

SCREENING OF *LAWSONIA INERMIS* ESSENTIAL OIL AGAINST FUNGI CAUSING DERMATOPHYTIC INFECTION IN HUMAN

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ABSTRACT

Objective: *Lawsonia inermis* belonging to family Lythreace commonly known as henna has been used in traditional herbal medicine from age. This study deals with the extraction of henna essential oil, separation of essential oil fractions and antidermatophytic behavior of oil and their fractions.

Methods: In present investigation, essential oil obtained from the leaves of *Lawsonia* through hydrodistillation method was screened for their antidermatophytic activity against selected dermatophytes through disc diffusion technique and minimal inhibitory concentration (MIC) by semisolid agar susceptibility testing methods. *Lawsonia* oil was further subjected in buchii glass oven equipment for the separation of the different fraction at different temperature interval. These fractions were labeled as LA_I, LA_{II}, LA_{III}, LA_{IV}, and LA_V.

Results: MIC of *Lawsonia* essential oil was ranging from 0.025 to 1.5 µl/ml against selected dermatophytes and other related fungi. MIC of these fractions were also studied which were ranging from 0.3 to <4 µl/ml. *Trichophyton rubrum* was found to be most susceptible fungus and *Candida albicans* was most resistant strain. Among all fractions studied LA_{II} was found to be the most effective fraction. Fraction LA_I was discarded because of very less amount which could not be applied for MIC.

Conclusion: The *L. inermis* demonstrating broad spectra of activity may help to discover new antibiotics that could serve as selective agents for the maintenance of animal or human health and provide biochemical tools for the study of infectious diseases. This versatile medicinal plant is the unique source of various types of chemical compounds, which are responsible of the various activities of the plant.

Keywords: Essential oil, Dermatophytes, *Trichophyton rubrum*, Minimal inhibitory concentration.

INTRODUCTION

Lawsonia is a monotypic genus represented by *Lawsonia inermis* Linn (syn. *Lawsonia alba*). The plant belongs to the family Lythreaceae and is a native of South West Asia and North Africa and nowadays is widely cultivated in India, Middle East and along the African coasts of the Mediterranean Sea [1,2] and commonly known as henna. Henna has been used medicinally and cosmetically for over 9000 years. Traditionally in India, mehndi is applied to hands and feet. Its use became popular in India because of its cooling effect in the hot Indian summers. Henna leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments [3-5]. The plant has been reported to have antiviral, antitrypanosomal, molluscicidal, cytotoxic, anti-inflammatory, analgesic, antifungal, tuberculostatic, antibacterial, anti-diabetic, hepatoprotective, and antioxidant properties [6-12]. It is considered as a source of unique natural product for development of medicines against various diseases and also for the development of industrial products. The development of new antimicrobial agents is a research area of the utmost importance.

Human infections, particularly those involving the skin and mucosal surface constitute a serious problem, especially in tropical and subtropical developing countries due to their prevailing moisture and temperature regimes. Dermatophytoses pose a serious concern to the sociologically backward and economically poor population of India [13]. In dermatophytic infections of skin, the fungus remains confined to stratum corneum while pathogenic changes are produced in the deeper layer of the epidermis and dermis. These fungi produced a ring-shaped lesion on skin. These infections are usually treated by topical antimycotic agents generally by the use of a drug belonging to the imidazole family like griseofulvin, itraconazole, ketoconazole, zacon, and miconazole. These drugs are mostly fungistatic, and a source of this agent is largely nonrenewable petroproducts that are non-

degradable and cause adverse side effects and residual toxicity. Besides long duration of the treatment High cost of this medicines and the development of several side effects, prevented these drugs from being accepted in every case.

Study of *L. inermis* essential oil and their fractions in respect of the treatment of tinea or dermatophytic infections are an untouched area of research till now.

These finding promoted us to explore *Lawsonia* essential oil and their fractions for the control of dermatophytes. Present studies deal with antidermatophytic activity of *L. inermis* essential oil and their fractions isolated from different temperature intervals.

METHODS**Oil extraction**

Leaves of *L. inermis* collected from the University Nursery were shade dried. The semi-crushed leaves were hydrodistilled in a Clevenger's apparatus for 4 hrs. Essential oil collected in tubes were dried with anhydrous sodium sulfate. Moisture free oil was then stored in amber colored bottles and kept in the refrigerator.

Essential oil of *L. inermis* was further subjected in glass bulbs of buchii glass oven equipment, purchase from Switzerland for the separation of their ingredients, at a different temperature.

Microorganism for *in vitro* studies

Lawsonia oil was evaluated for their antifungal properties against selected dermatophytes and related fungi. Among this *Trichophyton rubrum*, *Trichophyton verrucosum*, *Microsporum gypseum*, and *Candida albicans* were isolated from infected skin scrapings of tinea patients from SMS Hospital, Jaipur, while *Microsporum fulvum*, *Microsporum canis*, and *Fusarium verticillioides* were isolated from soil samples

through To. Ka.Va. hair baiting technique [14]. These fungi were maintained on Sabouroud's dextrose agar medium.

