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Antifungal activity of *Lawsonia inermis* leaf extract against dermatophytes species

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Abstract

Plant extracts and plant-derived compounds are valuable sources as folk medicine for the treatment and prevention of a wide range of diseases including infectious diseases; Henna represent one of the most effective traditional remedy against multiple diseases that includes ulcer, skin disorders and infection malignancies. This study was carried out using 51 clinical isolates of dermatophytes representing three different species *Trichophyton mentagrophytes*; *Microsporum canis* and *Trichophyton eurinacei*, the antifungal activity of *Lawsonia inermis* was determined by agar diffusion and henna was used as ethanolic extract, henna extract showed the high antifungal activity against all dermatophytes species (17 to 60mm inhibition zone).

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Introduction

Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness (Kamal. M, 2010; Ushimaru, P. I *et al.*, 2007). Despite the progress of modern medicine, the therapeutic traditions still exist. In Africa, more than 80% of people keep using traditional medicine to eliminate diseases. This large-scale usage is due to the accessibility and the availability of traditional medicine in emergent countries, and to the expensiveness and secondary effects caused by synthetic medicines (Goswani M, *et al.*, 2011).

Traditional healers have long used plants to prevent or cure infectious diseases, Almost 50% of current pharmaceuticals are derived from the plant kingdom plants are rich in a wide variety of secondary metabolites polyphenols, such as tannins, terpenoids, alkaloids and flavonoids which have been demonstrated to have in vitro antimicrobial properties (Gonzalez-Lamothe R. *et al.*, 2009).

Lawsonia inermis is a tall flowering shrub or tree about 5m in height, native to tropical and Subtropical regions of Africa, Southern Asia and Northern Australia in semi arid zone and oases in the Sahara (Singh YV, Kumar Sand Singh M, 2005; Muhammad, H. S and S. Muhammad, 2005).

Henna leaves are used as a remedy in skin diseases in the form of a paste or decoction against burns, bites and skin inflammation, leaves in the form of paste have been used as external application in headache and rubbed over the soles of the feet in burning feet (Bhuvanewari K *et al.*, 2002). A wide range of biological activities have been attributed to henna, including antifungal, antibacterial, virucidal, antiparasitic, anti-inflammatory, analgesic and anticancer properties, as well as hepatoprotective, immunomodulatory, anthelmintic, antitrypanosomal and antioxidant activities (Ruchi Badoni Semwal *et al.*, 2014). Dermatophytosis is a fungal infection caused by dermatophytes which are comprised of three genera, *Trichophyton*, *Microsporum* and *Epidermophyton*, capable of invading keratinized tissues of humans and animals (Kanbe T, *et al.*, 2003).

The dermatophytes cause lesions called dermatophytoses on the human being, these are the most frequent cutaneous mycoses. They appear in the skin (epidermis) and the dander (hair, fur, nails). Dermatophytoses take various clinical aspects which makes a mycological pre-levement and profound diagnostic very important (Chabasse D and Contet-Audonnet N, 2011). The study presented here is to enhance our knowledge about leaves of *Lawsonia inermis* in the form of the ethanolic extract against clinical dermatophyte species.

Material and methods

Sample collection

212 patients were subjected to a mycological pre-levement in the University Hospital center of Oran (CHUO) during 2015, the patients diagnosed with dermatomycosis were included in this study, the clinical specimens, including cutaneous skin seal, hair and nail scraping or scrapings were collected from all patients for mycological examination.

Laboratory procedures and identification

One part of each specimen was routinely subjected to direct microscopic examination for detection of fungal hyphae using potassium hydroxide (KOH 15%) solution, for culture examination, each specimen was cultured on Sabouraud gelose agar with chloramphenicol and other specific media for confirmation, all and incubated at 27°C for at least 03 weeks and checked twice weekly; Cultures were examined macroscopically for morphology, texture and color from the top and reverse sides of plate, then using a sterile straight loop, the colony was examined by placing a sample on a drop of lactophenol solution on a glass slide, the preparation was observed under the microscope for the presence of microconidia, macroconidia and other structures.

Plant material

Henna harvest has been made in Tmaskht kssar Tmaskhi (located in the wilaya of Adrar; Algeria) in April, 2015. The extraction has been made using fresh leaves of *Lawsonia inermis* L 25g each of the henna was suspended into 100ml of ethanol and extracted using rotavapor, the dried extracts were weighed and kept in the freezer until subjected to further analysis (Sharma V. K, 1990).

Antifungal activity

The antifungal activity of henna was determined by employing agar diffusion technique (Sharma KK *et al.*, 2011) for each isolate a suspension of mycelia from 9 days culture prepared in steril saline to a concentration of 10^6 cells/ml the suspensions were swabbed evenly on the surface of the petri plates containing solidified saboraud dextrose agar; 6mm diameter disks containing different concentration of the ethanolic extract of *Lawsonia inermis* have been place on a gelose surface and let one hour in ambient temperature for diffusion, petris boxes have then been incubated at 27°C during 3 to 7 days, the inhibition zone diameters around the disks have been measured and recorded. Miconazole and DMSO has been used as control.

