Assessment of Inhibitory Effect of Aqueous and Alcoholic Extract of *Lawsonia inermis* leaves Against Some Fungi Growth

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**ABSTRACT**

The effect of aqueous and methanol extracts of *Lawsonia inermis* leaves were studied on some isolated fungi from 20 patients suffering onychomycosis attending Dermatic Consultant Clinic in Al- Sadar Teaching Hospital in Al-Najaf. The fungal isolates were identified as two *Aspergillus* species; *A. terreus* and *A. flavus* none of them was a dermatophytes. The results show that the mean inhibitory diameter (MID) of methanol extract against *A. terreus* and *A. flavus* was (11.2 and 6.8 mm) respectively, while aqueous extract had a lower MID effect (6.8 mm) against *A. terreus* with no effect against *A. flavus*. The Ketoconazole (antifungal drug) had less MID effect against *A. terreus* and *A. flavus* than methanol extract (6.4 and 6 mm) respectively, but it had much more mean diameter inhibitory effect (6 mm) against *A. flavus* over aqueous extract which show no inhibitory effect, and the MID effect of Ketoconazole was little less (6.4 mm) against *A. terreus* than the aqueous extract (6.8 mm).

**Key words:** *Lawsonia inermis*, onychomycosis, aqueous extract, methanol extract, and mean inhibitory diameter (MID)


**INTRODUCTION**

Plants are a rich source of medicines and are used as chemical defense against infection [1]. There is currently a large demand for the use of herbs in developed countries and larger in developing countries being a folk treatment and for its effectiveness and low cost and a sense of safety in their use [2, 3]. Henna plant of genus *Lawsonia* has one specie is *Lawsonia inermis* family Lythraceae and has various common names in different world countries [4]. The original cultivation of Henna was in North Africa and South-East Asia and is now cultivated in all tropical regions. It is a 2-6 meter high shrub and is used for dyeing skin, hair, fingers, nails, silk and wool [5]. It is also used to treat fungal infections, leprosy, dysentery, anemia, bleeding, fever, cough, as diuretic and anti-inflammatory [6,7]. Onychomycosis is one of the most common nail diseases, accounting for more than half of nail disorders [8]. The pathogens of Onychomycosis include a variety of dermatophytes, some non-dermatophytes, and yeasts, the first is the major cause of infection in European countries and regions with temperate climates while the second is the most common causative in tropical and subtropical regions where high temperature and humidity [9]. Non-dermatophytes are usually found in nature as saprophytes and other are parasitic fungi which cause plant diseases; they cannot penetrate the keratin layers and live only on the non-keratinized parts or benefit from the broken of keratinized cells as a result of dermatophytes, trauma or other nail diseases [10]. The main constituent in the henna plant that gives the distinctive color is the alkaloid lawsone (2-hydroxy-1, 4 naphthaquinone), also containing glucose, fat, resin and gallic acid [11] and. The antifungal activity is attributed to lawsone[12].

**MATERIALS AND METHODS**

Preparation of alcohol extract:

Dry henna leaves (*Lawsonia inermis*) was purchased from the markets and were botanically classified at the University of Babylon. The extracts were obtained using methanol in a concentration of 98% as solvent at room temperature, where the dry leaves of the henna were ground to a fine powder using the mill, the powder was divided into sections where each 10 grams was mixed with 100 ml of the solvent in a 250 ml conical flask (3 samples were used), which were tightly closed. The three flasks were put on a 24-hour shaker, then left to settle...
for 5 hours. The mixture was first filtered with several layers of gauze, then centrifuged at 5000 rpm for 15 minutes, supernatant layer was collected [13]. The solvent was evaporated at a temperature of 45 °C with vacuum evaporator until a final volume equal to 1/5 of the original volume reached, then stored at 4 °C in sealed bottles for further examination.

Preparation of water extract:
Forty grams of leaves were crushed in a mortar to fine powder, mixed with 200 ml of sterilized distilled water for 24 hours at room temperature. The mixture was filtered with several layers of gauze, the filtrate was centrifuged at 3000 rpm for 10 minutes, supernatant layer and was taken and filtered again using the filter papers Whatman No.1. The filtrate was dried using a rotary evaporator, the residue was kept in sealed plastic bottles under freezing until use [14].

