	0
	50 ml	5	5
	100 ml	6	6
Control (DD)	50 ml	0	0
Standard (*Amphotericin B)* 10 mg/ml	100 ml	9	9

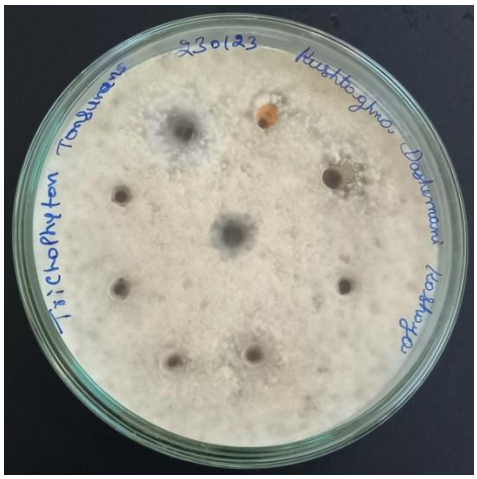


Fig.1 Result of Trichophyton tonsurans showing zones of inhibition after 7 days of observation.

Table no.4 - Invitro antifungal activity of *Kushtaghna dashemani Kashaya* against *M. canis.*

Sample	Volume	Zone of inhibition – (Radius in mm)	
Kushtaghnadashemani Kashaya-2B	25 ml	0	0
	50 ml	5	5
	100 ml	6	6
Control (DD)	50 ml	0	0
Kushtaghnadashemani Kashaya-5B	25 ml	0	0
	50 ml	6	6
	100 ml	7	6
Control (DD)	50 ml	0	0
Standard (*Amphotericin B)* 10 mg/ml	100 ml	9	9

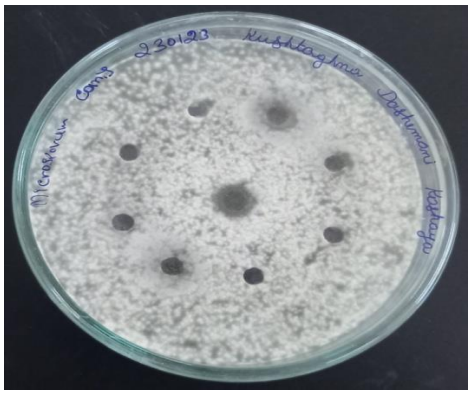


Fig.2 Result of Microsporum canis showing zones of inhibition after 10 days of observation.

Table no.5- Invitro antifungal activity of *Kushtaghna Dashemani Kashaya* against *T. rubrum.*

Sample	Volume	Zone of inhibition – (Radius in mm)	
Kushtaghnadashemani Kashaya -3C	25 ml	5	5
	50 ml	5	5
	100 ml	6	6
Control (DD)	50 ml	0	0
Kushtaghnadashemani Kashaya-6C	25 ml	0	0
	50 ml	5	5
	100 ml	6	6
Control (DD)	50 ml	0	0
Standard (*Amphotericin B)* 10 mg/ml	100 ml	10	10

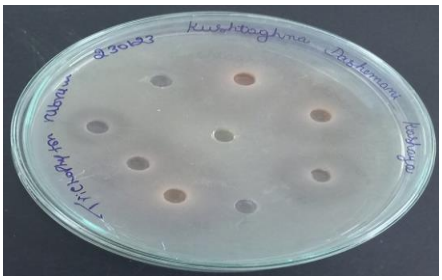
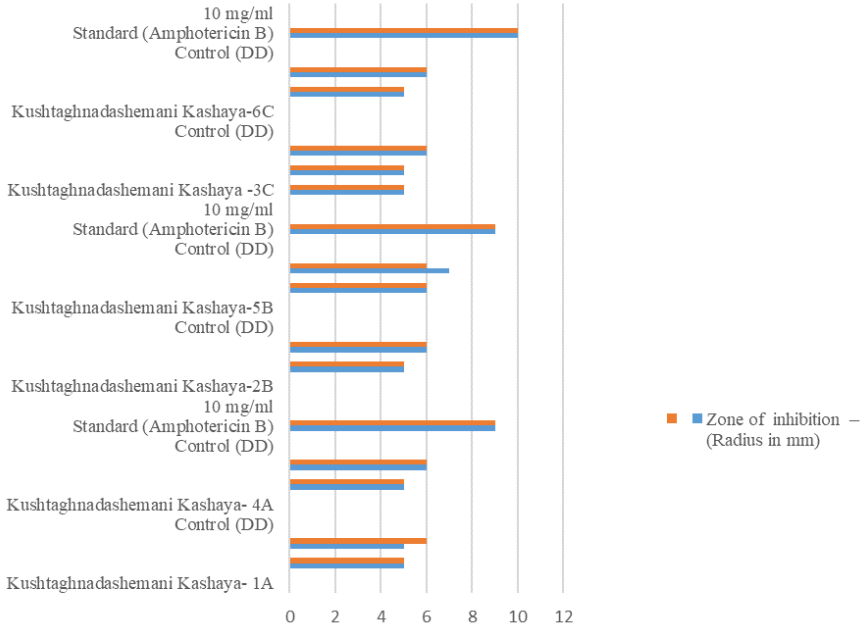


