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INTERNATIONAL JOURNAL
OF CURRENT RESEARCH

International Journal of Current Research
Vol. 7, Issue, 04, pp.14237-14240, April, 2015

ISSN: 0975-833X

RESEARCH ARTICLE

ANTIFUNGAL EFFECT OF MOROCCAN *LAWSONIA INERMIS* LEAF EXTRACTS ON THE GROWTH OF FILAMENTOUS FUNGI ISOLATED FROM HISTORICAL WOOD

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ARTICLE INFO

Article History:
Received 19th January, 2015
Received in revised form
15th February, 2015
Accepted 10th March, 2015
Published online 28thApril, 2015

Key words:

Antifungal activity, wood, biodeterioration, fungi, *Lawsoniainermis*.

ABSTRACT

Plant extracts have a long history of applications as antimicrobial agents, but their use as wood preservatives has rarely been reported. This study deals with the antifungal activity of the *Lawsonia inermis* extracts against four cellulolytic wood fungi, in order to find new bioactive natural products. The methodology used is based on a measuring the inhibition halos produced by discs impregnated with the extracts and establishing their Minimum Inhibitory Concentrations (MIC). The extracts, object of the survey, showed a strong antifungal activity of ethanolic extracts of leaves against all tested fungi. These results suggest the possible exploitation of the Lawsonia extracts as potential botanical effects in ecofriendly control of wood biodeterioration by fungi.

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INTRODUCTION

The monumental buildings of the Medina of Fez in Morocco consist mainly of wooden materials, however, this natural are continuously exposed to physical, chemical and biological degradation. Among biological agents, fungi have a critical importance in the deterioration of these historic buildings. Problems of wood biodeterioration in the Medina of Fez were reported by El Bergadi *et al*. (2011) and El Abed *et al*. (2010). Generally, the preservation of wood are isever, the use of these products for many indoor applications have been banned or limited in many countries, due to their harmful effects on human health and the environment. Development of natural alternatives that are user friendly and demonstrate negligible toxicity to humans is of interest. In the past ten years, the extracts herbaceous plants have evoked interest as sources wood protection agents to prevent mold and fungal growth on in-service wood (Clausen and Yang, 2007). In Morocco, Henna or *Lawsonia inermis* is described in the traditional pharmacopeia, known by its various cosmetic and therapeutic properties. It is abundantly collected in the areas of the south because it supports well the subtropical climate in Morocco. Henna leaves, flowers, seeds, stem bark and roots are attributed in India to have many medicinal (Lavhate and Mishra, 2007; Chetty, 2008) and cosmetic properties (Rahmoun *et al*., 2013). Many researchers have since demonstrated that the plant performs antimicrobial (Habbal *et al*., 2011; Gull *et al*., 2013), antifungal (Khan and Nasreen, 2010; Philip *et al*., 2011), virucidal (Mouhajir *et al*., 2001) and antiparasitic (Zhong Yao Za Zhi, 2013) activities in food applications, pharmaceutical researches and other areas. However, little has been published previously on the use of plant extracts as antimold agents on wood.

Hence, in the present study, the extracts of *Lawsonia inermis* have been screened for their antifungal activity against the fungi responsible of biodeterioration of wood.

MATERIALS AND METHODS

Lawsonia samples

The plant was collected from Errachidia regions (south of Morocco) during harvesting season. The leaves of plant were cleaned, air dried in the shade, then grounded to fine powder and stored in airtight bottles in the dark until extraction.

Microorganisms

In this study, four local wood degrading cellulolytic filamentous fungi *Aspergillus niger*, *Penicillium griseoroseum*, *Penicillium italicum* and *Lewia infectoria* were used as test organisms. These fungi were isolated from degraded wood samples and identified in our previous study by El Bergadi

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et al. (2011). The cultures were maintained in Malt extract agar medium at 25°C for 7 days before being used.

Preparation of material extracts

An aqueous extract was prepared by boiling 16g of the powdered plant in 100 ml of sterile distilled water for about 10 to 15 min. The macerate was first filtered twice using a Millipore filters (Millipore 3 mm) to remove particulate matter. The final filtrates were collected in wide mouthed evaporating bowls and dried under room temperature. The dried extracts were weighed to calculate the extractability percentage. The extracts were stored aseptically at 4°C for further use. However, the ethanolic and methanolic extracts blended respectively with 100 ml of ethanol and methanol then for each trial. The resultant extracts solutions were filtered using Whattman No 1 filter paper. The filtrates obtained were concentrated under reduced pressure at 50°C on a rotary evaporator to obtain the crude extract. Subsequently, the extracts were kept at 4°C until use.

