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Antimicrobial screening of the Algerian *Lawsonia inermis* (henna)

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ABSTRACT

Henna represents one of the most effective traditional remedy against multiple diseases that includes ulcer, skin disorders and infection malignancies. Identifying the active ingredient of Henna species grown in different regions of the world will definitely advance its therapeutic application. The in vitro antimicrobial screening of the Algerian henna leaves (Lawsonia inermis Linn.) was assessed against four strains of pathogenic bacteria, namely Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 29212, and one multidrug resistant yeast namely C.albicans ATCC 10231. The henna DMSO, ethanolic and ethyl acetate leaves extracts were found to be the most active fractions against gram positive bacteria S.aureus and E.faecalis and the yeast C.albican. Such data suggests that this activity is strongly related to the presence of Lawsone in henna leaves. Inhibition of the microorganisms' growth suggests that Algerian henna can be largely exploited in research of new antimicrobial drugs.

Keywords: Henna, Lawsone, antimicrobial activity, natural product.

INTRODUCTION

Many of today's modern drugs against multiple diseases have their origin in traditional plant medicines [1]. Moreover the natural products are a true source of synthetic and traditional herbal medicine [2]. The therapeutic efficacies of many indigenous plants in several disorders have been described by practitioners of traditional herbal medicines [3]. However, isolation of microbial agents less susceptible to regular antibiotics and recovery of resistant isolates during antimicrobial therapy is increasing throughout the world [4]. One of the measures to combat this increasing rate of resistance is to have continuous investigations into new, safe and effective

antimicrobials as alternative agents to substitute for less effective ones. In this regard, research is being carried out to investigate ethnobotanical uses of plants prevailing among native people. The henna plant is one such plant known since with healing attributes, and is now the subject of intense scientific studies.

The *Lawsonia inermis* plant, commonly known as Mehendi or henna, is native to a number of tropical regions in Asia, Northern Africa and Australia. It is naturalized and cultivated in the tropics of America, Egypt, India and parts of the Middle East [5].

It is a glabrous highly branched shrub with a greyish brown bark. The leaves are opposite, subsessile and elliptic or broadly lanceolate, entire, acute or obtuse, 2-3 cm long and 1-2 cm wide. The leaves of *L. inermis* contain soluble matter, Lawsone, 2-hydroxy-1,4-naphthoquinone, tannin, gallic acid, glucose, mannitol, fat, resin and mucilage. The color matter is attributed to the quinone. Lawsone is the chief constituent responsible for the dyeing properties of the plant, known to be the major bioactive constituent. The dried powdered leaves of *Lawsonia* contain about 0.5–1.5% Lawsone [6]. *L.inermis* has long been used for many medicinal purposes including as an astringent, an antihemoragic agent and for its cardio-inhibitory, hypotensive, and sedative effects. It has also been used as a folk remedy for amoebiasis, headache, jaundice and leprosy [7].

Screening of organic and aqueous extracts from *L.inermis* plant is the common approach to identify compounds with biomedical importance, for example new anticancer agents [8], antibacterial [9,10] and antifungal effect [11,12]. It was reported that the bioactive constituent (Lawsone) has significant activity as antimicrobial agent [13].

In this report, we describe the screening of different extracts from leaves *L.inermis* specie for antibacterial and antifungal activities as alternative sources for the development of new treatments for the emergence of resistance to antimicrobial therapy, which leads to greater research and interest for optimal and new antimicrobial agents.

MATERIALS AND METHODS

Biological materials

The henna plant "*L.inermis*" was provided by the National Institue of Agronomic Research (NIAR, Adrar-Algeria). It was harvested in the area of Adrar in March 2008 and stored in the dark at ambiant temperature in our laboratory.

1.1 Preparation of *L. inermis* extracts

Plant crude extracts were prepared according to the method of Sharma [10], 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 hours. At the end of each extraction the extract was passed through Whatman filter paper N^{o} 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. Dimethylsulfoxide (DMSO) and water extracts were not evaporated because of their high boiling point, and were used without further treatment. All extracts were stored at 4°C until further use. The influence of heat on the extraction yield was studied by comparison with maceration in cold water.

