In vitro evaluation of antifungal effects of *Lawsonia inermis*, *Pistacia lentiscus* and *Juglans regia*

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Abstract: The antifungal activity of the hydro alcoholic extracts of *Lawsonia inermis*, *Juglans regia* and *Pistacia lentiscus*, was studied in vitro. Effects of these three plants, largely used in traditional medicine, were compared with that of Griseofulvine. *Trichophyton rubrum*, *Trichophyton mentagrophyte*, *Microsporum canis* and *Candida albicans*, 4 stocks dermatophytes and yeast were selected for this study. By the method of diffusion in solid medium, all the three plants appeared active with concentration of 35mg/ml as compared to control. The hydro alcoholic extract of *Pistica lentiscus* was most active by inducing diameters of growth inhibition of 17±1,00 mm and 16,67± 0,58 mm on *Trichophyton mentagrophyte* and *Microsporum canis* respectively, except the effect of the 3 extracts on the stock *Trichophyton rubrum* is comparable. Whereas for the stock *Candida albicans*, the extract of *Lawsonia inermis* was most active with a diameter of inhibition of 18,87± 0,58mm. By the method of dilution in liquid medium, *Pistacia lentiscus* and *Lawsonia inermis* showed antifungal effects on *Trichophyton mentagrophyte* with one MIC =0,068 mg/ml and on *Candida albicans* with one MIC =0,034 mg/ml respectively. The study concludes that the hydroalcoholic extracts of *L. inermis*, *J. regia* and *P. lentiscus* possess antifungal activities against the tested strains with variable degrees of sensitivity.

Keywords: *Lawsonia inermis*; *Pistacia lentiscus*; *Juglans regia*; antifungal activity; MIC.

Introduction

Dermatophytes are a group of fungi adapted to keratin of human and animal skins; they are very contagious and responsible for numerous outbreaks. Statistics show an increase in fungal diseases and increased resistance of many pathogens to current treatments (Balkis et al. 2002; Dinesh Babu and Subhasree 2009). This phenomenon of resistance and limited diagnostic tools of mycological clinical research lead us to look for new antifungal compounds. The present study was undertaken to evaluate the antifungal activity of three widely used medicinal plants in Algerian folk medicine.

*Lawsonia inermis* (Lythraceae) characterized by its multiple uses as a poultice against eczema, fungal, astringent, antiseptic, healing wounds and injuries and ointment against burns (Hseini et Kahouadji 2007). *Pistacia lentiscus* (Anacardiaceae) known for its anti-inflammatory, antiviral, antibacterial, antifungal properties and used in the treatment of eczema (Benhammou et al. 2008; Yarikamarani et al. 2007). *Juglans regia* (Juglandaceae) show a significant therapeutic use in the care of the backbone, astringent, antiseptic, antifungal and antibacterial and antifungal keratinizing (Benhammou et al. 2008; Hseini et Kahouadji 2007).

In the present study by using two different techniques viz., diffusion technique on solid medium and the dilution method in liquid medium, we evaluated the antifungal properties of these three plants against four strains of dermatophytes.

Materials and Methods
Plant material

Collection and conservation of plant material

Plants were collected from their natural habitat or were purchased from local herbalist. They were identified by the plant taxonomist and their vouchers specimens (No. AB04-xx) were deposited in the herbarium of the Botany Laboratory, Pharmacy Department, Faculty of Medicine, Mentouri Constantine University (Algeria).

Preparation of plant extracts

The plant material used consisted of leaves of *Pistacia lentiscus* and *Lawsonia inermis* and bark of *Juglans regia* (Table 1). The harvested and dried plant materials were sprayed using a grinder electric knife. The extract was prepared as described by Zirihi et al. (2007) and Bagre et al. (2007). The powder obtained (100 g) was macerated in methanol: water (70: 30, v/v, 500 mL) and stirred on a magnetic hot plate for 24 h. This operation was repeated three times with renewal of the solvent. After filtration, the extract was concentrated using a Rotavapor and lyophilized.

