

Isolation and Identification of Dermatophytes From Clinical Samples and Antidermatophytic Activity of *Lawsoniainermis* (Henna plant)

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ABSTRACT

Dermatophytes are a group of closely related fungi that have the capacity to invade the keratinized tissue (skin, nail and hair) of human and other animals to produce cutaneous infection called dermatophytosis commonly referred to as ring worm infections or tinea. In this study, to isolate and identify the dermatophytes from clinical samples and to study the antidermatophytic activity of *Lawsoniainermis* (henna plant). Six different isolates, *Trichophyton rubrum*, *Trichophyton everrucosum*, *Trichophyton tonsurans*, *Trichophyton equinum*, *Microsporum canis*, *Microsporum gypsum* were identified. The chloroform extract of henna plant showed maximum zone of inhibition against *Microsporum canis* (41mm) when compared to other isolates.

Keywords: Dermatophytes, Keratin, Dermatophytosis, Non dermatophytic fungi.

INTRODUCTION

Dermatophytes are fungi that require keratin for growth. These fungi can cause superficial infections of the skin, hair and nails. Dermatophytes are spread by direct contact from other people, animals and soil, as well as indirectly from fomites. (Barry et al., 2003). Some dermatophytes are spread directly from one person to another (anthropophilic organisms), others live in and are transmitted to humans from soil (geophilic organisms), and still others spread to humans from animal hosts (zoophilic organisms). Transmission of dermatophytes also can occur indirectly from fomites (eg., upholstery, hair brushes and hats). Infections are commonly referred as ring worm or tinea include tinea capitis (head), tinea barbae (face and neck), tinea corporis (body), tinea cruris (groin), tinea inguinum (nail) and tinea pedis (athlete's foot).

The dermatophytes are hyaline and septate molds with more than hundred species described. 42 species are considered valid and less than half are associated with human diseases. These are divided into three main anamorphic genera depending on their morphological characteristics, *Trichophyton* (24 species), *Microsporum* (16 species), *Epidermophyton* (2 species). The teleomorphic states of only 23 dermatophytes have been described which either belong to genus *Trichophyton* or *Microsporum* as the sexual

state of *Epidermophyton* has not yet been described. (Jadish chander, 1995). Ira Salkin et al., 1997 reported that, a medium, Dermatophyte identification medium (DTM) was specifically developed to eliminate problems of false – positive results associated with commercially marketed media, such as Dermatophyte Test Medium (DTM). DTM culture was an inexpensive, rapid and accurate method for the presumptive identification of dermatophytes in the clinical mycology laboratory.

Traditional medicinal practice has been known for centuries in many parts of the world for the treatment of various human ailments. Medicinal plants are part and parcel of human society to combat from the dawn of civilization. According to the report of World Health Organization (WHO) 80% of the world population mainly on traditional therapies which involve the use of plant extracts or their active substances. (Daljit et al., 2007). The antimicrobial properties of the medicinal plants are reported from all over the world recently and used in the treatment of many diseases. *Lawsonia* (Henna) is a small shrub frequently cultivated in India, Persia and along the African coast of the Mediterranean sea. Powdered leaves of this plant in the form of a paste are used both as cosmetic and as remedy for boils, wounds and some mycotic infection in certain countries of the

Middle East. Antifungal screening of higher plants, the leaves of *Lawsoniainermis* were found to be exhibit strong fungi toxicity where naphthaquinones were found to be active factor. (Tripathi and Dixit, 1978).

MATERIALS AND METHODS

Collection of samples

50 samples (skin scrapping, nail and hair) were collected from clinically suspected cases of dermatophytosis. The collected samples were transported using sterilized sample collector vials to laboratory for the microbiological examination.

Direct microscopic observation by KOH mount (singh, 2003)

A drop of 10 % KOH (in case of skin and hair) and 40 % (in case of nail clipping) was kept on a clean, grease free glass slide. Then the sample (Skin scrap and hair) was mixed gently with the KOH drop and the slide passed through a burner flame to hasten keratolysis (keratolysis softened the sample). This preparation was covered by a clean glass cover slip without trapping any air bubbles. After that the mount was observed under high power objective. In case of nail sample, the nail clippings in KOH were kept for overnight. Then the mount was observed under high power objective.

