

Demonstration of the antifungal activity of *Lawsonia inermis* (henna) on dermatophytes and yeasts of medical interest

Mise en évidence de l'activité antifongique de la *Lawsonia inermis* (henné) sur les dermatophytes et les levures d'intérêt médical

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ABSTRACT

Lawsonia inermis (henna) is an ancestral plant long used by women in cosmetics, but it was also used as a topical treatment for some onychopathies and ringworm of the scalp. This

led us to investigate in vitro the antifungal activity of this plant on the main fungi responsible for these conditions (dermatophytes and yeasts). The objective of our study was to confirm or refute the antifungal effect of *Lawsonia inermis* on dermatophytes and yeasts. This is a prospective study including mycological samples from our laboratory with isolated species (*Candida albicans*, *Candida glabrata*, and *Trichophyton rubrum*), which were inoculated on petri dishes with sterilized henna paste. The yeasts were insensitive to the henna paste, as evidenced by their growth on the paste itself. The isolated *Trichophyton rubrum* strains were sensitive to henna paste (with inhibition diameters ranging from 25 mm to 40 mm); however, some *Trichophyton rubrum* cultures were contaminated with molds (*Penicillium* spp.). This involuntary contamination allowed us to identify the sensitivity profile of this mold to henna, revealing complete insensitivity. Henna did not show activity on the studied yeasts or isolated mold; however, it would probably be effective against the *Trichophyton rubrum*, which is responsible for most onychomycosis and intertrigo. Therefore, the traditional use of henna in these cases could be partly justified by our study. Thus, any mycological sampling should not be done on a Lawsonian pigmentation in order not to distort the final result.

Keywords: *Lawsonia inermis*, henna, antifungal activity, onychopathies, dermatophytes, yeasts.

RÉSUMÉ

Introduction : La *Lawsonia inermis* (henné) est une plante ancestrale utilisée depuis longtemps par les femmes en cosmétique, mais elle était utilisée aussi comme traitement topique de certaines onychopathies et teignes du cuir chevelu. Cela nous a poussé à investiguer in vitro l'activité antifongique de cette plante sur les principaux champignons responsables de ces affections (dermatophytes et levures).

Objectif : Confirmer ou infirmer l'effet antifongique de la *Lawsonia inermis* sur les dermatophytes et les levures d'intérêt médical.

Méthodes : Etude prospective incluant des prélèvements mycologiques de notre laboratoire avec comme espèces isolées (*Candida albicans*, *Candida glabrata* et *Trichophyton rubrum*) qui ont été ensemencés sur des boîtes de pétri en présence de la pâte du henné stérilisée.

Résultats : Les levures étaient totalement insensibles à la pâte du henné comme en témoigne leur croissance sur la pâte elle-même. Les souches de *Trichophyton rubrum* isolées étaient sensibles à la pâte du henné (avec des diamètres d'inhibition allant de 25 mm à 40 mm), cependant, certaines cultures de *Trichophyton rubrum* ont été contaminées avec une moisissure (*Penicillium* spp.), cette contamination involontaire nous a permis d'identifier le profil de sensibilité de cette moisissure au henné, révélant une insensibilité totale.

Conclusion : Le henné n'a pas montré d'activité sur les levures étudiées ni sur la moisissure isolée, par contre il serait probablement efficace contre le dermatophyte étudié (*Trichophyton rubrum*) qui est responsable de la plupart des onychomycoses et des intertrigos,

Mots clés : *Lawsonia inermis*, henné, activité antifongique, onychopathies, dermatophytes, levures.

