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Research Article

IN-VITRO ANTIFUNGAL EVALUATION OF *KUSHTHAGHNA MAHAKASHAYA* PLANT EXTRACTS AGAINST DERMATOPHYTES: A STUDY ON *EPIDERMOPHYTON FLOCCOSUM, MICROSPORUM CANIS AND TRICHOPHYTON MENTAGROPHYTES*

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ABSTRACT

Now days skin diseases are very common. In society, the sufferer faces physical, emotional and socioeconomic embarrassment. In Ayurveda, skin diseases have been comprehended under the heading of *Kushtha* which causes vitiation as well as discoloration of the skin. In contemporary medical science, *Dadrukushtha* is correlated to dermatophytosis which is caused by mainly three fungal strains i.e. Trich*ophyton, Microsporum* and *Epidermophyton. Acharya Charak* mentioned the *Kushthaghna mahakashaya* for the management of *Kushtha*. In this study, anti-fungal activities of *Kushthaghna mahakashaya* plants were tested against these three common pathogenic fungal strains. **Material and Methods:** These plants were subjected to solvent extraction using hot solvent of increasing polarity into ethanol using Soxhlet apparatus. The antifungal activity of crude extracts was screened against the test organisms by agar well diffusion method. **Result**: Out of these tested plants, three plant extracts *Semicarpus anacardium, Embellica officinalis* and *Curcuma longa* showed significant antifungal activity against all three fungal strains. Five plant extracts did not show activity against *E. floccosum* but showed activity against *M. canis* and *T. mentagrophytes*. Largest ZOI (18 mm) was produced by *Embelia ribes* against *M. canis*. **Conclusion**: Based on this study, it can be concluded that some plants of *Kushthaghna mahakashaya* group have significant antifungal activity against selected fungal strains. Their significant ZOIs prove that *Kushthaghna mahakashaya* play important role in treating fungal diseases (*Kushtha*).

INTRODUCTION

All the skin diseases in Ayurveda have been discussed under the broad headings of *Kushtha*. The disease which causes discolouration of skin and the putrific changes happen in skin after a long period of time called *Kushtha*, whereas '*Ghna'* means destroying or killing.[1] Thus the drugs which are used in the treatment of all kind of skin diseases (*Kushtha*) are called *Kushthaghna Mahakashaya*[2] mentioned in *Charak Samhita*.

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Description of microorganisms involved in this study

Epidermophyton floccosum

It is a filamentous fungus that causes infections in the skin and nails of humans. This fungus, which primarily infects humans, can result in conditions such as athlete's foot (tinea pedis), jock itch (tinea cruris), ringworm of the body (tinea corporis), and nail infections (onychomycosis).[16]

Microsporum canis

It is a pathogenic, asexual fungus belonging to the Ascomycota phylum. It mainly infects the outer, dead layers of the skin of domestic cats, and can also affect dogs and humans occasionally.[17]

Trichophyton mentagrophytes

It is a species in the fungal genus *Trichophyton*. It ione of the three most common fungi that cause

ringworm in pets. It is also the second most frequent fungus responsible for zoonotic skin diseases, meaning diseases that can spread between different species.[18]

The details of the drugs of *Kushthaghna Mahakashaya* are described in Table **1**.

Table 1: Description of *Kushthaghna Mahakashaya* plants

Drug	Botanical Name	Family	Chemical constituents	Pharmacological Properties
Harad	*Terminalia chebula* Retz.	*Combretaceae*	Tannin, chebulagic acid, carbohydrates, gum	Antioxidant, wound healing, anti-diabetic hypolipidemic anti-bacterial[3] anti-fungal[4] anti-inflammatory[5], immunomodulatory, purgative Hepatoprotective, anti-carcinogenic.
Amalaka (*Aanvala*)	*Embellica officinalis* Gaertn.	*Euphorbiaceae*	Tannin, Vit. C, fat, carbohydrates	Antioxidant anti-diabetic immune-modulatory hypolipidemic,[6] protective role for skin,[7] hepatoprotective chemoprotective role anti-venom effect.
Haldi	*Curcuma longa* Linn.	*Zingiberaceae*	Curcumin, Vit.A, carbohydrates	Anti-inflammatory,[8] immunomodulatory, hepato-protective anti-microbial, anti-allergic, anti-carcinogenic, protective role in skin diseases.
Arushkara (*Bhilaawa*)	*Semecarpus anacardium* Linn.	*Anacardaceae*	Semecarpol, bhilwanol	Immunomodulatory anti-inflammatory anti-microbial[9] anti-carcinogenic
Saptaparni	*Alstonia scholaris* R.Br.	*Apocynaceae*	Ditamine, echitamine, echitanine	Anti-oxidant,[10] immunomodulatory, anti-microbial,[11] wound healing anti-carcinogenic hepatoprotective.
Aaragvadha (*Amaltaas*)	*Casia fistula* Linn.	*Fabaceae*	Anthraquinone, gluten, tannins	Laxative, hypoglycaemic, anti-inflammatory[12] wound healing anti-microbial[13]
Vidanga	*Embelia ribes* Burm.f.	*Myrsinaceae*	Embelin, christembine, volatile oil, tannin, fixed oil	Wound healing anthelmintic anti-microbial[14]
Jatipravala	*Jasminum officinale* Linn.	*Oleaceae*	Salicylic acid, jasminine	Anti-bacterial[15] anti-viral analgesic and antispasmodic.

