

Research Article

***In Vitro* Activity of *Lawsonia inermis* (Henna) on Some Pathogenic Fungi**

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The present study was conducted to investigate antifungal activity of *Lawsonia inermis* (Henna plant). Leaf samples of the plant were collected from Eastern Nile of Khartoum State, Sudan. Ethanol and petroleum ether extracts were obtained by maceration (cold method). The extracts were bioassayed *in vitro* to know their bioactivity to inhibit the growth of tested fungi. The cup-plate agar diffusion method was adopted to assess the antifungal activity of the extracts against tested yeasts, while agar incorporated method was used for other molds. Both extracts revealed antifungal activity against all yeast strains except *Pichia fabianii* which was found resistant to both ethanol and ether extracts. The results displayed antifungal activity against tested fungi. Minimum inhibition concentration (MIC) of 5, 7.5, and 10 mg/mL was found to inhibit the growth of tested dermatophytes. The obtained results revealed antifungal activity of Henna leaves extracts which support the traditional use of Henna in therapy of fungal infections. The possibility of therapeutic use of Sudanese Henna as antifungal agent is recommended.

1. Introduction

Plants have been used to treat humans, animals, and plant diseases from time immemorial. Herbal medicines have been known to man for centuries [1]. Henna plant, *Lawsonia inermis* Linn, is such a plant known for healing attributes and is now the subject of intense scientific study [2]. Though it was used for various purposes, the antifungal property was not yet investigated. Thus the present study was conducted to evaluate its antifungal potential. *Lawsonia inermis* (Henna) is an ornamental evergreen plant cultivated in the tropics [3]. It belongs to the family Lythraceae. In Sudan it is traditionally used to develop a red or black coloring to hands, feet, and hair in some occasions such as weddings and religious festivals.

The phytochemical analysis of the plant leaves revealed the presence of anthraquinones as major constituents which are commonly known to possess antimicrobial activity [4]. Ethyl acetate extract of *Lawsonia inermis* was found to be active against all bacteria tested [5]. Screening of the barks of 30 plant species against *Microsporum gypseum* and *Trichophyton mentagrophytes* showed that only *L. inermis*

L. inermis extract exhibited absolute toxicity. The extract showed broad fungicidal spectrum when tested against 13 ring worm fungi [6]. The present study was conducted to evaluate the antifungal potential of *Lawsonia inermis* leaf extracts.

2. Materials and Methods

2.1. Henna Plant. Home-grown (*Lawsonia inermis* Linn) Henna leaves were collected from El Bakrab village east of the Blue Nile, Khartoum State, Sudan. The plant was identified and authenticated by the experts of Medicinal and Aromatic Plants Research Institute (MAPRI). Voucher specimen has been deposited at the Herbarium of MAPRI.

2.2. Microorganisms. *Candida albicans* (ATCC 7596) was obtained from National Centre for Research (Sudan) and the clinical isolates of *Pichia fabianii*, *Saccharomyces cerevisiae*, *Aspergillus flavus*, *Scopulariopsis brevicaulis*, *Trichophyton mentagrophytes*, *Trichophyton violaceum*, *Trichophyton verrucosum*, and *Phialophora richardsiae* were obtained from

the Mycology Department, Central Veterinary Research Institute, Soba, Khartoum, Sudan.

2.3. Preparation of Crude Extracts. Leaves of *Lawsonia inermis* (Henna) were dried in the shade. 150 g of powdered leaves was extracted in soxhlet apparatus with petroleum ether 60–80°C and 170 g of powdered leaves was extracted with 80% ethanol using maceration method [7]. Serial dilutions of the extracts were prepared to determine MIC. Dimethyl sulfoxide (DMSO) and petroleum ether were used as negative control and ketoconazole and Nystatin were used as positive control.

2.4. Preparation of Stock Drug Solution (Ketoconazole). Stock drug solution (1280 mg/liter) was prepared by adding 50 mL of dimethyl sulfoxide to 64 mg of ketoconazole and allowed to stand for 30 min. It was then dispensed in small amount and stored at –20°C until used [8].