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INTRODUCTION

Lawsonia inermis (henna) is an ancestral plant used for a long time by women in traditional cosmetics, but also as a topical treatment to treat some benign pathologies, such as onychopathies or ringworm of the scalp, with convincing results (1). Phytochemical studies have shown that henna contains the following components: flavonoids, coumarins, naphthalene derivatives, xanthenes, phenolic glycosides, quinoids, lupeolgaic acid, luteolin, betulin, and β -sitosterol glucosides (2). The substance responsible for the orange-red coloration of henna is the 2-hydroxy-1,4-naphthoquinone, also called lawsone, which is

present at concentrations of 0.4 to 1.5% in the dried leaves (3). Given its

traditional medicinal uses, we came up with the idea of verifying in vitro the antifungal activity of this plant on the main fungi responsible for these conditions (onychomycosis and ringworm), especially since the sensitivity of these fungi to conventional antifungals has decreased (4). In human pathology, dermatophytes are mainly responsible for onychomycosis, ringworm, and skin lesions, and *Candida* is responsible for candidiasis, localized or generalized (5,6). The purpose of our study is to verify the presumed antifungal activity of *Lawsonia inermis* on dermatophytes and yeasts.

METHODS

This is a prospective study including mycological samples taken in the Parasitology-Mycology Laboratory of the Mohammed VI University Hospital in Oujda with 5 isolates of *Candida albicans*, 2 isolates of *Candida glabrata*, and 20 isolates of *Trichophyton rubrum*. We ground the henna leaves to obtain a green powder, then we mixed this powder with physiological serum to obtain a henna paste inside a sterilizable glass petri dish. Finally, we replaced the lid of the petri dish, and we sterilized this dish at 121°C for 15 min in order to eliminate molds and different contaminants (7). We previously prepared Sabouraud + chloramphenicol media in the laboratory in petri dishes, and we created 1cm diameter wells in the middle of each dish in which we put 1g of sterilized henna paste. Then we prepared the strains to be studied by taking the colonies from the original media and mixing them with physiological serum in appropriate tubes. Finally, we inoculated the petri dishes with the isolated strains, and after inoculation, we placed 1g of henna paste in the corresponding wells. The petri dishes were incubated respectively at 37°C for 48 hours for yeast cultures (*Candida albicans* and *Candida glabrata*) and at 30°C for 5 days for dermatophyte cultures (*Trichophyton rubrum*).

RESULTS

After incubation at 37°C for 48 hours, we observed the yeast cultures (*Candida albicans* and *Candida glabrata*), and we found that both *Candida* species appeared to be

insensitive to the henna paste, with even growth of these yeasts on the henna (Figure.1).

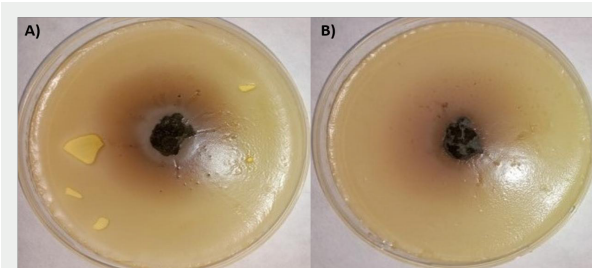


Figure 1. Antifungal activity test using the well diffusion method. vWells were filled with sterilized henna paste. (A) *Candida albicans*: growth is visible on the culture medium and rectly on the henna paste(varrow), indicating resistance. (B) *Candida glabrata*: a similar resultvis observed, with fungal growth on the henna paste.

Regarding the dermatophyte (*Trichophyton rubrum*), the petri dishes were incubated at 30°C for 5 days. After this period, we observed the cultures, and we found that the isolated *Trichophyton rubrum* strains were probably sensitive to the henna paste, with inhibition diameters ranging from 25 mm to 30 mm with an average of 27.7 mm and a standard deviation of 1.75 mm (Table 1) (Figure 2) (7). Some *Trichophyton rubrum* cultures were contaminated by a mold (*Penicillium* spp.), thus limiting the interpretation of the results of this study. Nevertheless, this accidental contamination allowed us to study the sensitivity profile of this mold to henna, revealing a complete insensitivity to it (Figure 3).