MATERIALS AND METHODS

This study was conducted to know the antifungal activity of *Kushthaghna Mahakashaya* plant extracts against 03 common fungal strains i.e., *E. floccosum M. canis* and *T. mentagrophytes*. The extraction of these plants was done at lab of Research & Innovation Department, SKAU, Kurukshetra, while antifungal activity was conducted at lab of UIET, Kurukshetra University, Kurukshetra.

Collection of medicinal plants

The eight selected plants were purchased from local market. Fresh leaves of *Cassia fistula* were collected from university campus, Kurukshetra, Haryana, India.

Processing of the sample

a. Washing and chopping

The root, bark and other underground parts were washed carefully to remove soil and other unwanted materials such as parts of the same plant, grass, herbs, or other impurities. Both the samples collected from the campus and those bought from the market were cut into small pieces using a clean knife.

b. Drying of the sample

After cutting, the samples were placed in the shade at room temperature until fully dried. The samples were flipped at least twice a day to help dry quicker.

c. Grinding

The dried samples then proceed to grinding by means of machine to obtain fine sample powder.

Extraction of plant material

The finely ground and shade-dried medicinal plants were continuously extracted using absolute ethanol (99%) in a Universal Soxhlet apparatus (Buchi) to obtain the crude extract.

A known weight of 70 grams of the dried powder was placed in a sterile filter paper and loaded into a clean and dry thimble of the Soxhlet apparatus. The extraction was carried out for three hours. The resulting solution was then evaporated using a rotary vacuum evaporator under reduced pressure to obtain the crude mass.

Preparation of stock/working solution

A stock solution of 50mg/ml for each crude extract was prepared by dissolving 500mg of the plant extract in 10ml of DMSO (dimethyl sulfoxide) in clean, capped test tubes.

The extract was dissolved in DMSO by shaking. The test tubes were then capped, sealed, and stored in a refrigerator (2–8°C) until they were used.

Collection of standard cultures

The standard fungal cultures used in this study were obtained from MTCC, IMTECH, Chandigarh. After obtaining the cultures, the test organisms were spread on potato dextrose agar plates and kept in an incubator at 28°C ± 2°C for 2–5 days.

The test microbes used in this study are:

1) *Epidermophyton floccosum* (MTCC 7880)

2) *Microsporum canis* (MTCC 2820)

3) *Trichophyton mentagrophytes* (MTCC 7687)

Preparation of standard culture inoculum

Two to three similar appearance colonies from NA plate were picked up using an inoculating loop in aseptic conditions. The colonies were then transferred into a tube that contained 2ml of sterile potato dextrose broth. The tube was then compared with a turbidity standard (McFarland Nephelometer standard tube 0.5) as recommended by WHO (1991) for antimicrobial susceptibility testing.

Screening and evaluation of antifungal activity

The agar well diffusion method was used in this study to screen and evaluate the anti-fungal activity of medicinal plant extracts. In this method, the diameter of the zone of inhibition (ZOI) formed by the plant extract on a specific microorganism was measured to estimate the antifungal activity of the medicinal plant extracts.