Preparation of inoculums

After incubation of the Malt extract medium at 25 °C for 7 days. Spores of each fungus were collected 7 days as described by Dao *et al.* (2008) by flooding the surface of the plates with NaCl (0.9%). The sporangial suspension concentration was estimated using a Malassez cell and the spores were adjusted to 10^6 spores mL-1. The fungal spore suspensions were stored in 20% glycerol at –40°C.

Assay of antifungal activity

Antifungal activity of the extracts was determined *in vitro* by the agar-diffusion assay method as described by Gautam *et al.,* (2007). Sterile discs of filter paper 16 mm in diameter were impregnated with 50 µL of the extracts obtained and they were placed on Malt extract Agar Petri dishes, which had previously been surface-plated with 100 µl of a spore suspension of tested fungi. The dishes were incubated for 72 h at 25°C. Controls were observed using Petri dishes with results and the inhibition halos formed were determined by measuring their total diameter in millimeters (mm). The distilled water and ketoconazole (200 mg/mL) were used as the negative and positive controls respectively. The test was carried out three times to assure repeatability.

Effect of different concentration

The antifungal activity of leaf ethanolic extract was determinated at different concentration 25, 50, 75 and 100% (V/V%)

Determination of Minimal Inhibitory Concentration (MIC)

MIC values of the ethanol extract against tested microorganisms were performed using the macrobroth dilution method (Warnock, 1989). Ethanol plant extracts were first diluted to the highest concentration to be tested (30 mg/mL), and then serial two-fold dilutions were made in a concentration range from 9 to 1.5 mg/mL in test tubes containing Sabouraud's broth. The tubes were then inoculated with 10 µL of cultures (10^6spores mL-1). The tubes were then incubated at 25°C for 3 days. The MIC was defined as the lowest concentration of plant extracts that caused growth inhibition more than 90% after the period of incubation.

RESULTS AND DISCUSSION

Physical characteristics of aqueous and ethanol extracts of the leaves of *Lawsonia inermis* are depicted in Table 1.

Table 1. Physical properties of *L. inermis* leave extracts

	Aqueous extract	Ethanolic extract	Methanolic extract
Colour	Dark green	Dark green	Brownish
Weight of dry powder (g)	16	16	16
Weight of dry extracts (g)	1.8	3.9	3.1
Percentage yield (%)	11.2	24.4	19.3

Aqueous extract was dark green in colour and about 11.2% was recovered after the extraction processes. Ethanolic extract was also dark green colored, and 24.4% was recovered after the extraction. Methanolic extract was brownish in colour, and have as percentage yield 19.3%. Therefore, it can be observed that the powdered leaf of *Lawsonia inermis* contained chemical constituents that are more soluble in ethanol than methanol and water.

Table 2 shows the diameters of the inhibition halos produced by the aqueous, ethanolic and methanolic extracts of *Lawsonia inermis* when in contact with *L. infectoria*, *A. niger*, *P. italicum* and *P. griseoroseum.*

Table 2. Inhibition halos produced by aqueous, ethanolic and methanolic extracts of *Lawsonia inermis* when in contact with cellulolytic fungi

Microoganisms	Inhibition (mm)		
	Aqueous	Ethanolic	Methanolic
L. infectoria	11.00±1.00	14.00±2.00	10.33±0.57
A. niger	13.00±1.00	14.33±2.08	8.66±1.52
P. italicum	10.33±0.57	13.33±0.57	8.66±2.08
P. griseoroseum	9.66±1.15	11.66±0.57	7.66±1.52
Control	10.00±1.00	12.00±1.00	11.33± 0.57

The antifungal effects of *L. inermis* extracts on the tested fungi were compared with the control (Table 2). The results obtained show that the leaves extracts of this plant reduced colony growth of the fungus, however the inhibitory effect varied between tested extracts. In fact, ethanol extract are more effective on *L. infectoria, A. niger, P. italicum* and *P. griseoroseum* than aqueous and methanolic extracts. The better activity of ethanol extracts of *L. inermis* have already been reported in several studies (Choubey *et al*., 2010; Khan and Nasreen, 2010; Philip *et al*., 2011). Our results were in agreement with the findings of other investigations which demonstrated that for the antifungal activity of alcoholic Henna extracts was more effective than the water based extract. This may be due to the lack of the solvent properties which plays an important role in antimicrobial efficacy (Arun *et al*., 2010) and the solubility of the active principle present in Henna (lawsone) (Khan and Nasreen, 2010).

Also of interest, we found that the diameter of inhibition zones produced by ketoconazole (positive control) was relatively smaller than that produced by the aqueous and ethanolic extracts on all the organisms used in this study except on *P. griseoroseum*. Therefore, the ethanolic extract of *L. inermis* has a very promising potential against wood fungi.