1.2 Microorganisms and media

Evaluation of the antimicrobial activity of the *L.inermis* extracts and commercial Lawsone (Purchased from Sigma-Aldrich and used without further purification) was performed against four strains of pathogenic bacteria, namely *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, and one multidrug resistant yeast namely *C.albicans* ATCC 10231.

The evaluation was carried out by disc diffusion on agar medium, based on the recommendations of the National Clinical Committee Laboratory Standards [14,15]. The disc diffusion methodology yields a quantitative result. The activity evaluation was thus determined by measurement of the inhibitory zone diameter after incubation at 37°C during 24 hr. Ciprofloxacin [16] and Gentamycin [17] were used as positive controls. The choice of these drugs was based on the broad range of antibiotic activity with respect to the strains used.

Antifungal activity against *Candida albicans* (ATCC 10231) was measured according to a standardized disc diffusion Method for yeasts [18]. Amphotericine B was used as positive control [19].

The extracts and Lawsone were subjected to an evaluation for their level activity using the broth microdilution method to estimate the Minimal Inhibitory Concentration (MIC). This evaluation was based on the recommendations of the NCCLS [15,20]. A serie of dilutions of the plant extracts on DMSO (5%) were prepared. The effect of DMSO at the concentration of 5% was checked and eliminated. The data points constitute the mean of three replicates. The concentration with a prominent decrease in turbidity was visually determined as the MIC.
One of the strong points in the NCCLS recommendations is reproducibility, thing that we have also checked with other compounds. The results were in complete agreement with those described in literature.

1.2.1 The disc diffusion method

The screening of antibacterial activity of the crude *L.inermis* extracts was carried out with agar disc diffusion method using Mueller Hinton agar medium. The bacteria inoculum was prepared by suspending colonies from 24 hr culture in 9 ml of sterile distilled water saline. The cell density of each inoculum was adjusted with a spectrophotometer (DO=0.08-0.1/ λ = 625nm) in order to obtain a final concentration of approximately 10^8 CFU (0.5 McFarland standard). In this method, the inoculum of 10^8 CFU is spread over the surface of a Mueller-Hinton agar, 3-4 mm of thickness. After drying (no more than 15 minutes), Wattman paper discs N°3 of 6mm diameter and containing 10µl of crude *L.inermis* extracts dissolved in100% of DMSO were applied in the plate. The activity is determined by the measurement of the inhibitory zone diameter in mm after incubation at 37°C for 24hr.

The screening of antifungal activity was carried out with the same way as bacteria except that the culture medium used is Mueller Hinton + 2% glucose + 0.5µg/ml methylene blue/ pH 7.4. The cell density of the inoculum was adjusted with a spectrophotometer (DO=0.12-0.15/ λ= 530 nm).The reading is realized as described for the bacteria.

Ciprofloxacin, Gentamycin, and Amphotericine B were used as reference antimicrobial. Pure solvent was used as negative control. The antimicrobial activity is considered starting from a diameter of 6 mm or higher, and is classified as follows:

Very Strong activity: diameter ≥ 30 mm; Strong activity: diameter between 21-29 mm; medium activity: diameter between 16-20 mm; Weak activity: diameter between 11-15 mm; Small or no activity: diameter ≤ 10 mm.

1.2.2 Broth Microdilution method:

A serie of dilutions of the crude plant extracts dissolved in DMSO (up to 5% final DMSO concentration) were prepared in a 96 well sterile microplat. To each well we introduced 100µl of the dilution extract to be tested. Subsequently, 100µL of an inoculum containing 10^6 CFU was added to each well. The well used as a negative control was prepared using the inoculum alone. Ciprofloxacin and Gentamycin were used as antibacterial reference whereas Amphotericin B was used as antifungal reference. The plate was incubated at 37°C for 24hr. Minimal Inhibitory Concentration (MIC) was considered as the weakest concentration to which no turbidity was eye-observed.

RESULTS AND DISCUSSION

Emergence of multi-drug resistance in human and animal pathogenic microbes as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin.