Results and discussion

This study has been made on 51 patients on who hair and nail skin dermatomycose has been confirmed. There were 43.14% children, 39.21% women and

17.65% men from whom we have isolated three different spicies of dermatophyts (Fig. 1).



Fig. 1. spicies of dermatophyts *M. canis*; *T. mentagrophytes*; *T. eurinacei*.

The present study has studied the antifongic activity of the ethanolic extract of Henna on three dermatophyts by using the agare diffusion method, the inhibition zones present *Lawsonia inermis* extract's effect on dermatophyts species.

This effect is shown in the table1 and table 3 The Henna extract has shown a high antifongic activity on the studied dermatophyts strain the inhibition zones were (17 at 32mm) for the *Microsporium canis*; (19 at 56mm) for *Trichophytum eurinacei* and (17 at 60mm) for *Trichophytum mentagrophytes*

Table 1. Diameter of the inhibition zone of the ethanol extract.

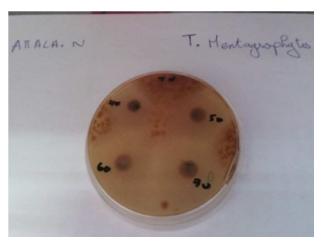
Dermatophytes Strains	Diameter of the zone* (mm) concentrations of the ethanol extract (%)										
	2	10	20	30	40	50	60	70	80	90	100
<i>M. canis</i>	17 ±0.32	19 ±0.29	20 ±0.35	22 ±0.38	30 ±0.20	30 ±0.19	32 ±0.22	32 ±0.23	32 ±0.25	32 ±0.18	32 ±0.15
<i>T. Mentagrophytes</i>	17 ±0.62	17 ±0.56	18 ±0.79	30 ±0.41	30 ±0.35	35 ±0.39	40 ±0.29	44 ±0.09	50 ±0.11	55 ±0.26	60 ±0.31
<i>T. eurinacei</i>	19 ±0.08	31 ±0.15	30 ±0.20	33 ±0.05	18 ±0.17	35 ±0.25	19 ±0.21	35 ±0.67	44 ±0.71	55 ±0.55	56 ±0.47

(*)Diameter of the inhibition zone produced around the disc by the addition of 15µl of extract, the values represent the average of 3 measurements ± SD.

Table 2. Diameter of the inhibition zone of the miconazol and DMSO.

Dermatophytes strains	Diameter of the inhibition zone (mm)	
	DMSO	MICONAZOL(500 µ g/l)
<i>Microsporium canis</i>	0	29 ±1.52
<i>Trichophytum mentagrophytes</i>	0	20±0.95
<i>Trichophytum eurinaci</i>	0	22±1.31

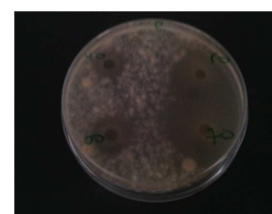
Table 3. sensitivity of the tested strains to ethanolic extract.



Sensitivity of *T. mentagrophytes* to ethanol extract



Sensitivity of *M. canis* to ethanolic extract



Sensitivity of *T. eurinacei* to ethanolic extract

In this study the ethanol extract showed strong antifungal activity against *M. canis*; *T. mentagrophytes* and *T. eurinacei* at all the concentrations. Mansour *et al* (Mansour-Djaalab H *et al* 2012) reported in their research that *L. inermis* leaves extract have developed a fungicidal effect against *T. mentagrophytes* and *C. albicans*.

Lawsonia inermis is a tropical and subtropical shrub; cultivated in the Middle East, along the African coast of the Mediterranean sea, artifacts that definitively prove henna use by humans can be dated to pre-dynastic Egypt, where it was used to mask the appearance of aging, greying hair and cosmetics (Raja W *et al.*, 2013). Henna extract showed a high antifungal activity against all dermatophytes species in this study. Henna is widely used throughout arabia including Algeria in addition to its use as a cosmetic henna leaves are also used as a cosmetic, henna leaves are also used for fevers as a local anesthetic, antiinflammatory and for treating mouth ulcers (Habbal O *et al.*, 2011) and tumor (Priya, R *et al.*, 2011).

Henna has been used to treat skin infection such as tinea and is known to have antimicrobial properties which have been attributed to naphthoquinones; including *Lawsonia* the major bioactive constituent in *Lawsonia inermis* (M. N. Rahmoun *et al*, 2010). The ethanol extract of the whole plant of *Lawsonia inermis* showed antifungal activity against *T. mentagrophytes*, *M. canis* and *T. eurinacei*. Our results indicate the presence of antifungal agents in the plant which were found effective in inhibiting the growth of *T. mentagrophytes*; *M. canis* and *T. eurinacei*. The results of this study confirm the great potential of *Lawsonia inermis* and are useful for rationalizing the use of medicinal plants in primary health care. Moreover, according to the previous results, all the concentration of our extract have proved an antimicrobial effect on all the strains tested with strong inhibiting power.

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