Fig.3 Result of Trichophyton rubrum showing zones of inhibition after 7 days of observation.

Fig.4 Illustration of results of all the test samples



DISCUSSION

Kushtaghna dashemani is a well-known formulation with the drugs having *kushta, krimighna* properties in it. The present study was taken up to evaluate the Anti-dermatophyte action of it in the dermatophyte species.

Criteria for selection of dermatophyte species: The criteria behind selection of the species were as per the prevalence rate and availability of Standard MTCC Strains.

Reason for selection of different concentrations: The selection of different concentrations of *Kashaya* was done with the aim of validating the concentration of *Kashaya* with the maximum potency. It also led to explore the dose-response relationship, drug development, efficacy evaluation and to know various Clinical applications.

Mode of action on dermatophytes:

Prakriti vighata is a unique and known treatment in case of *Krimi* / Infectious agents. *Prakriti* is the 'normal flora' and *Vighata* means warding off. Hence it refers to the changing the atmosphere in such a manner that it is not favourable to the growth and development of *krimi*. The drug possesses *tikta, kashaya* and *katu rasa* with *Laghu,Ushna, ruksha guna* predominantly with *krimighna karma* are known for doing *prakriti vighata.*

Anti-dermatophyte activity: Antidermatophytic activity is a technique in which response of an organism to a particular anti- dermatophytic agent is established. Many methods are employed for evaluation of anti-dermatophytic activity of a drug. In the present study well diffusion metjod was selected. It is simple and relatively inexpensive which makes it still the method of choice for the average laboratory.

Each kind of Dermatophytes has specific growth requirements, most of the microbes can be grown in culture medium in the laboratory. In the present study, Saboraud's dextrose agar media was used. Growth of the organism was confirmed by turbidity of the media and Agar is universally used as a solidifying agent.

The test drug *Kushtaghna dashemani Kashaya* was used in different concentrations against the taken Dermatophyte species, Standard Antifungal drug Amphotericin B 10 mg/ml concentration the results are expressed by determining the zone of inhibition measuring in mm by using Vernier calliper.

Results of Antidermatophyte activity:

Anti-dermatophyte effect and efficacy of different concentration of *Kushtaghna dashemani Kashaya* was seen at the different volume used against the species Trichophyton tonsurans, Microsporum canis and Trichophyton rubrum.

CONCLUSION

The above study can conclude that *guda* plays a vital part in the excretory process of the human body. It helps in the excretion of *mala* and *apan vayu* or excretory products of our body. Along with these functions, it has significant surgical importance in the human body. Its structural relevance should be kept in mind while performing any surgical or parasurgical procedures. Otherwise, it can be a life-threatening condition. *Kushtaghna dashemani Kashaya* is having numerous pharmacotherapeutic properties. Among them krimighnata of the drug was studied with respect to Antidermatophyte effect in *Dadru kushta* (Dermatophytosis) used against the species Trichophyton tonsurans, Microsporum canis and Trichophyton rubrum. with different concentrations of *Kashaya*, have shown good results and thus *Kushtaghna dashemani* can definitely be an alternative to the standard antifungal drug Amphotericin B.

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