2.5. In Vitro Assay of Antimicrobial Activity of the Extracts

2.5.1. The Cup-Plate Agar Diffusion Method. The cup-plate agar diffusion Hadecek and Greger [9] method was adopted with some minor modifications to assess the antifungal activity of the prepared extracts. One gram of each extract was weighed and dissolved in 5 mL of the solvent used for the extraction to give 200 mg/mL. The microorganisms were standardized according to the 0.5 McFarland scale. 100 μ L of the stock solution was thoroughly mixed with 20 mL molten sterile Sabouraud dextrose agar and maintained at 25°C. The agar plates were left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (Number 4) and agar discs were removed. Cups were filled with 0.2 mL of each extract using automatic microlitre pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 2-3 days. Activity of each extract was tested in triplicate. The diameters of zones of inhibition were measured in millimeter using a transparent well calibrated ruler, averaged and the mean values were tabulated. Results of the qualitative screening were recorded as the average diameter of the growth inhibition zone surrounding the wells containing the extract [10].

2.5.2. Agar Incorporation Technique. The antifungal susceptibility of the extracts and the standard drugs ketoconazole and Nystatin were determined using agar incorporation technique. Concentrations of 50, 100, and 200 mg/mL of the extracts were tested. The test organisms were inoculated onto Sabouraud dextrose agar (SDA) supplemented with chloramphenicol (0.05 mg/mL) for yeasts and SDA supplemented with chloramphenicol and cycloheximide (0.5 mg/mL) for dermatophytes. Each plate was inoculated with the test organism. The control group was inoculated with the same organism. All plates were incubated at 25°C for three to seven days except for dermatophytes which were incubated for up to three weeks at the same temperature [8]. Plates were observed for the presence or inhibition of growth. Negative control

(ethanol and petroleum ether) and positive control (Nystatin and ketoconazole) were run in the same manner parallel to the treatment. The test was performed in triplicate.

2.6. Minimum Inhibitory Concentration (MIC) Determination. The MIC of the ethanol extract against tested microorganisms was determined by the macrobroth dilution method [8]. Stock solution of the extract at a concentration of 200 mg/mL was prepared by serial two-fold dilutions in Sabouraud's broth. The tubes were then inoculated with 100 μ L of cultures (10^7 cells). Uninoculated tubes containing growth medium and extract were used as controls. The tubes were then incubated at appropriate temperatures for 48 hours. The MIC was defined as the lowest concentration that completely inhibited the growth of the organism.

2.7. Statistical Analysis . Results were expressed as mean \pm SD. The significant difference between means was determined using one-way analysis of variance (ANOVA). Statistical significance was established at $P < 0.05$.

3. Results

The current investigation showed that Henna plant possesses good antimicrobial activity against tested fungi. The obtained results demonstrated antifungal activity of both extracts. The cup-agar diffusion method revealed antifungal activity of the extract against yeasts and mould demonstrated by area of inhibition zone around the wells, while inhibition of growth revealed antifungal activity against dermatophytes. The ethanol extract has shown significant activity against yeasts compared to petroleum ether extract. The inhibition zone induced by ethanol extract at the concentration of 10 mg/mL was found to be 26.3 ± 3 mm against *Saccharomyces cerevisiae* and 17 ± 0 mm against *Candida albicans*.

Petroleum ether extract exhibits antifungal activity to *S. cerevisiae* with mean inhibition zone equal to 25.3 ± 0.6 mm at a concentration of 10 mg/mL and 22.7 ± 0.6 mm to *C. albicans* which is greater than that shown for the commercial Nystatin (17 ± 0 mm). It was noted that both extracts showed no antifungal activity to *Pichia fabianii* (Table 1, Figures 1 and 2).

Significant antifungal effect expressed as MIC of ethanol extract against tested dermatophytes was shown (Table 2). *Trichophyton mentagrophytes* and *T. violaceum* were found more sensitive to ethanol extract with MIC of 7.5 mg/mL. However, all *Trichophyton* species were found sensitive to ethanol at a concentration of 10 mg/mL (Figure 3).