In recent years, several studies have been focused on screening of plant extracts to develop new antifungal compounds that can be used to control fungi responsible in biodeterioration of wood (Chebli *et al*., 2003; El Ajjouri *et al*., 2008 ; Hussain *et al*., 2013), and number of studies demonstrate significant antifungal activity of *L. inermis* leaves (Jeyaseelan *et al*., 2012; Kannahi and vinotha, 2013), flowers and fruits (Jeyaseelan *et al*., 2012), but the activity of this plant against wood fungi are the first time reported. Therefore, the results of the present work bring additional data on the antifungal activity of *L. inermis.*

The study of antifungal effect of leaf ethanol extract with different concentrations is represented in Table 3.

Table 3. Inhibition halos produced by ethanolic extract of *L. inermis* at different concentrations

Microorganisms	Inhibition (mm)			
	25%	50%	75%	100%
L. infectoria	11.66±0.57	14.00±2.00	15.00±1.00	16.33±0.57
A. niger	14.00±1.00	14.33±2.08	17.33±0.57	18.33±0.57
P. italicum	11.66±1.15	13.33±0.57	14.33±0.57	16.33±0.57
P. griseoroseum	11.00±1.00	11.66±0.57	13.00±1.00	15.00±1.00
Control	13.66±0.57	12.00±1.00	17.66±1.52	17.66±0.57

The results indicate that the ethanolic extract influence the mycelium growth of all tested fungi at different concentrations. In fact, the activity of ethanol extract of leaves against most on the test fungi showed minimum activity at 25% and maximum activity at 100%. The activity of ethanol extract of *L. inermis* against *A. niger* was more effective (18.33±0.57 at 100%) than that produced by control (17.66±0.57 at 100%). These results are in line with those obtained by Jeyaseelan *et al*. (2012) and Rajashri *et al.* (2014), which showed respectively that the inhibition zones of *A. niger and A. flavus* increased with the increasing of the concentration of plant extract. However, Kannahi and Vinotha (2013) reported that the inhibition halo of *A. niger* decreased with the increasing of the concentration. Significant antifungal effect expressed as MIC of ethanol extract against tested wood fungi was is shown in Table 4.

Table 4. Minimum Inhibitory Concentration (MIC) of the ethanolic extract of *Lawsonia inermis* on cellulolytic fungi

Microorganisms	Concentration (mg/mL)					
	1.5	3	4.5	6	7.5	9
L. infectoria	+	+	+	+	—	—
A. niger	+	+	+	—	—	—
P. italicum	+	+	+	+	—	—
P. griseoroseum	+	+	+	+	+	—

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

The data in Table 4 indicated that *A. niger* was more sensitive to ethanol extract with MIC of 6 mg/mL. *L. infectoria* and *P. italicum* were found sensitive ethanolic extract at a concentration of 7.5 mg/mL. However, *P. griseoroseum* was found to be more resistant to extract with 9 mg/ mL as a value of MIC.

Conclusion

Moroccan henna from Saharan region demonstrates high *in vitro* antifungal activity against some fungi isolated from historical wood. Therefore, the present study will be helpful in the realistic approach for the development of ecofriendly fungicides and thus in the effective management of biodeteriorated fungi. A further study will evaluate the bioactive compounds present in ethanolic extracts of *L. inermis.*

REFERENCES

Arun, P., Purushotham, K., Jayarani, J. and Kumari, V. 2010. In vitro Antibacterial activity and Flavonoid contents of Lawsonia inermis (Henna). *Int. J. PharmTech. Res.,* 2(2) :1178-1181.

Chebli, B.C.H ., Achouri, M., Hassani, L.M.I . and Hmamouchi , M. 2003. Chemical composition and antifungal activity of essential oils of seven Moroccan Labiatae against Botrytis cinerea Pers: *Fr. J. Ethnopharmacol.,* 89 :165-169.

Chetty, K. 2008. Flowering plants of Chittoor. Andhra Pradesh; 1st edition.

Choubey, A., Ojha, M., Mishra, A., Mishra, S. and Patil, U.K. 2010. Hypoglycemic and antihyperglycemic effect of ethanolic extract of whole plant of Lawsonia inermis (henna) in streptozotocin induced diabetic rats. *Int. J. Pharm. Sci. Res.,* 1(8):74-77.

Clausen, C.A. and Yang, V. 2007. Protecting wood from mould, decay, and termites with multi-component biocide systems. *Inter. Biodeterior. Biodegrad.,* 59(1):20-24.

