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SHORT COMMUNICATION

Antifungal activity of the Algerian *Lawsonia inermis* (henna)

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Abstract

Context: *Lawsonia inermis* Linn. (Lythraceae) or henna has been used since the earliest times as a medicine, preservative, and cosmetic. It has long been recommended in traditional medicine as an astringent, purgative, and abortifacient.

Objective: Lawsone and six extracts of *L. inermis* plant, used by Algerian traditional healers to treat infectious diseases, were screened for their antifungal activity against filamentous fungi.

Materials and methods: Water and five organic extracts – DMSO, ethanol, chloroform, ethyl acetate, and di-ethyl ether – of *L. inermis* leaves, collected in the area of Adrar (Algeria), were prepared by soaking 25 g of powdered plant in 100 mL of solvent. The extracts were screened for antifungal activity using the poisoned food technique against five filamentous fungi.

Results: Results demonstrated that the best yield (8.03%) was obtained with the ethanol extract. The commercial lawsone showed potentially interesting MICs against the strains *Fusarium oxysporum* (12 µg/mL) and *Aspergillus flavus* (50 µg/mL). The ethanol extract showed the only interesting MIC (230 µg/mL of crude extract) against the strain *F. oxysporum* compared with other extracts.

Discussion and conclusion: These results suggest that the Algerian *L. inermis* plant has antifungal activity that can be related to the presence of lawsone in the leaves plant. The results can be exploited largely in research of new antifungal drugs.

Keywords: Antimicrobial agents, natural product, lawsone, bioactive compound

Introduction

Herbal remedies used in traditional folk medicine provide an interesting and still largely unexplored source in the creation and the development of potential new drugs for chemotherapy which might help to overcome the growing problem of resistance and the toxicity of the currently available commercial antibiotics (Spellberg et al., 2008). The traditional medicinal method, especially the use of medicinal plants, still plays a vital role to cover the basic health needs in the developing countries. Therefore, it is of great interest to carry out screening of these plants to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents. According with the ethnobotanical literature (Ali et al., 1999; Mostefa-Kara et al., 2010; Rahmoun et al., 2010), a great number of medicinal plants are being used to treat microbial infections, particularly in the rural areas of Algeria where the traditional folk medicine remains a major source to cure minor ailments. Up-to-date, very little research was done to investigate these traditionally medicinal plants (El-Fiky et al., 1995).

Lawsonia inermis Linn. (Lythraceae) or henna, a traditional plant with religious associations, has been widely used over centuries for medication and cosmetics in some regions of the world especially in the Middle East, Africa and Asia (Al-Tufail et al., 1999).

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Phytochemical investigations have shown the presence of the following constituents: β-sitosterolglucosides, flavonoids, quinoids, naphthalene derivatives, luteolin, betulin, lupeol galic acid, coumarins, xanthones, and phenolic glycosides (Lal & Dutta, 1933; Agarwal et al., 1959; Bhardwaj et al., 1977; 1978; Atal et al., 1978). The plant, *L. inermis*, contains 25–33% water soluble matter; aqueous solutions are orange in color and show a green fluorescence. The principal coloring matter is lawsone, 2-hydroxy-1,4-naphtoquinone which is present in dried leaves in a concentration of 0.4–1.5%. The two pentacyclic triterpenes, namely, 3β,30-dihydroxylup-20(29)-ene (hennadiol) and (20S) 3β,30-dihydroxylupane have been isolated from the bark (Chakrabarty et al., 1982).

It has been found that the ethanol extract of the whole plant has antifungal activity (Misra & Dixit, 1979) and antitubercular activity (Bhatnagar et al., 1961). The decoction of bark and leaves has been found to inhibit peptic enzymes (Prasad & Gupta, 1967) and showed anti-inflammatory activity (Singh et al., 1982). Moreover, henna naphthoquinones derivatives have been reported to have pharmacological potential, particularly as antifungal and antibacterial agents (Rahmoun et al., 2010; Villemin et al., 2010).

This paper reports the screening of lawsone and six different extracts of the leaves of *L. inermis*, for their antifungal properties against five filamentous fungi.

Materials and methods

Biological materials

The proposed material for the study was provided by the National Institute of Agronomic Research (NIAR, Adrar-Algeria). It was identified and authenticated by us in collaboration with the service for plant authentication of NIAR and was submitted as *L. inermis* leaf. It was harvested in the area of Adrar in March 2008, dried and stored at room temperature in the dark. Commercial lawsone (L) was purchased from Sigma-Aldrich and was used without further purification.