The ethanol extract displayed fungicidal activity to tested moulds at a concentration of 5 mg/mL and fungi static activity to *Aspergillus flavus* at a concentration of 10 mg/mL (Table 3, Figures 4, 5, and 6).

4. Discussion

The antifungal activity of *L. inermis* leaf extracts has been evaluated *in vitro* against *C. albicans*, *S. cerevisiae*, and some selected dermatophytes. The effect was found to be dose

TABLE 1: Antimicrobial activity of *Lawsonia inermis*, ethanol, and petroleum ether leaf extracts against yeasts.

Extract	Concentration (mg/mL ⁻¹)	<i>S. cerevisiae</i>	<i>C. albicans</i> Diameter of inhibition zone (mm)	<i>P. fabianii</i>	Nystatin
Ethanol	100	23.3 ± 1.5	16.7 ± 0.6	0	
Ethanol	200	26.3 ± 3.2	17 ± 0	0	17 ± 0
Petroleum ether	100	24.7 ± 0.6	21.3 ± 0.6	0	
Petroleum ether	200	25.3 ± 0.6	22.7 ± 0.6	0	

Data are presented as mean ± SD of zone of inhibition (mm); inhibition zones are the mean of three replicates; S (*Saccharomyces*); C (*Candida*); P (*Pichia*).

TABLE 2: MIC of ethanol extract of *Lawsonia inermis* leaf against dermatophytes.

Extract	Concentration (mg/mL)	<i>T. mentagrophytes</i>	<i>T. verrucosum</i>	<i>T. violaceum</i>	Ketoconazole
	2.5	+	+	+	—
Ethanol extract	5.0	+	+	—	—
	7.5	—	+	—	—
	10	—	—	—	—

Symbols (+) indicates growth of fungi; (—) indicates inhibition of growth.



FIGURE 1: Inhibition zone of *C. albicans*. C: ethanol as negative control; R: ether extract; E: ethanol extract.



FIGURE 2: Inhibition zone of *S. cerevisiae*. C: ethanol as negative control; R: ether extract; E: ethanol extract.

dependent. Petroleum ether extract showed a wide antifungal spectrum compared to ethanol extract against tested yeasts. It exhibited inhibition zone wider than the commercial Nystatin. Therefore, petroleum ether is a better solvent for consistent extraction of antifungal substances from *L. inermis* plant. Inhibition of growth of *C. albicans* shown in the present

study was consistent with that reported by Maher and his collaborators [11] where ethanol extract of *Ruta chalepensis* revealed similar inhibition zone of 17 mm. But *S. cerevisiae* was found to be more sensitive to ethanol extract at the concentration of 10 mg/mL compared to *C. albicans*. This shows species susceptibility to the extract used. Furthermore, all tested moulds were found to be sensitive to ethanol extract at a concentration of 5 mg/mL except *Aspergillus flavus*. This indicates less sensitivity of the extract to *A. flavus*.

In the present study, all tested dermatophytes showed great sensitivity to ethanol extract where complete inhibition of growth was observed. This finding is similar to those obtained in some previous studies [12] where 100% inhibition of *Trichophyton mentagrophytes* growth was observed on using 10 mg/mL of *L. inermis* ethanol extract. This indicates great sensitivity of dermatophytes to *L. inermis* plant. Thus further research on mode of action of such novel plant is recommended.

The phytochemical screening of Sudanese Henna plant revealed naphthoquinone as an active ingredient that might display the antifungal activity against tested fungi [4]. Previous studies [6, 13] disclosed the same result when they screened the antifungal activity of higher plants; leaves of *L. inermis* were found to exhibit strong fungicidal effect due to naphthoquinone which was found to be the main active ingredient. Furthermore, the presence of tannin might play a vital role as antimicrobial agent as suggested by Banerjee and his collaborators [14].