Culturing of dermatophytes

To a sterile petridish, 20ml of sterile (sabouraud dextrose agar and potato dextrose agar) medium was poured. To the solidified medium, the sample (hair, skin scrapping or nail clipping) was placed under sterile conditions. The plates were incubated at 25-27°C for 3 weeks, and observed for growth.

Selective medium for dermatophytes dtm (dermatophyte test medium)

The positive cultures from sabouraud dextrose agar and potato dextrose agar were inoculated in the dermatophyte test medium (DTM), selective medium for dermatophytes. The bottles were incubated at 25-27°C for 7-9 days. After incubation, the grown dermatophytic fungus was identified by Lacto phenol cotton blue mount (LPCB).

Lactophenol cotton blue mount

A drop of lacto phenol cotton blue was placed on the clean grease free glass slide, and a tuft of fungal filament was picked up from the culture plate using teasing needle. Then the filaments were transferred to the lacto phenol stain and gently teased and a clean cover glass was carefully placed over the

preparation without any air bubbles. This preparation was examined under high power objective. The hyphae, spore structure and their arrangement were observed.

Antidermatophytic activity of *Lawsoniainermis* (Henna Plant)

Collection of Henna Plant

The leaf portion of henna plant were collected and dried in shade.

Preparation of solvent extraction of henna plant

20 g of henna leaf were suspended in 50 ml of distilled water in 100 ml flask for 24 hrs at room temperature. The mixtures were then sieved through a fine muslin cloth followed by filtration using filter paper to trap the finer particles that went through the cloth. The filtrates were then mixed with the solvents (chloroform, petroleum ether) in separating funnel and the mixture was shaken well until separation was observed in form of two layers; the water and the solvent extract. The different layers were run out into separation beakers and placed in oven to dry at 50°C.

Preparation for henna plant extract disc

The sterile discs were loaded with 50 µl of extracts dissolved in Dimethylsulfoxide (DMSO) and were left to dry for 6 to 10 hours in 37°C in sterile condition. Prepared discs were stored at 4°C in the refrigeration till use. To avoid any condensation the discs were kept at room temperature for 1 hour before use.

Agar disc diffusion assay

Sabouraud's dextrose agar were prepared and sterilized. Then 0.1 ml of spore suspensions of each clinical isolates were aseptically transferred into sterile petriplates and 20 ml of the cooled molten Sabouraud's dextrose agar was poured to each plate and the plates were rotated clockwise and anti-clockwise for uniform mixing of fungal spore suspensions. After solidification, the discs impregnated with henna plant extract were placed on inoculated SDA plate using sterile forceps. Antifungal antibiotics such as nystatin, and amphotericin B act as a control. Then the plates were incubated at 25°C for 3-5 days. After incubation, the zone of inhibition around the discs were measured.

RESULTS AND DISCUSSION

Isolation and identification of dermatophytes

In this study, A total of 50 clinical samples (skin, hair and nail) were collected from 22 men (44%) and 28 women (56%) and all the

collected samples were plated on dermatophytes test medium (DTM). The most of the dermatophytic fungi were isolated from skin samples than hair and nail. Based on the colony morphology and microscopic observations, 6 different species were identified namely, *Trichophytonrubrum*, Flat to slightly raised velvet, yellow brown to red pigment on reverse side of the medium and tear-drop macro conidia, abundant micro conidia as small pyriform observed under microscope. *Trichophytonverrucosum*, heaped, deeply folded and white on reverse side of the medium and macro conidia as long, thin, smooth walled, many chlamydospore chains, micro conidia as pyriform observed under microscope. *Trichophyton tonsurans*, flat with raised growth, powdery to cream, yellow brown on reverse side of the medium and abundant microconidia, thick walled, irregular, much branched, macroconidia observed under microscope. *Trichophytonequinum*, white to buff colored, dark red pigment at the center of the medium and abundant micro conidia, macro conidia as clavate, thin and smooth observed under microscope. *Microsporumcanis*, flat, white to cream colored with a dense cottony surface, brownish yellow on reverse side of the medium and macro conidia as spindle shape, rough and thick walled, few micro conidia as pyriform to round shape observed under microscope. *Microsporum gypsum*, powdery, tawny buff surface, yellow brown on reverse side of the medium and abundant, thin walled macroconidia with 4-6 septa observed under microscope. All isolated dermatophytes belongs to the Genus *Trichophyton* and *Microsporum*. Among 6 different isolates, *Trichophytonrubrum*, *Trichophytonequinum*, *Microsporumcanis* and *Microsporum gypsum* were found frequently.