Preparation of *L. inermis* extracts

Plant extracts were prepared according to the method of Sharma (1990) with minor modification. Extraction was carried out from the crushed dry leaves. Briefly, 25 g of powdered plant material were soaked in 100 mL of solvent. Various extractions were carried out using six different solvents. Each mixture was stirred for 24 h. At the end of each extraction, the extract was passed through Whatman filter paper No. 1 (Whatman, UK). The volatile filtrates obtained (ethanol, chloroform, ethyl acetate and di-ethyl ether filtrates) were concentrated under vacuum on a rotary evaporator at low temperature 30°C. Dimethyl sulfoxide (DMSO) and water extracts were not evaporated because of their high boiling point, and were used without further treatment. The extracts were all stored at 4°C until further use. The influence of heat on the extraction yield was studied by comparison with maceration in cold water.

The rates of extracts were calculated as follows:

$$\text{Yield} = \frac{m_0}{m_1} \times 100$$

Where, $m_{0:}$ evaporated extract mass, and $m_{1:}$ initial vegetal mater mass.

Microorganisms

A panel of five pathogenic filamentous fungi was used in this study. Two tested filamentous fungi (*Aspergillus flavus* 994294 and *Fusarium oxysporum* 963917) were obtained from the collection of the Musée National d'Histoire et Nature de Paris (MNHNP), while the other filamentous fungi (*Aspergillus niger*, *Rhizopus stolinifer* and *Penicillium* sp.) were isolated and obtained from the Antibiotics Antifungal Laboratory: Physical Chemistry, Synthesis and Biological Activity, Department of Biology, Faculty of Sciences, (Tlemcen University) Algeria. The identities of these filamentous fungi were checked by colonial and microscopic morphology before being tested (Larone, 2002). The tested strains were all maintained on Sabouraud's Agar at 28°C.

Fungitoxic assay

The fungitoxic activity of lawsone and all extracts was evaluated against molds by the poisoned food technique of Perrucci (Perrucci et al., 1994; Singh et al., 2008).

Saboraud dextrose agar (SDA) medium was prepared in the flasks and sterilized. To this medium, a quantity of samples of lawsone, amphotericin B, and extracts were added. Lawsone and amphotericin B were prepared in order to get desirable final concentrations 150 μg/mL. The samples were thoroughly mixed by stirring. A negative control was also prepared in the same way using solvent DMSO. The medium was then poured. The mycelial discs, taken from the cultures of the fungi test previously grown on SDA medium petri plate for seven days, were used for inoculation in the center of Petri plate aseptically. Suitable controls were kept where the cultured discs were grown under the same conditions on SDA medium without any sample. The plates were inoculated at 28°C. The efficacy of each sample was determined by measuring the radial mycelial growth. The radial growth of the colony was measured in two directions at right angle to each other, and the average of two replicates was recorded in each case. Data were expressed as percent inhibition over control from the size of the colonies.

The percentage inhibition that is given in the Tables was calculated using the formula (Pinto et al., 1998):

$$\text{Percentage inhibition} = \left[(C-T)\times 100\right] \Big/ C$$

Where, C = diameter of the fungus colony in the control plate after 96 h; T = diameter of the fungus colony in the tested plates after the same period.

Results and discussion

Henna has been used for more than 4000 years as a cosmetic by Mediterranean, Middle Eastern, and Asian cultures. In many circumstances, the dye is applied over extensive areas of the body to create a variety of designs, and henna is frequently applied to newborn infants for ceremonial purposes (Kandil et al., 1996). The widespread use of henna clearly shows that it is considered to be safe for external application.

Yield

Various extractions of the *L. inermis* plant were carried out for the antifungal screening. Thus, water and five organic solvents were used: DMSO, ethanol, ethyl acetate, chloroform, and di-ethyl ether. The results of the various yields (Table 1) showed that the best yield was obtained with ethanol than ethyl acetate, chloroform, and finally with di-ethyl ether extract.

DMSO and water are solvents difficult to be evaporated. Yields in these cases are deferred compared to the initial vegetable matter and cannot be compared with those of other solvents.