Screening of oil

The filter paper disc method of Wannisorin *et al.* [15] was used for screening the essential oils against dermatophytes. Standard size Whatman no. 1 filter paper discs 6.0 mm in diameter, sterilized by dry heat at 140°C in an oven for 1 hr were used to determine antifungal activity. 20 ml sterilized Sabouraud's dextrose agar medium was taken in each autoclaved Petri dish and allowed to solidify. Fungal spore suspension was prepared in sterilized distilled water by transferring a loopful of 15-day-old culture. 1 ml of spore suspension of approximately 0.5 to 5×10^4 (cfu/ml) was spread over the respective agar medium plates. Sterilized filter paper was soaked in neat undiluted (100%) as well as in diluted oil (25%, 50%, 75% concentration). Dilution has been done in acetone. An oil saturated disc was placed on an agar plate containing fungal spore suspension. Ketoconazole was used as a standard drug. These plates were incubated. Five replicates were kept in each case, and the average values were determined and inhibition zone (IZ) was observed. The antifungal activity was determined by measuring the IZ around the disc. The activity of oil was measured by the following formula:

$$\text{Activity index} = \frac{\text{IZ of the sample}}{\text{IZ of the standard}}$$

Semisolid agar antifungal susceptibility (SAAS) method

SAAS testing method for end point determination minimal inhibitory concentration (MIC) was carried out in brain heart infusion agar (BHIA) (Himedia). BHIA was prepared according to manufacturer's instruction.

Inoculum preparation

Sterile swab dipped into sterile tween 80 was used to pick the pure colony of yeast. This was then suspended in 3-4 ml of sterile normal saline and vortexed. The turbidity of the homogenous suspension was adjusted to ~ 0.5 McFarland standard. Similarly, inoculum was prepared for filamentous fungi (3-7 days old slant at 37°C on potato dextrose agar). By swabbing the pure colony (mixture of conidia and hyphal fragments) was suspended in 3-7 ml of sterile saline. The mixture was vortexed and heavy particles were allowed to settle. The homogenous suspension was adjusted to 0.5 McFarland standard.

Inoculation of drug-containing tubes

The semisolid agar tubes containing known concentrations of test oils as well as oil-free controls, prepared in duplicate, were inoculated with one loopful (Himedia Flexiload 4) of 0.5 McFarland adjusted culture by inserting the loop deep within the semisolid agar. The tubes were incubated at 37°C for 48 hrs (96 hrs for dermatophytes). A loopful of the inoculum suspension was streaked onto Sabouraud dextrose agar to check for purity and viability.

End point determination

End point determination was done according to the NCCLS/CLSI guidelines, M27-A, and M38-A. Growth was compared to that of oil-free control and scored by visual inspection as follows: +4: Growth same as control; +3: Slight decrease in growth; +2: Significant reduction in growth reduction 80% in yeast and 50% in filamentous; +1: Slight growth or few visible hyphal fragments; 0: No growth.

RESULTS

L. inermis is very well known plant generally used in dye industries. Major constituents of essential oil are 1, 8-cineole α -pinene, p-cymene, eugenol, hexadecanoic acid, phytol, α -terpinol and etherphenylvinyl. It is clear from the results that *lawsonia* oil exhibited excellent anti-dermatophytic activity against dermatophytes (Table 1). It was also observed from the results that disc diffusion technique was not so effective as compared to semisolid agar susceptibility method of MIC.

The maximum zone of inhibition was found against *M. gypseum* (10 ± 0.57 mm) followed by *C. albicans* (9 ± 1.00 mm) and *M. canis* (8 ± 0.57 mm) and *F. verticilloides* (8 ± 1.00 mm). Data incorporated in Table 2 shows MIC of *L. inermis* essential oil against test fungi. *Lawsonia* oil exhibited excellent result against *Trichophyton* spp., e.g., *T. rubrum* (0.025 μ l/ml) and *T. verrucosum* (0.05 μ l/ml) followed by *M. fulvum* (0.3 μ l/ml), *M. gypseum* (0.4 μ l/ml), *M. canis* (1.1 μ l/ml) and *F. verticilloides* (1.5 μ l/ml). *F. verticilloides* was found less susceptible to this oil.

By using glass oven equipment five fractions were obtained at different temperature interval. LA_I was very less in amount (μ l) so MIC was not screened. During the screening of LA_{II} fraction (Table 3), *T. rubrum* (0.3 μ l/ml) was found to be most susceptible fungus. MIC against *M. gypseum* was found to be 0.5 μ l/ml and 0.9 μ l/ml against *M. canis* and *C. albicans*. *T. rubrum* was also found susceptible to LA_{III} (0.4 μ l/ml) and LA_{IV} (0.3 μ l/ml) fractions, while *C. albicans* was found most resistant fungus for all the fractions tested (above 3 μ l/ml). LA_V fraction was found to be the least effective fraction as compared to rest of the fractions of *Lawsonia* oil.