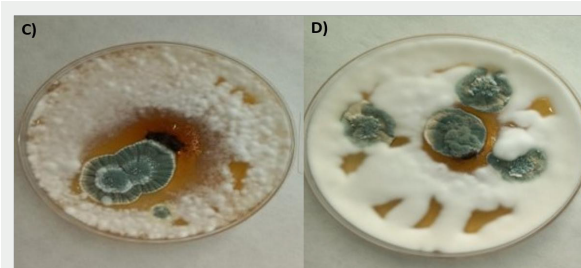


Figure 2. Antifungal activity of henna against *Trichophyton rubrum*. (C and D) Clear zones of inhibition (halos) are visible around the wells containing henna paste, indicating sensitivity. Diameters ranged from 25 to 30 mm.

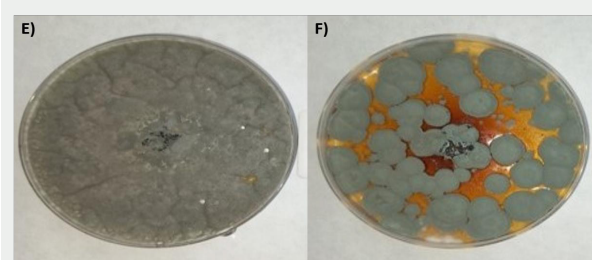


Figure 3. Accidental contamination of a *T. rubrum* culture by *Penicillium* spp. (E and F). The mold (arrow) is growing on the henna paste and the surrounding medium, demonstrating its insensitivity to henna.

Table 1. Inhibition diameters of 20 samples of *Trichophyton rubrum*.

Dermatophyte (n=20)	Inhibition diameter	Mean	Standard Deviation
<i>Trichophyton rubrum</i> (Sample N°. CHUO 311)	27 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 318)	25 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 323)	28 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 328)	26 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 331)	27 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 336)	30 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 339)	26 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 344)	29 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 352)	30 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 358)	30 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 361)	26 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 367)	28 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 370)	25 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 372)	30 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 376)	27 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 379)	29 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 381)	30 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 384)	26 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 389)	28 mm	27.7 mm	1.75 mm
<i>Trichophyton rubrum</i> (Sample N°. CHUO 392)	27 mm	27.7 mm	1.75 mm

DISCUSSION

The presumed activity of henna on dermatophytes and yeasts has been the subject of several studies with varying degrees of conclusiveness, but generally this activity has been clearly demonstrated in most of these studies (7–9). Concerning the yeast strains studied in our experiment, henna did not show activity on these strains nor on the mold that grew on henna itself, while other studies have found that some strains of *Candida* were sensitive to henna, while other strains of the same yeast and in the same studies were not sensitive to henna as was the case in our study (10–12). However, *Trichophyton rubrum* appeared to be sensitive to the action of henna, as was the case in other studies (9,13,14), thus justifying the traditional use of henna paste in folk medicine. Henna is also traditionally used to treat ringworm of the scalp, hence the interest in extending the study to dermatophytes responsible for

ringworm, such as *Microsporum canis*, *Trichophyton violaceum*, or *Trichophyton verrucosum*. Finally, given the small number of strains tested, the conclusions drawn from this study will be preliminary observations that need to be supported by studies of larger cohorts in order to arrive at more relevant and statistically acceptable conclusions. Furthermore, a major limitation of this preliminary study is the absence of positive (e.g., a standard antifungal agent) and negative controls, which prevents direct comparison of the inhibition diameters with established antifungal drugs.

CONCLUSION

This preliminary study provides preliminary evidence of the presumed antifungal activity of henna on *Trichophyton rubrum*, which could be an interesting avenue for further and more methodical research, by testing henna on a larger cohort of samples or, preferably, by extracting the active ingredients from henna and testing them directly on these fungi. This study also raises the issue of the potential interference of the traditional use of henna with mycological sampling, which can give false negative results. Therefore, henna should be considered as a topical treatment and respecting a therapeutic window (disappearance of Lawsonian pigmentation) before sampling the patient to obtain a reliable result and adequate management.

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