The choices of solvents correlate with certain work which showed that the primary antimicrobial activity screening must be started by the aqueous and ethanol crude extracts followed by less polar solvents (Cowan, 1999). According to Zhang and Lewis (1997), the active components identified for their antimicrobial activity were aromatic compounds or organic saturated compounds and were obtained with methanol or ethanol extraction.

Fungitoxic activity evaluation of lawsone and *L. inermis* extracts

Data in Table 2 reveal that lawsone (150 μg/mL) showed a high degree of inhibition against the tested molds (*F. oxysporum*, *A. niger*, *A. flavus*, and *Penicillium* sp.) except the strain *R. stolinifer*. The minimum inhibitory concentration was examined at different concentrations of lawsone using the poisoned food method and the lowest values were recorded against the strains *F. oxysporum* (12 μg/mL) and *A. flavus* (50 μg/mL) (Table 2).

Results of amphotericin B fungitoxic activity at the concentration of 150 μg/mL were similar to lawsone against four strains: *F. oxysporum*, *A. niger*, *A. flavus*, and *Penicillium* sp. *R. stolinifer* showed a sensitivity for amphotericin B.

Evaluation of fungitoxic activity of different extracts of *L. inermis* (Table 3) showed that the ethanol extract presented the best activity comparing to the other tested extracts, in particular with an inhibition rate of 72% (3.7 μg/mL) of the growth of *F. oxysporum*. However, the other extracts did not show any interesting activity against the strains used in this study. It could be explained by the low mass of the vegetable matter used to carry out the extraction, or by the resistance of the strain used in this screening. The activity of the ethanol extract remains very interesting until the concentration of 230 μg/mL, in the study of the MIC against the only strain *F. oxysporum* (Table 4).

Some studies showed that the dying proprieties of the henna were used also as a drug to prevent hands and feet against microbial infections (Cowan, 1999; Mostefa-Kara et al., 2010). Ethanol extract of the whole plant was found in some studies to have antifungal activity (Misra & Dixit, 1979). Also, Muhammad and Muhammad (2005) achieved a study where they tested the activity of *L. inermis* leaves against the hospital strain originary: *A. niger* and *F. oxysporum*. The study showed that the aqueous crude extract was active until the concentration of 80 mg/mL against *A. niger* and until 30 mg/mL against *F. oxysporum*.

Aqueous extract of *L. inermis* leaves was tested for antifungal activity against eight important isolated species of *Aspergillus* from sorghum, maize, and padcdy seed samples. *A. flavus* recorded high susceptibility and hence solvent extracts, viz., petroleum ether, benzene, chloroform, methanol, and ethanol extract of the plant showed significant antifungal activity (Raveesha et al.,

Table 1. Extraction yields of volatile solvents.

Solvent	Ethanol	Ethyl acetate	Chloroform	Di-ethyl ether
Rate %	8.03	4.75	3.25	1.25

Table 2. Inhibition† rate (%) of different concentrations of lawsone.

	Strains				
Lawsone concentrations (μg/mL)	*F. oxysporum*	*A. niger*	*A. flavus*	*Penicillium* sp.	*R. stolinifer*
300	88.3	87.47	90.62	69.02	00.00
200	88.3	87.47	90.62	69.02	00.00
150	69.30	87.47	61.32	69.02	00.00
100	42.35	00.00	35.28	00.00	00.00
50	17.12	00.00	5.12	00.00	00.00
25	7.36	00.00	00.00	00.00	00.00
12	5.34	00.00	00.00	00.00	00.00
6	00.00	00.00	00.00	00.00	00.00
Standard	00.00	00.00	00.00	00.00	00.00
Amphotericin B‡	88.1	88.15	88.12	79.64	68.82

†Average of three replicates.
‡The commercial fungicide, amphotericin B (150 μg/mL) was used for the comparison of activity.

Table 3. Inhibition† rate (%) of different *L. inermis* extracts using five fungi.