Table 1: Comparative analysis of *Lawsonia inermis* essential oil with standard drug

Fungi	Activity of oil and standard drug		
	<i>L. alba</i> IZ (mm)	Ketoconazole IZ (mm)	AI
<i>M. canis</i>	8 ± 0.57	14 ± 1.80	0.57
<i>C. albicans</i>	9 ± 1.00	28 ± 1.73	0.32
<i>F. verticilloides</i>	8 ± 1.00	20 ± 1.00	0.40
<i>M. gypseum</i>	10 ± 0.50	37 ± 0.50	0.27

IZ: Inhibition zone including 6 mm diameter of filter paper disc, AI: Activity index, *M. canis*: *Microsporium canis*, *C. albicans*: *Candida albicans*, *F. verticilloides*: *Fusarium verticilloides*, *M. gypseum*: *Microsporium gypseum*

Table 2: MIC (μ l/ml) of essential oil against selected dermatophytes

Fungal species	Oil
	<i>L. inermis</i>
<i>C. albicans</i>	0.7
<i>M. gypseum</i>	0.4
<i>M. canis</i>	1.1
<i>T. rubrum</i>	0.025
<i>T. verrucosum</i>	0.05
<i>M. fulvum</i>	0.3
<i>F. verticilloides</i>	1.5

L. inermis: *Lawsonia inermis*, *M. canis*: *Microsporium canis*, *C. albicans*: *Candida albicans*, *F. verticilloides*: *Fusarium verticilloides*, *M. gypseum*: *Microsporium gypseum*, *T. rubrum*: *Trichophyton rubrum*, *T. verrucosum*: *Trichophyton verrucosum*, *M. fulvum*: *Microsporium fulvum*, MIC: Minimal inhibitory concentration

Table 3: MIC (μ l/ml) of *Lawsonia inermis* fractions against dermatophytes

Fungi	Fraction				
	LA_I	LA_{II}	LA_{III}	LA_{IV}	LA_V
<i>C. albicans</i>	-	0.9	<3	<3	4
<i>M. gypseum</i>	-	0.5	0.9	0.6	0.6
<i>M. canis</i>	-	0.9	0.3	3.2	<4
<i>T. rubrum</i>	-	0.3	0.4	0.3	1

C. albicans: *Candida albicans*, *M. gypseum*: *Microsporium gypseum*, *M. canis*: *Microsporium canis*, *T. rubrum*: *Trichophyton rubrum*, MIC: Minimal inhibitory concentration

DISCUSSION

The emergence of antifungal-resistant strain of various fungi such as dermatophytes, *Candida* spp., and *Cryptococcus neoformans* has prompted research into developing new strategies for fighting fungal infections [16] which may be less toxic to man. This study was conducted to evaluate the antidermatophytic activity of *L. inermis* used by Indian peoples to show that therapeutic properties. The antidermatophytic activity was expressed at varying degrees with the activity being all the pathogens and method depended. In this study, the results were encouraging, as the *L. inermis* oil appeared to contain substances that have antimicrobial properties. SAAS method of Provine and Hadley [17] was found to be effect for determination of MIC. It was ranging from 0.025 to 1.5 µl/ml against test fungi. Singh and Pandey [6] studied the bark extract of *L. inermis* against ring worm fungi *Trichophyton mentagrophytes* and *M. gypseum*. Various work [1,18-22] has been done for the screening of antifungal activity of *Lawsonia* plant extracts but antidermatophytic of essential oil of *L. inermis* is a new aspect. During present investigation, *T. rubrum* was found to be the most susceptible fungus. While *C. albicans* was found to be most resistant test organism. A similar result was obtain by Sagar and Vidyasagar [23] during evaluation of different solvent extracts of leave of *L. inermis* against skin pathogenic fungi through broth dilution method. *T. rubrum* was reported as most susceptible fungus. In these studies, we also separated the fraction of oil through glass oven equipment through different temperature interval and checked the activity of these fractions against test pathogens and found excellent results. No work has been reported on antidermatophytic activity of fractions of essential oils. Fraction LA_{II} was found to be most effective against all the test fungi as compared to other fractions. LA_V (0.6-4 µl/ml) fraction was least effective.

This study is a preliminary evaluation of antimicrobial activity of the henna plant. It indicates that several plants have the potential to generate novel metabolites. The plants demonstrating broad spectra of activity may help to discover new antibiotics that could serve as selective agents for the maintenance of animal or human health and provide biochemical tools for the study of infectious diseases. This versatile medicinal plant is the unique source of various types of chemical compounds, which are responsible of the various activities of the plant. Hence, extensive investigation is needed to exploit their therapeutic utility to combat diseases. A drug development program should be undertaken to develop modern drugs with the compounds isolated from henna. Hence, extensive investigation is needed to exploit their therapeutic utility to combat diseases.

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