Nasreen Khalid Thebo et al., (2006) reported that a total of 13 species of dermatophytes viz., *Aspergillusniger*, *Aspergillusfumigatus*, *Aspergillusflavus*, *Trichophytonmentagrophytes*, *Trichophytonrubrum*, *Trichophytonverrucosum*, *Trichophyton tonsurans*, *Trichophytonmagnini*, *Microsporumcanis*, *Microsporum gypsum*, *Microsporumadouini*, *Candida albicans* and *Epidermophytonfloccosum* were isolated and identified from 26 cases from civil hospital, Karachi and 40 cases from the Liaquat university of medical and health sciences, Jamshoro. In this study, we observed that the most common *Trichophyton* and *Microsporum* was more in skin samples than

hair and nail which were collected from females were found to have dermatophytic infections than males. Kannan et al., (2006) reported that more isolates could be obtained from skin and hair compared to nail specimens. More exposure and more surface area of skin may be the reason for dermatophytic infection.

In our study, anti dermatophytic activity of *Lawsoniainermis* (henna) against 4 isolates such as *Trichophytonrubrum*, *Trichophytonequinum*, *Microsporumcanis*, *Microsporum gypsum* which were obtained frequently in all the samples were detected. The chloroform extraction of henna plant showed maximum zone of inhibition against *Microsporumcanis* (41 mm) followed by *Trichophytonequinum* (30 mm), *Trichophytonrubrum* (27 mm) and *Microsporumgypseum* (24 mm) than the petroleum ether extraction of henna plant showed high inhibition zone in *Microsporumgypseum* (14 mm), followed by *Trichophytonequinum* (13 mm), *Trichophytonrubrum* (13 mm) and *Microsporumcanis* (11 mm). The clinical isolates were sensitive to nystatin and amphotericin B which were used as control. (table-2).

Chaudhary et al., (2010) who reported that during screening of barks of 30 plant species against dermatophytes, only *Lawsoniainermis* (Henna) extract exhibited absolute toxicity. The extract showed broad fungitoxic spectrum when tested against 13 ring worm fungi. *Lawsoniainermis* exhibited absolute toxicity against ring worm causing fungal species such as *Microsporum gypseum*, *Trichophytonmentagrophytes*, (Singh, 2003).

In this study, the medicinal plant, *Lawsoniainermis* (henna) extract was prepared by chloroform and petroleum ether. The chloroform extraction of henna plant showed maximum zone of inhibition against 4 tested dermatophytes. Among 2 solvents, chloroform extraction of *Lawsoniainermis* (henna) has given good results.

CONCLUSION

The antimicrobial properties of the medicinal plants are reported from all over world and used in the treatment of many diseases. Medicinal plants are the best source to obtain a variety of newer herbal drugs. From this study, *Lawsoniainermis* (henna) has given good results against dermatophytes and it may be recommended for dermatophytic infection.

Table 1: Sex Distribution of Samples

TYPE OF SAMPLE	MALE	FEMALE
Skin	15	20
Nail	5	7
Hair	2	1
Total	22 (44%)	28 (56%)

Table 2: Antidermatophytic activity of solvent extraction of *Lawsoniainermis* (henna) plant

S.No	Name of the Dermatophytes	Zone of inhibition in mm			
		Solvent extraction of henna plant		Antifungal antibiotics (control)	
		Chloroform	Petroleum ether	Nystatin	Amphotericin B
1	<i>Trichophyton rubrum</i>	27	13	09	13
2	<i>Trichophyton equinum</i>	30	13	14	12
3	<i>Microsporum canis</i>	41	11	10	13
4	<i>Microsporum gypsum</i>	24	14	20	25

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