Extracts	Inhibition rate† (%)				
	F. oxysporum	*A. niger*	*A. flavus*	*R. stolinifer*	*Penicillium* sp.
Commercial lawsone (150 µg/mL)	88.29 ± 0.4	87.47 ± 1.6	90.62 ± 0	00.00 ± 0	69.02 ± 3.2
DMSO	18.96 ± 0.6	00.00 ± 0	00.00 ± 0	00.00 ± 0	21.19 ± 1.4
Aqueous	00.00 ± 0	00.00 ± 0	00.00 ± 0	00.00 ± 0	00.00 ± 0
Ethanol	71.95 ± 0.7	00.00 ± 0	09.46 ± 0.4	00.00 ± 0	25.01 ± 2.9
Chloroform	10.65 ± 0	00.00 ± 0	03.95 ± 0.5	00.00 ± 0	14.31 ± 2.4
Ethyl acetate	16.31 ± 1.3	00.00 ± 0	00.00 ± 0	00.00 ± 0	10.01 ± 1.7
Di-ethyl ether	14.73 ± 1.3	00.00 ± 0	00.00 ± 0	00.00 ± 0	18.44 ± 2.4
Amphotericin B‡	88.29 ± 0.4	87.47 ± 1.6	90.62 ± 0	00.00 ± 0	69.02 ± 3.2

†Average of three replicates.
‡The commercial fungicide, amphotericin B (150 µg/mL) was used for the comparison of activity.

Table 4. Minimum inhibitory concentration of ethanol extract against *F. oxysporum*.

Concentration in 10^3 µg/mL	14.72	7.36	3.7	1.84	0.9	0.46	0.23	0.11
Inhibition rate %	89.01	89.01	71.95	55.75	25.45	15.90	09.20	00.00

2007). In a study realized against the strains *Trichophyton mentagrophytes*, *T. rubrum*, *T. tonsurans*, *T. violaceum*, *T. verrocosum*, *T. schoenleinii*, *Epidermophyton floccosum*, *Microsporum ferrugineum*, *M. canis*, *Sporotrichum schenckii*, sensitivity toward henna was strong. Ethanol, methanol, and aqueous extracts of *L. inermis* leaves were involved also in defensive mechanism against spore germination of *Drechslera oryzae* (Natarajan & Lalitha, 1987). Also, during screening of 30 plant species barks for activity against *Microsporum gypseum* and *Trichophyton mentagrophytes*, the extract of *L. inermis* exhibited absolute toxicity. The extract showed broad fungitoxic spectrum when tested against 13 other dermatophytes (Singh & Pandey, 1989).

Some authors report the origin of the antimicrobial activity of *L. inermis* leaves to gallic acid or naphthoquinones (lawsone) (Cowen, 1999; Ahmed et al., 2000). When comparing the activities of ethyl acetate and water phases to the ethanol extracts, it seems that the active compounds could belong to the lipophilic group rather than to the hydrophilic. From Tables 3 and 4, and the literature (Cowan, 1999; Wichtl, 1999; Khare, 2007), we think there is a role of polyphenols in the antifungal activity showed by the ethanol extract. The polyphenols isolated from the ethanol extract are: naphthoquinones derivatives, lawsoniaside, luteolin, acacetin, cosmosiin, lalioside, lawsoniaside, syringinoside, daphneside, daphnorin, agrimonolide pyranoside derivatives, and isoscutellarin (Takeda & Fatope, 1988; Cuong et al., 2010).

It was reported that lawsone isolated from the leaves of *L. inermis* has shown a significant antifungal effect (Dixit et al., 1980). During antifungal screening of higher plants, the leaves of *L. inermis* were found to exhibit strong fungitoxicity. The antifungal factor was found to be lawsone (Tripathi et al., 1978). However, it should not be assumed that lawsone, the most active compound, accounts only for the bulk of the activity of the ethanol extract. It was reported that the most active compound found is not necessarily responsible for the major part of the effect. It is important to determine the concentration of each compound and correlate it with its dose-response characteristics in the test system (Houghton et al., 2007).

Finally, our finding suggested that henna extract could be used as alternative source of antifungal agents for protection of humans or plants against fungal infection. Lawsone is one of the bioactive compounds that warrants consideration because of the interesting *in vitro* activities. It is important later to reinforce further our investigation in more than one model of fungal species in order to establish a basis for taking an extract or preparation into *in vivo* or clinical tests.

Conclusion

These results enable us to conclude that the ethanol extract of the *L. inermis* plant has interesting antifungal activity against the strain *F. oxysporum* (230 µg/mL). This activity can be related to the presence of lawsone. Further studies are needed to investigate the effect of the ethanol extract on other strains such as the systemic dermatophytes or mycoses. Studies of cytotoxicity data from human or animal cells in tissue culture are needed in order to establish that the ethanol extract, particularly lawsone, has selective antimicrobial activity and therefore may be a realistic prospect for future clinical use in humans.

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Declaration of interest

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