<table>
<thead>
<tr>
<th>Plant species (Family)</th>
<th>Part used</th>
<th>Code</th>
<th>Location</th>
<th>Voucher specimen No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lawsonia inermis</em> L. (Lythraceae)</td>
<td>Leaves</td>
<td>LIN</td>
<td>Herbalist – Souk</td>
<td>AB04 – 111</td>
</tr>
<tr>
<td><em>Pistacia lentiscus</em> L. (Anacardiaceae)</td>
<td>Leaves</td>
<td>PLE</td>
<td>Coast mountainous</td>
<td>AB04 – 116</td>
</tr>
<tr>
<td><em>Juglans regia</em> L. (Juglandaceae)</td>
<td>Bark</td>
<td>JRE</td>
<td>University campus – Chihani Bachir</td>
<td>AB04 – 109</td>
</tr>
</tbody>
</table>

Strains tested

Antifungal activities were assessed on strains from clinical isolates that have been provided by the laboratory of Parasitology, University Hospital of Constantine, Algeria (Table 2).

In vitro antifungal test

Method of diffusion on solid medium

Diffusion method in solid consists of depositing paper discs of 6 mm diameter soaked with 25 l of extract to be tested, on the surface of agar Sabouraud chloramphenicol actidione, poured in Petri dishes, previously seeded by flooding of a suspension of microorganisms from $10^6$ units / ml grown in physiological saline to dermatophytes and in nutrient broth YPG for *Candida albicans* (Collins et al. 1995). Incubation of Petri dishes for 18 to 24 h at 37 °C for yeasts and lasts 7 days for dermatophytes. Antidermatophyte activity was determined by measuring the inhibition zone diameter that appeared clearly around each disc (Vander Bergh and Vlietink 1991).

Method of dilution on liquid medium

Dilution method in liquid medium was used to determine the minimum inhibitory concentrations (MIC). 200 l of methanol was distributed in a series of tubes which were added 100 l of different dilutions of half in half from plant extracts (a concentration range spanning as follows: 35, 17.5, 8.75, 4.38, 2.19, 1.09, 0.54, 0.27, 0.14, 0.068, 0.034 and 0.017 mg / ml) with the exception of the first to serve as a negative control, then removed 100 l of the mixture that was added to 5 ml of nutrient broth and 200 l of mycelial suspension, incubated and reading was done visually (Kamagate Kone et al. 2001).

Statistical data

Results were expressed as mean of three repetitions with standard deviation (Mean ± SD), they were analyzed by Origin software, version 6.0, by applying the Student t-test to compare populations. The significance level was set at P <0.01.

Results

In this study, we evaluated in vitro antidermatophyte activity of hydro alcoholic extracts of 3 plants *Juglans regia, Lawsonia*
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inermis and Pistacia lentiscus on growth of four strains Trichophyton rubrum, Trichophyton mentagrophyte, Microsporum canis and Candida albicans.

Analysis of experimental data showed a significant antifungal activity of all plant extracts compared to Griseofulvin and control.

At a concentration of 35 mg / ml of plant extracts LIN, JRE, PLE and GRISEO at 0.6 micrograms / ml had a significant effect on the strain of Trichophyton rubrum tested against the control, with diameters of growth of: LIN (14.67 mm), JRE (13.67 mm) and PLE (14.67 mm) (Figure 1). No significant differences have been noted between extracts of LNI, JRE and PLE.

Figure 1: Antifungal activity of the vegetable extracts on Trichophyton rubrum.

Abbreviations: LNI: Lawsonia inermis, JRE: Juglans regia, PLE: Pistacia lentiscus, CONTR: Controls Methanol / Water (70%, 30%), GRISOE: Griseofulvin. The inhibition zone diameter is expressed as the average of three repetitions (Mean ± SD). ** Significant at P <0.01) [extracts vs. control].

At a concentration of 35 mg / ml of plant extracts LIN, JRE, PLE and GRISEO at 0.6 micrograms / ml had a significant effect on the strain of Microsporum canis tested against the control, with diameters of growth of LIN (15.33 mm), JRE (14.67 mm) and PLE (16.67 mm) (Figure 3). A significant difference was noted for PLE extract (P <0.01) compared to extracts LIN and JRE, by cons no significant differences between extracts of LIN and JRE were noted.

Figure 3: Antifungal activity of the vegetable extracts on Microsporum Canis.

Abbreviations same as in figure 1. The inhibition zone diameter is expressed as the average of three repetitions (Mean ± SD). ** Significant at (P<0.01) [PLE vs LNI & JRE].
At a concentration of 35 mg/ml of plant extracts LIN, JRE, PLE and GRISO at 0.6 micrograms/ml had a significant effect on Candida albicans strain tested compared to control, with diameters of growth of LIN (18.67 mm), JRE (16.67 mm) and PLE (17.33 mm) (Figure 4). A significant difference of the extract LNI (P <0.01) was noted compared to the JRE and PLE extracts, by contrast no significant difference were noted between JRE and PLE extracts.