Qualitative screening and determination of antifungal activity

The antifungal activity of crude extracts from medicinal plants was tested against the selected test organisms using the agar well diffusion method as described by Dingle et al. (1953). Sterile Potato Dextrose Agar (PDA) plates of about 4mm thickness were prepared. A total of three plates were made. Before use, the plates were dried under hot air at a suitable temperature to remove any excess moisture from the surface of the medium. A fresh inoculum that matched the turbidity standard was prepared. Then, 100 ml of the test microorganisms in PDB were poured onto the plates using Pasteur pipettes and spread evenly using sterile cotton swabs. Excess inoculum was removed by pressing and rotating the swab against the inner wall of the tube above the liquid level, and the swab was carefully applied over the entire surface of the plates. The plate was rotated by 90 degrees after each swab. Finally, the swab was passed around the edges of the agar surface. The inoculated plates were left to dry for a few minutes at room temperature with the lid closed. Three wells were made on each plate using a sterile cork borer no. 8, resulting in a well diameter of 6mm. 200ml of the extract was added to each well using a micropipette. The plates were then left for half an hour with the lid closed so that the extract could diffuse into the medium. The plates were incubated overnight at 28±2°C for 2–5 days. After proper incubation, the plates were examined for the presence of fungal growth inhibition, which was indicated by a clear zone surrounding the wells. The size of the zones of inhibition (ZOI) was measured and the antifungal activity was expressed in terms of the average diameter of the inhibition zone in millimeters. The absence of a zone of inhibition was interpreted as a lack of activity. The ZOI measurements were taken using a scale and the mean was recorded.

RESULT

The antifungal activity was assessed by measuring the diameter of zone of inhibition (ZOI) produced by *Kushthaghna Mahakashaya* plants extract on particular microorganism i.e., E. floccosum, M. canis and T. mentagrophytes. [Table 2] and [Figure 1]

Table 2: Antifungal efficacy of *Kushthaghna Mahakashaya* constituent herbs against *E. floccosum, M. canis* and *T. mentagrophytes*

S.No.	Medicinal Plant extract (Absolute ethanol)	Anti-fungal activity ZOI (in mm) against tested Microbes		
		E. floccosum **(MTCC 7880)**	***M. canis*** **(MTCC 2820)**	***T. mentagrophytes*** **(MTCC 7687)**
1.	*Alstonia scholaris*	-	13	16
2.	*Terminalia chebula*	-	10	13
3.	*Embelia ribes*	-	18	17
4.	*Semecarpus anacardium*	10mm	12mm	13
5.	*Jasmin officinale*	-	14	10
6.	*Emblica officinalis*	10	16	16
7.	*Cassia fistula*	-	-	16
8.	*Semecarpus anacardium* (after purification)	-	-	15
9.	*Curcuma longa*	12	10	12