Isolation and diagnosis of fungi:
A total of 20 patients suffering from the onychomycosis attending Dermatic Consultant Clinic in Al-Sadar Teaching Hospital in Najaf from the first of October to the thirtieth of November 2017. The infected toes and fingers were cleaned and sterilized by 70% alcohol, each sample was collected using a nail clipper, in addition, parts of the tissue under the nails is also taken in glass containers with a lid. Part of each sample was mixed with 20% potassium hydroxide solution to be examined under a microscope to identify fungus type. The other part of the sample was cultured twice in a Sabourud dextrose agar (SDA) cultured media mixed with chloramphenicol in a ratio of 0.05 mg / ml [15]. The culture was incubated at room temperature of 27 °C and examined during the first 5-7 days and then through 3-4 weeks for the growth of fungi, the fungus isolates were refined and stored in (slant) culture to be used for investigation. The isolated fungi were identified in the Prevention Department at the Faculty of Agriculture / University of Kufa.

Antifungal efficacy test:
The method used was agar-well diffusion method. Part of each identified fungus with a four days age in sterile conditions was taken and put in a tube containing 10 ml of sterilized water and was vigorously shaken and then diluted 10 times to be used for testing [16] The SDA media was prepared and poured in the Petri dishes where they were inoculated with 1 ml of suspension containing the fungus before it hardened. Using a glass sterile Pasteur pipette, drill holes in the media with a diameter of 5 mm. Half gram of each of water and methanol extract was dissolved in 2 ml of water and methanol respectively with a final concentration of 250 mg / ml, then 25 μL of each extract was taken and put in the agar holes using micropipette, the dishes were incubated at room temperature for 5 days, water was used as negative control. Each dish was examined and the diameter of the inhibition, including the diameter of the hole, was measured in millimeters [17]. The antifungal ketoconazole disk (2mg / disk) was used as positive control and placed on inoculated dishes for comparison. Disk Diffusion method was used, mean diameter of inhibition was calculated in three replications.

**RESULTS**
The isolated fungi from patients with onychomycosis was examined and found to be *Aspergillus flavus* (*A. flavus*) and *Aspergillus terreus* (*A. terreus*) which are nondermatophyte. Methanol and aqueous extracts of *L. inermis* were tested for antifungal activity using Ketoconazole as positive control and sterile water as negative control. The table show that the mean inhibition diameter (MID) of methanol extract of *L. inermis* was 11.2 mm and 6.8 mm against *A. terreus* and *A. flavus* respectively and were highest than both Ketoconazole and aqueous extract. On the other hand the aqueous extract was a little more efficient than Ketoconazole against *A. terreus*, but was inactive against *A. flavus*.

<table>
<thead>
<tr>
<th>Fungus type</th>
<th>MID (mm) Aqueous extract</th>
<th>MID (mm) methanol extract</th>
<th>MID (mm) Ketoconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. terreus</em></td>
<td>6.8</td>
<td>11.2</td>
<td>6.4</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>0</td>
<td>6.8</td>
<td>6</td>
</tr>
</tbody>
</table>

**DISCUSSION**
The results showed that the isolates of fungi taken from the samples of patients attending to the Dermatic Consultant Clinic at Al-Sadr Teaching Hospital were not dermatophytes, which were consistent with a study in

Brazil where 38.4% of onychomycosis were caused by nondermatophytes [18] and was identified as \textit{A. flavus} and \textit{A. terreus}, which is close to the results of Hwang and his group, which showed that the percentage of onychomycosis caused by \textit{Aspergillus} was 83% where 20% Of which were \textit{A. flavus} and \textit{A. terreus}. The methanol extract has an antifungal effect more than the water extract, being a good solvent for the active substances in henna leaves to eliminate fungus. This is consistent with [19, 20, and 21]. About the antifungal effect of Henna plant which was attributed to lawsone and 2-Hydroxy-1, 4-naphthaquinones presence in leaves. The results showed that \textit{A. flavus} was not affected by the water extract of henna.

REFERENCES