The antimicrobial activity of the leaves extracts *L.inermis* and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter respectively as given in Table 1. The effects against gram positives bacteria were more pronounced than those against the gram negatives. The disc diffusion method showed that beside the positive controls and the commercial Lawsone, only DMSO, ethanol and ethyl acetate extracts showed a medium activity against the two gram (+) bacteria, *S. aureus* and *E. faecalis*. The yeast *C.albicans* was sensitive toward tow extracts: DSMO, ethanol. However, no antimicrobial activity was found in our study for the prepared aqueous, chloroform, and di-ethyl ether extracts.

It seems clearly that the active compounds are belonging to the lipophilic group rather than to the hydrophilic one. According to Cowan [21], coumarins, flavonoïds and tannins compounds are not responsible of antimicrobial activity of *L.inermis* extracts. Starting from the composition of the ethanolic fraction, we can suggest that the mainly activity is due to polyphenolic compounds (naphtoquinones derivatives). Lawsone, the major bioactive constituent in *L.inermis*, is known for its antibacterial activity [7, 13,21] and possesses a wider spectrum of activity, as shown in Table 1.

Table 1. Screening of the antibacterial activity of different *L.inermis* extracts using the disc diffusion method

Extracts	Inhibition zone diameter (mm)				
	P. aeruginosa	*E. coli*	*S. aureus*	*E. faecalis*	*C.albicans*
Commercial Lawsone	06,0 ±0 [a]	20,5 ± 0,7	16,8± 0,7	13,5± 0,7	08,0 ± 0 [b]
DMSO extract	08,0 ±0,8	06,0 ± 0[a]	16,0 ±1,4	21,6 ± 1,3	11,7 ± 0,8
Cold aqueous extract	06,0 ±0 [a]	06,0 ±0[a]	06,0 ±0 [a]	06,0 ± 0 [a]	06,0 ± 0 [a]
Hot aqueous extract	06,0 ±0 [a]	06,0 ±0[a]	06,0 ±0 [a]	06,0 ± 0 [a]	06,0 ± 0 [a]
Chloroform extract	06,0 ±0 [a]	06,5 ±0,8	07,0 ±1,4	07,0 ± 0 [a]	06,0 ± 0 [a]
Ethanol extract	08,0 ±0,4	08,8 ±0,8	17,0 ±0,9	16,0 ± 0 [a]	10,0 ± 0,5
Ethyl acetate extract	06,0 ±0 [a]	06,0 ±0[a]	16,1 ± 0,6	19,1 ± 0,6	06,0 ± 0 [a]
Di-ethyl ether extract	06,0 ±0 [a]	06,0 ±0[a]	09,5 ±0,7	06,0 ± 0[a]	06,0 ± 0 [a]
Ciprofloxacin(5 x 10^{-3} mg/disc)	31,5 ± 0,7	33,6 ± 1,7	28,8± 0,4	30,0 ± 0	-
Gentamycin(15 x 10^{-3} mg/disc)	20,5± 0.50	24,0 ± 00	25,5 ± 0.21	28,0 ± 1,1	-
AmphotericinB(200 x 10^{-3} mg/disc)	-	-	-	-	22,20

[a] : no inhibitory zone was observed / [b] : Lawsone at 200 x 10^{-3} mg/disc

It has already been reported that the Agar and broth tests could show considerable discrepancies. On this basis, we screened all compounds for MIC on liquid medium (Table 2).

The data in Table 2 of the antibacterial screening showed a similar degree of activity between the different crud extracts against the microbial strains. Two extracts: DMSO and ethanol showed interesting MIC against all strains used. Ethyl acetate extract showed activity only against the bacterial strains. The extracts: di-ethyl ether, chloroform, cold aqueous and hot aqueous, did not show any activity against all the strains and they are probably completely inactive against the stains used in the test.

The Lawsone's MIC does not seem to be very distant from those obtained with the DMSO, ethanol and ethyl acetate extracts. Knowing that the rate of Lawsone in Henna leaves is 0.5–1.4% [5], we can suggest that the antimicrobial activity Henna leaves is largely influenced by the action of Lawsone. However, Lawsone is not the only compound which is responsible for the totality of this activity, at least in the free form, knowing that Lawsone is an heteroside form in the leaves [22].