5. Conclusions

Ethanol and petroleum ether leaf extracts of *L. inermis* (Henna) revealed antimycotic activity against tested fungi. With the ever increasing resistant strains of microorganisms to the already available and synthesized antifungal drugs, the naturally available *Lawsonia inermis* (Henna) could be a potential alternative. Since our study was carried out using

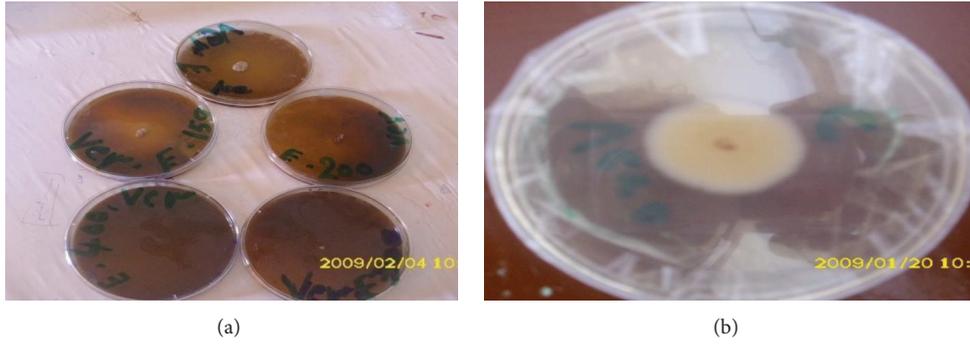


FIGURE 3: Control plate (b) showing growth of *T. verrucosum* and growth inhibition of *T. verrucosum* in ethanol extract of *L. inermis* leaves (a) at different concentrations after 10 days of incubation at 37°C (x40).



FIGURE 4: Control plate of *Scopulariopsis brevicaulis* (a) and ethanol-treated plate (b) at a concentration of 10 mg/mL ethanol extract of *L. inermis* after 7 days of incubation at room temperature (x40).

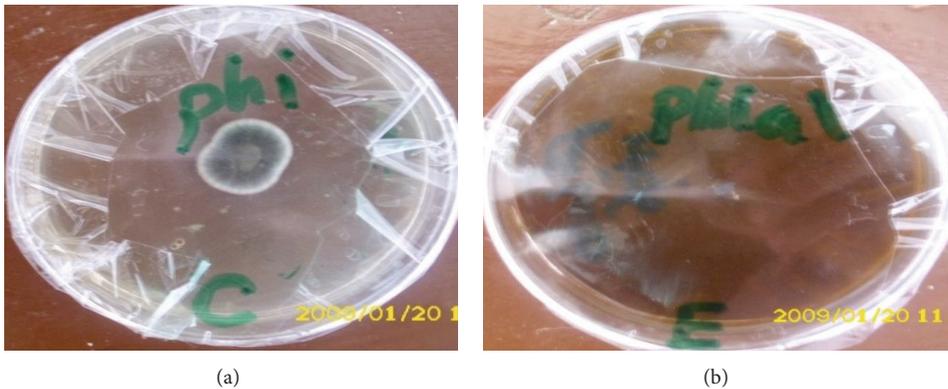


FIGURE 5: Control plate of *Phialophora richardsiae* (a) and ethanol-treated plate (b) at a concentration of 10 mg/mL ethanol extract of *L. inermis* after 7 days of incubation at room temperature (x40).

TABLE 3: Minimum Inhibition Concentration (MIC) of ethanol extract of *Lawsonia inermis* Leaf against moulds.

Extract	Concentration (mg/mL)	<i>Aspergillus flavus</i>	<i>Scopulariopsis brevicaulis</i>	<i>Phialophora richardsiae</i>	Dimethyle Sulphoxide
Ethanol	2.5	+	+	+	—
	5.0	+	—	—	—
	7.5	+	—	—	—
	10	—	—	—	—

Symbols (+) indicates growth of fungi; (—) indicates inhibition of growth.



FIGURE 6: White colony of *A. flavus* on SDA at a concentration of 10 mg/mL ethanol extract of *L. inermis* showing fungi static activity (vial) and green colony of the control (plate) after 7 days of incubation at room temperature ($\times 40$).

crude extract, it is necessary to purify the active principle and evaluate its bioactivity for therapeutic application.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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