**Figure 4**: Antifungal activity of the vegetable extracts on Candida albicans.

Abbreviations same as in figure 1. The inhibition zone diameter is expressed as the average of three repetitions (Mean ± SD). ** Significant at P <0.01) [extracts vs. control], ☀️☀️ significant at (P<0.01) [LNI vs JRE & PLE].

By the dilution method on liquid medium, we evaluated the minimum inhibitory concentration of LIN having developed a fungicidal activity with a significant difference compared with extracts of JRE and PLE with respect to C. albicans. The MIC value obtained was 0.034 mg/ml. Similarly, PLE has developed a fungicidal activity with a significant difference compared to the other two extracts (LIN & JRE) against Tr. Mentagrophyte; the MIC value obtained was 0.068 mg/ml (Table 3).

**Table 3**: Minimal inhibitory concentration of the most active vegetable extracts.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC of plant extracts (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LIN</td>
</tr>
<tr>
<td>Trichophyton mentagrophyte</td>
<td>ND</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>0.034</td>
</tr>
</tbody>
</table>

**Discussion**

Currently, few effective drugs are available for treating fungal infections, especially for dermatomycoses. Since knowledge of the active ingredients contained in higher plants, which are endowed with pharmacological properties, several authors have turned to look for plants with antifungal activity. In this study we tested the sensitivity of four fungal strains to the action of extracts from three plants well known in Algerian folk medicine: Lawsonia inermis, Pistacia lentiscus and Juglans regia.

The results showed that the three plants have antifungal properties, which justifies the use of these plants in traditional medicine.

Concerning L. inermis, Misra and Dixit (1979) reported that the ethanol extract of this plant has antifungal activity. Phytochemical analysis of this plant revealed the predominance of phenolic compounds (coumarins, flavonoids, and naphthalene derivatives of gallic acid) (Siddiqui et al. 2003). Other compounds such as triterpenoids, steroids and aliphatic carbohydrates have also been isolated (Siddiqui and Kardar 2001; Siddiqui et al. 2003). Abulyazid et al. (2010) reported the richness of the plant by naphthoquinones in which the main components are the Juglone, lawsone and plumbagone. Studies made in the natural naphthoquinones (Binutu et al. 1996; Gafner et al. 1996; Brigham et al. 1999) and those obtained by synthesis (Bogdanov et al. 2001; Riffel et al. 2002) have shown that these molecules inhibit growth of fungal strains and bacterial pathogens. In view of these studies, we might attribute the antifungal activity of L. inermis to naphthoquinones essentially.

As for Juglans regia, it has been shown by Pereira et al. (2008) and Oliveira et al. (2008) that the fruit extracts of this plant have antifungal activity against Candida albicans and Cryptococcus neoformans. The same antimicrobial activity was demonstrated using extracts from the bark (Alkhawajah 1997). Phytochemical analyzes made on different parts of this plant showed its richness in phenolic compounds which are represented mainly by naphthoquinones and flavonoids (Li et al. 2008).
These molecules are known by their power in inhibiting fungal growth.

For his part, *P. lentiscus* was the subject of several studies. Phytochemical analyzes have focused on different parts of this plant polyphenols have been identified in leaves (Romani et al. 2002). The essential oil of the leaves is mainly characterized by its high α-Pinene, γ-terpinene and terpinene-4-ol (Ben Douissa et al. 2005; Duru et al. 2003). Flavonoids have also been isolated from the aerial part of this plant (Kawashty et al. 2000). A study of Duru et al. (2003) on the resin of *P. lentiscus* showed antifungal activity against *Rhizoctonia solani*. Similarly, leaf extracts inhibited the growth of *Phythium Rhizoctania solani* and *Pythium ultimum* (Kordali et al. 2003). The results of this study confirm this pharmacological property and show the sensitivity of other fungal strains (*Trichophyton mentagrophyte, Trichophyton rubrum, Candida albicans* and *Microsporum canis*) to the leaf extract of *P. lentiscus*.

**Conclusion**

The results from these tests showed that hydroalcoholic extracts of the studied plants inhibit *in vitro* the growth of the strains tested with variable degrees of sensitivity. The extracts of *P. lentiscus* L. and *L. inermis* have developed a fungicidal activity with a minimal inhibitory concentration of 0.068 mg / ml and 0.034 mg / ml respectively against *Trichophyton mentagrophyte* and *Candida albicans*. The positive results of current study encourage us to perform extensive phytochemical analyzes to characterize the natural molecules responsible for this antifungal activity.

**References**


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