These findings correlate with the study carried out by Malekzadeh and Shabestari [23], the aqueous extract of *L.inermis* leaves was find to be active *in vitro* against the following strains: *Bacillus anthracis*, *B.cereus*, *B.subtilis*, *Enterobacter aerugenes*, *E.coli* O128-B7, *E.coli* O55-B5, *E coli* O127-B8, *Proteus mirabilis*, *P.vulgaris*, *Pseudomonas aeruginosa*, *S. paratyphi*, *Sarcina*, *Sarcina*, *Shigella dysenteriae* type 1, *S. dysenteriae* type 7, *S. dysenteriae* type 9, *S.aureus hemolyticus*, *S. citreus* and *S. epidermidis*. According to the same authors, the antimicrobial activity shown by the crud extracts returns to the presence of Lawsone compound. However, according to Abd-el-Malek *et al*.,[24], the ethanolic extract of henna leaves has an antibacterial effect against a broad range of bacterial strains such as: Staphylococcus, Streptococcus, Brucella and Salmonella, whereas the strains *P.aeruginosa* resists strongly to this extract. This result was confirmed by a recent study carried out by Ahmad and Begs [25], with 45 plants of Indian origin where the alcoholic extract of *L.inermis* leaves showed antimicrobial activity with broad spectrum against the multi-drug resistant strain: *S.aureus, B.subtilis, E.coli, S.paratyphi, S.dysenteriae and C.albicans.* This activity was regarded as being interesting.

Table 2. Minimal Inhibitory Concentrations (MIC) of *L.inermis* extracts.

Compound	MIC (mg/ml)				
	P. aeruginosa	*E. coli*	*S. aureus*	*E. faecalis*	*C.albicans*
Commercial Lawsone	2,05	1,02	0,51	2,05	0,51
DMSO extract	4,69	3,12	3,12	3,12	7,81
Cold aqueous extract	- [b]	- [b]	- [b]	- [b]	- [b]
Hot aqueous extract	- [b]	- [b]	- [b]	- [b]	- [b]
Chloroform extract	- [b]	- [b]	- [b]	- [b]	- [b]
Ethanol extract	3,45	2,30	2,30	2,30	11,53
Ethyl Acetate extract	3,50	2,62	2,62	2,62	- [b]
Di-ethyl Ether extract	- [b]	- [b]	- [b]	- [b]	- [b]
Ciprofloxacin	2.10^{-3}	$0,5.\ 10^{-3}$	$0,25.10^{-3}$	8.10^{-3}	
Gentamycin	$0,5.10^{-3}$	$0,008.10^{-3}$	$0,25.10^{-3}$	$0,5.10^{-3}$	
Amphotericin B	-	-	-	-	0,008

[b]*: non MIC was observed*

Some authors report the origin of *L.inermis* leaves antibacterial activity, against *S.aureus* strain, to the gallic acid [21], while others join this activity to naphtoquinones compounds (Lawsone) against *Mycobacterium tuberculosis* [12]. From studies quoted in the bibliography, we strongly think to the significant Lawsone role in the antibacterial activity of the *L.inermis* leaves.

Finally, our antimicrobial screenings come to justify the traditional uses of these plants in various application including infectious diseases. Study of the synergistic interaction of active phytocompounds with antibiotics can be researched to exploit the plant extracts potential in the combination therapy of infectious diseases caused by multi drug-resistant organisms.

CONCLUSION

Henna has been used since earliest times as a medicine, preservative and cosmetic. It has long been recommended in traditional Eastern medicine as an astringent, purgative and abortifacient [26]. In This work, Algerian *L.inermis* leaves were assessed for their antimicrobial activity by realizing different extractions based on polarity difference. The active compounds, founded to be belonging to the lipophilic group, showed a very interesting data against bacterial strains. Our results come to reinforce the work completed on this plant and extend the fact that the Algerian *L.inermis* plants have an antibacterial capacity that can be exploited. Lawsone appears to be responsible of the antimicrobial activity showed.