Dao, T., Bensoussan, M., Gervais, P. and Dantigny, P. 2008. Inactivation of conidia of Penicillium chrysogenum, P. digitatum and P. italicum by ethanol solutions and vapours. *Int. J. Food. Microbiol.,* 122:68-73.

El Abed, S., Hamadi, F., Latrache, H., Iraqui, H.M. and Ibnsouda Koraichi, S. 2010. Adhesion of Aspergillus niger and Penicillium expansum spores on Fez cedar wood substrata. *Ann. Microbiol.,* 60(3): 377-382.

El Ajjouri, M., Satrani, B., Ghanmi, M., Aafi, A., Farah, A., Rahouti, M., Amarti, F. and Aberchane, M. 2008. Activité antifongique des huiles essentielles de Thymus bleicherianus Pomel et Thymus capitatus (L.) Hoffm. & Link contre les champignons de pourriture du bois d'œuvre. *Biotechnol. Agron. Soc. Environ.,* 12 :345-351.

El Bergadi, F., Elabed S., Laachari F. and Ibnsouda S.K. 2011. Penicillium italicum, Penicillium griseoroseum and Pseudomonas trivialis are the novel species that degrade cedar wood in historical Moroccan sites. *J. Pure. Appl. Microbio.,* 5(2):501-510.

Gautam, R., Saklani, A. and Jachak, S.M. 2007. Indian medicinal plants as a source of antimycobacterial agents. *J. Ethnopharmacol.,* 110:200-234.

Gull, I., Sohail, M., Aslam, M.S. and Athar, M.A. 2013. Phytochemical, toxicological and antimicrobial evaluation of Lawsonia inermis extracts against clinical isolates of pathogenic bacteria. *Ann. Clin. Microbiol. Antimicrob.,* 12:36-42.

Habbal, O., Hasson, S.S., El- Hag, A.H., Al-Mahrooqi, Z., Al-Hashmi, N., Al-Bimani, Z., Al- Balushi, M.S., and Al-Jabri, A.A. 2011. Antibacterial activity of Lawsonia inermis Linn (Henna) against Pseudomonas aeruginosa. *Asian. Pac. J .Trop. Biomed.,* 1(3):173-176.

Hussain, A., Shrivastav, A. and Jain, S.K. 2013. Antifungal activity of essential oils against local wood degrading cellulolytic filamentous fungi. *Adv. Biores.,* 4 :161-167.

Jeyaseelan, E.C., Vinuja, T., Pathmanathan, K. and Jeyadevan, J.P. 2012. Control of Plant Pathogenic Fungi using Organic Solvent Extracts of Leaf, Flower and Fruit of Lawsonia inermis L. *Int. J. Pharm. Biol. Arch.,* 3(4) :783-788.

Kannahi, M. and Vinotha, K. 2013. Antimicrobial activity of Lawsonia inermis leaf extracts against some human pathogens. *Int. J. Curr. Microbiol. App. Sci.,* 2(5):342-349.

Khan, Z.S., Nasreen, S. 2010. Phytochemical analysis, antifungal activity and mode of action of methanol extracts from plants against pathogens. *J. Agr. Tech.,* 6(4) :793-805.

Lavhate, M. and Mishra, S. 2007. A review: nutritional and therapeutic potential of Ailanthus excels. *Phcog. Rev.,* 1 (1):105-113.

Mouhajir, F.J.B., Hudson, G.H.N. and Rejdali, Towers, G.H.N. 2001. Multiple antiviral activities of endemic medicinal plants used by berber peoples of Morocco. *Pharmaceut. Biol.,* 39(5):364-374.

Philip, J.P., Madhumitha, G. and Mary, S.A. 2011. Free radical scavenging and reducing power of Lawsonia inermis L. seeds. *Asian Pac. J. Trop. Med.,* 4(6) :457-461.

Rahmoun, N., Boucherit-Otmani, Z., Boucherit, K., Benabdallah, M. and Choukchou-Braham, N. 2013. Antifungal activity of the Algerian Lawsonia inermis (henna). *Pharm. Biol.,* 51 (1):131-135.

Rajashri, R.T., Sachin, A.N. 2014. Antifungal activity of Nigella sativa and Lawsonia inermis and its comparative study. *World. J. Pharm. Res.,* 3(4) :1468-1472.

Warnock, D.W. 1989. Methods with antifungal drugs. In : Evans EGV, Richardson MD (eds) Medical mycology a practical approach, chapter 11, 1st edition, IRL Press, Oxford. UK, pp. 235-247.

Zhong Yao Za Zhi Z. 2013. Advances in studies on chemical constituents and biological activities of Lawsonia inermis. *Pub. Med.,* 38(6):795-799.

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