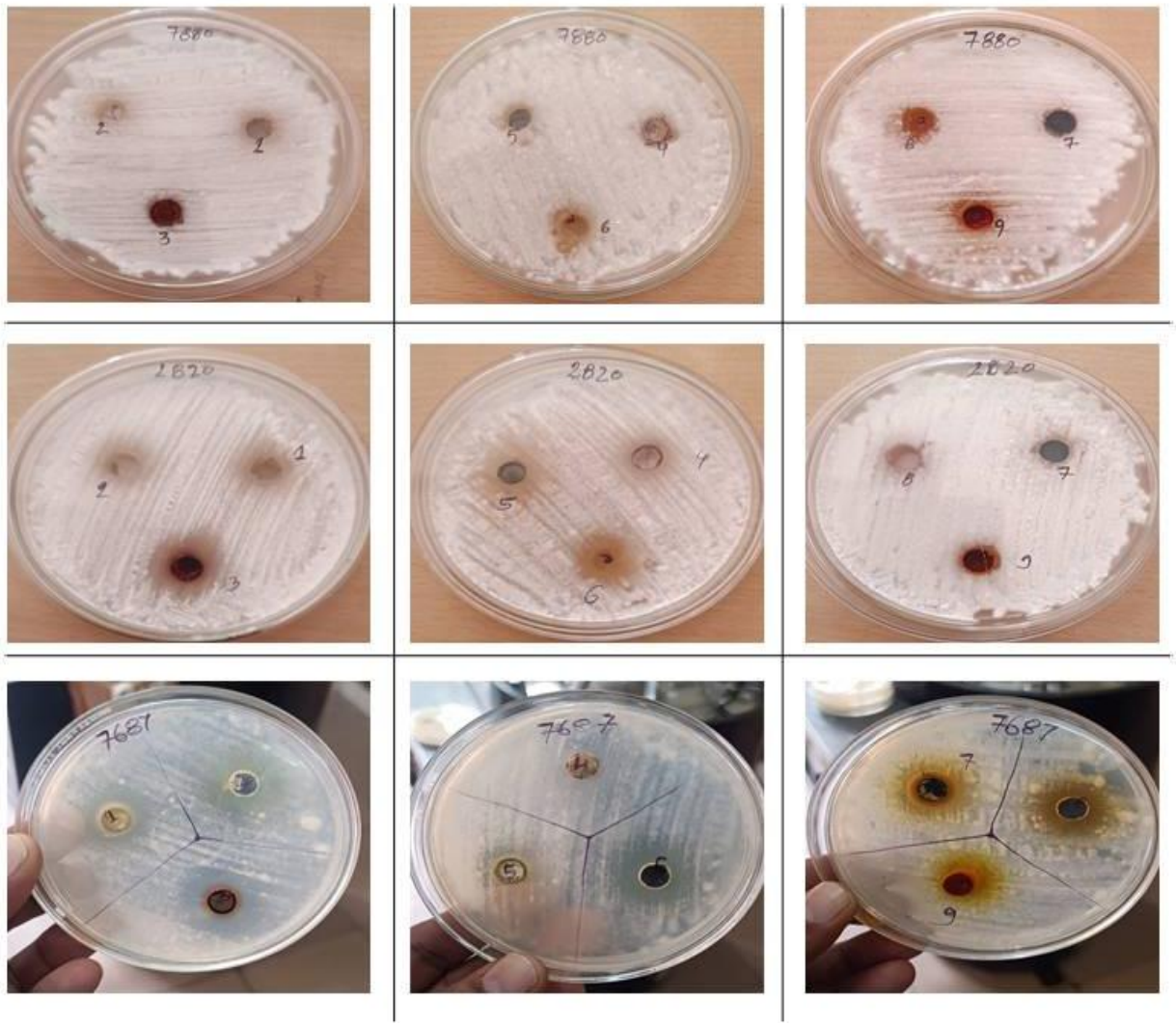

Figure 1: Zone of inhibition (ZOI) of *Kushthaghna Mahakashaya* constituent herbs ethanolic extracts against *E. floccosum* (MTCC 7880), *M. canis* (MTCC 2820) and *T. mentagrophytes* (MTCC 7687)

DISCUSSION

In this study, nine constituent herbs of *Kushthaghna Mahakashaya* were evaluated for their in-vitro antifungal efficacy.

- The antifungal potential of the selected *Kushthaghna Mahakashaya* drugs demonstrates promising activity, particularly against *Trichophyton mentagrophytes* and *Microsporum canis*. Among the tested drugs, *Embelia ribes* showed the most potent antifungal activity with a ZOI of 18mm against *Microsporum canis* and 17mm against *Trichophyton mentagrophytes*, suggesting strong broad-spectrum efficacy. This aligns with previous studies that highlight its bioactive compound embelin, known for antimicrobial and antifungal properties.
- Both *Emblica officinalis* and *Alstonia scholaris* also showed good antifungal activity, with a ZOI of 16 mm against Trichophyton mentagrophytes. Additionally, *Emblica officinalis* showed 10mm and 16mm ZOI against *E. floccosum* and *M. canis* respectively, indicating a consistent and balanced antifungal profile. These results reinforce the traditional claims of *Amla* (*Emblica officinalis*) in treating skin conditions associated with fungal infections.
- *Semecarpus anacardium*, both in its raw and purified forms, displayed moderate antifungal action. The raw extract inhibited all three fungi, with the highest ZOI being 13mm against *T. mentagrophytes*, whereas the purified extract retained activity only against *T. mentagrophytes* (15mm). This suggests that purification may selectively enhance or diminish antifungal components, possibly due to the removal of certain phytochemicals during *Shodhana*.
- *Jasminum officinale* and *Terminalia chebula* showed limited activity, with ZOIs ranging between 10–14 mm, indicating mild to moderate efficacy. Interestingly, *Curcuma longa* demonstrated consistent inhibition across all three organisms, though the ZOIs (10–12mm) were relatively modest. This is in accordance with the known antifungal and anti-inflammatory actions of *curcumin*, though its moderate potency may be attributed to its solubility or extract concentration in ethanol.
- *Cassia fistula*, on the other hand, showed activity only against *T. mentagrophytes* (16mm), indicating a targeted effect rather than broad-spectrum antifungal activity.
- For *Epidermophyton floccosum*, none of the herbs tested showed a significant ZOI, except for *Semecarpus anacardium, Emblica officinalis,* and *Curcuma longa*. This implies that *E. floccosum* is less sensitive to these plant extracts, which may mean that higher concentrations or different methods of extraction are needed to improve their effectiveness.

CONCLUSION

The findings of this study indicate that *Embelia ribes, Emblica officinalis* and *Alstonia scholaris* are the most promising herbs from the *Kushthaghna mahakashaya* group in terms of antifungal activity. Their significant ZOIs, especially against *Trichophyton mentagrophytes* and *Microsporum canis*, support their traditional role in managing *Kushtha* (skin disorders).

Based on this study, it can be concluded that some of the herbs in the *Kushthaghna mahakashaya* group have notable antifungal activity against commonly occurring fungal strains. Since these fungal strains are responsible for dermatophytosis, the herbs studied play an important role in treating fungal diseases.

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