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REFERENCES

[1] T. Blanks, S. Brown, B. Cosgrave, J. Woody, V. Bentley, N. O'Sullivan and N. Graydon, *Ebury Press London*, **1998**, 173.
[2] A. Singh and D.K. Singh, *Indian J. Exp. Biol*., **2001**, 39, 263.
[3] V. Natarajan, P.V. Venugopal and T. Menon, *Indian J. Med. Microbiol*., **2003**, 21, 98.
[4] S.G.H. Bonjar, *Asian J. Plant Sci*., **2004**, 3, 310.
[5] H.S. Muhammad and S. Muhammad, *African Journal of Biotechnology*, **2005**, 4, 934.
[6] A.F. Chadwick and L.E. Craker. Herbs: An Indexed Bibliography. 1971-1980. The Scientific Literature on Selected Herbs, and Aromatic and Medicinal Plants of the Temperate Zone. Archon Books, **1984**, 770 pp., Hamden, CT.
[7] N.B. Shivananda, G. Isitor, E.M. Davis and G.K. Pillai, *Phytotherapy Research.*, **2007**, 21, 827.
[8] R.M. Botros, A.B. Farid, T.M. Galal and M.A.A. Mohamed, *Z. Naturforsch*., **2004**, 59c, 468.
[9] A.T. Bakkali1, M. Jaziri, A. Foriers, Y. Vander Heyden, M. Vanhaelen, and J. Homes, *Plant Cell, Tissue and Organ Culture,* **1997**, 51, 83.
[10] V.K. Sharma, *Tubercle,* **1990**, 71, 293.
[11] H.H. Kandil, M.M. Al-Ghanem, M.A. Sarwat and F.S., Al-Thallab, *AnnTrop Paediatr.*, **1996**, 287.
[12] S. Ahmed, A. Rahman, A. Alam, M. Saleem, M. Athar and S. Sultana, *J. Ethnopharmacol*., **2000**, 69, 1574.
[13] B. Mostefa-Kara, C. Ziani-Cherif, M. Benabdallah, N. M. Rahmoun, D. Villemin, N. Choukchou-Braham and K. Boucherit, *Der Pharma Chemica*, **2010**, 2, 14.

[14] National Clinical Committee Laboratory Standards (NCCLS), *Performance Standards for Antimicrobial Disc Susceptibility Tests; Approved Standard-Eight Edition*, **2003**, 23. M2-A8.
[15] P. Cos, A.J. Vlietinck, D. Vanden Berghe and L. Maes, *Journal of Ethnopharmacol.*, **2006**, 106, 290.
[16] M. Nath, X. Song, G. Eng and A. Kumar, *Spectrochimica Acta Part A*, **2008**, 70, 766.
[17] R.K. Tiwari, D. Singh, J. Singh, V. Yadav, A.K. Pathak, R. Dabur, A.K. Chhillar, R. Singh, G. L. Sharma, R Chandraa and A.K. Verm, *Bioorganic & Medicinal Chemistry Letters*, **2006,** 16, 413.
[18] A. Epsinel-ingroff, *Clinical Microbiology Newsletter*, **2007**, 29, 97.
[19] M. A. Pfaller, L. Boyken, S. A. Messer, S. Tendolkar, R. J. Hollis and D. J. Diekema, *Journal of Clinical Microbiology*, **2004**, 42, 4977.
[20] National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, M7-A4. National Committee for Clinical Laboratory Standards, Wayne, PA, **2000**.
[21] M.M. Cowan, *Clin. Microbiol. Rew*., **1999**, Oct, 564.
[22] J. Bruton, Pharmacognosie Phytochimie : plantes médicinales. Technique et documentation–Lavoisier, **1993**.
[23] F. Malekzadeh and P.P. Shabestari, *Journal of Sciences, Islamic Republic of Iran*, **1989**,1 ,7.
[24] Y. Abd-el-Malek, M.A. El-Leithy, F. A. Reda and M. Khalil, *Landwirtschaftliche und Technische Mikrobiologie*, **1973,** 128, 61.
[25] I. Ahmad and, A.Z. Beg, *J. Ethnopharmacol*., **2001**,74, 113.
[26] A. Monem. Islamic Medicine,1bn al-Kaim Josia (1292-1350). International Office for Research, Beirut, **1983**, p. 87.