Original article

In vitro study of the effects of henna extracts (*Lawsonia inermis*) on *Malassezia* species

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Abstract

Introduction and objective: Today cutaneous fungal infections of man include a wide variety of disease. *Malassezia* are normal flora of skin and cause pityriasis versicolor and foliculities under suitable conditions. The aim of this study was to investigate the antifungal effects of chloroformic, methanolic and aqueous extracts of henna leaves on *Malassezia*.

Materials and methods: One hundred grams of dried and powdered henna leaves were extracted using distilled water, methanol and chloroform solvents, separately. The used solvents were removed under reduced pressure. The extracts with different concentrations were mixed mycobiotic agar and covered with a layer of olive oil. In each tube, skin scales of patients with pityriasis versicolor were inoculated. In order to study the inhibitory effects of each henna extracts, the culture tubes were kept at 37^{o}C for about 14 days and the growth of *Malassezia* colonies were checked every 3, 7 and 14 days after culture.

Results: Results have shown that chloroformic extract of henna at 3 and 4 (V/V%) completely inhibit the growth of *Malassezia*. Methanolic extract of henna at 0.25 and 3 (V/V%) inhibit the growth of *Malassezia*. Aqueous extract of henna at 0.25, 0.5, 4 (V/V%) completely inhibit the growth of *Malassezia*. Miconazole nitrate as standard antibiotic in almost all concentrations has completely inhibitory effect on *Malassezia.*

Conclusion: The results demonstrated that henna has antifungal activity against *Malassezia.* In addition aqueous extract is more effective on *Malassezia* than methanolic and chloroformic extracts.

Keywords: Henna extract, *Lawsonia inermis*, *Malassezia*, Susceptibility test

Introduction

Because of good geographical conditions, a wide variety of plants are grown in Iran. The majority of these are effective in the treatment of many different diseases. Nowadays adverse drug effects occur in people and clinicians using traditional medicine instead of chemical drugs. The natural products that are abundant throughout our country are used to cure patients. *Malassezia* species are normal flora of skin and cause pityriasis versicolor and foliculities under suitable conditions [1,2].

Henna has different names in all over the world. The plant is grey-white and has spines. The length of the leaves of this plant is 2-3cm. The chemical components of henna are not well- known now but it has a color agent (Lawson), with chemical formula ($C_{10}H_6O_3$), which is the most effective component of henna [3,4]. Henna has been reported to have many different healing effects, antibacterial effects specially for gram positive bacteria, anti tumoral effects in rat, antifungal activity against dermatophytes and wound healing [5,6].

During screening the barks of 30 plant species against *Microsporum gypseum* and *Trichophyton mentagrophytes*, only *Lawsonia inermis* exhibited absolute toxicity. The Lawsonia bark extract was found to possess fungistatic nature at its maximum inhibitory dilution of 1:30 (W/V) against both the test pathogens but, become fungicidal at 1:10 (W/V). The extract showed broad fungitoxic spectrum when tested against 13 ring worm fungi [6], but there is no evidence about antifungal effect against *Malassezia* in literature and in the internet, so we decided to study about this effect of henna. In this investigation we have studied the antifungal effects of chloroformic, methanolic and aqueous extracts of henna leaves (*Lawsonia inermis*) on *Malassezia* in mycobiotic agar.

Materials and methods

Producing extracts and mixing with media

In this research, at first, leaves of henna (*Lawsonia inermis*) were gathered and dried. The plant extracts were prepared as follows: Aqueous extract: 100g of the chopped, dried henna leaves were extracted with 500ml distilled water by the soxhlet apparatus (Yousef Namdar, Iran). Methanolic extract: The same amount of leaves was extracted with 500ml methanol by the soxhlet apparatus. Chloroformic extract: 100g of the chopped, dried henna leaves were extracted by perculation method (3). The solvents used for obtaining these extracts were then removed under reduced pressure. Then extracts were mixed with mycobiotic agar (Merck, Germany) covered with a layer of olive oil with different concentrations (0.25, 0.5, 1, 2, 3 and 4 V/V%) and placed in culture tubes.

Methods for culture of fungi

In each tube, skin scales of patients with pityriasis versicolor were inoculated. In order to study the inhibitory effects of each henna extracts the culture tubes were kept at (37^oC) for about 14 days and the growth of *Malassezia* colonies were checked every 3, 7 and 14 days after culture.

Positive and negative control

We used miconazole nitrate as a standard antibiotic for positive control for evaluation of fungistatic effects of herbal extracts. For negative control, we used tubes containing mycobiotic agar with olive oil mixed with water, chloroform and methanol, separately. Each different concentration of henna extracts was cultured twice. Further, the fungistatic of extracts was unaltered at high temperature, on autoclaving.

Results and discussion

In this investigation we used henna leaves extracts against *Malassezia* which is a superficial fungi flora that can be pathogen in some conditions. *Malassezia* have been shown to be a common endogenous saprophyte of the normal skin. The disease commonly known as dandruff is caused by numerous host factors in conjunctions with the normal flora yeast [1,4,7]. The prevalence of disease is varying in the world with a rate of 5-50% [8].

Henna (*Lawsonia inermis*) from the family of Lythraceae that is a dwarf shrub native to Africa, southern Asia, and northern Australasia and well known worldwide for cosmetic use of the coloring material that is present in the leaves. The plant has been shown to have strong fungicidal as well as anti-inflammatory, analgesic, antibacterial, virucidal, antiparasitic, anticancer and possible anti-sweating properties. The chemical constituents of this plant include: naphthalene derivatives, quinoids, β-sitosterol glycoside, xanthones, flavonoids, galic acid, coumarins, and lawsoniasides. Lawsone, 2-hydroxy-1,4-naphthoquinone is responsible for henna's fungicidal activity [9]. Antipyretic and analgesic effects of henna in rats have also been reported [10].

The effectiveness of henna extracts is shown in figure 1 and table 1. Aqueous extracts are more effective on *Malassezia* than methanolic and chloroformic extracts. Miconazole nitrate as standard antibiotic in almost all concentrations has completely inhibitory effect on *Malassezia* (Table 2). These findings demonstrate that these extracts have antifungal activity *in vitro* against the yeast which causes pityriasis versicolor, pityrosporum foliculities and dandruff. Because of the side effects of chemical agents and some fungi resistance to them, herbal healer can be a proper substitution. In some instances they are cheaper and more efficient.

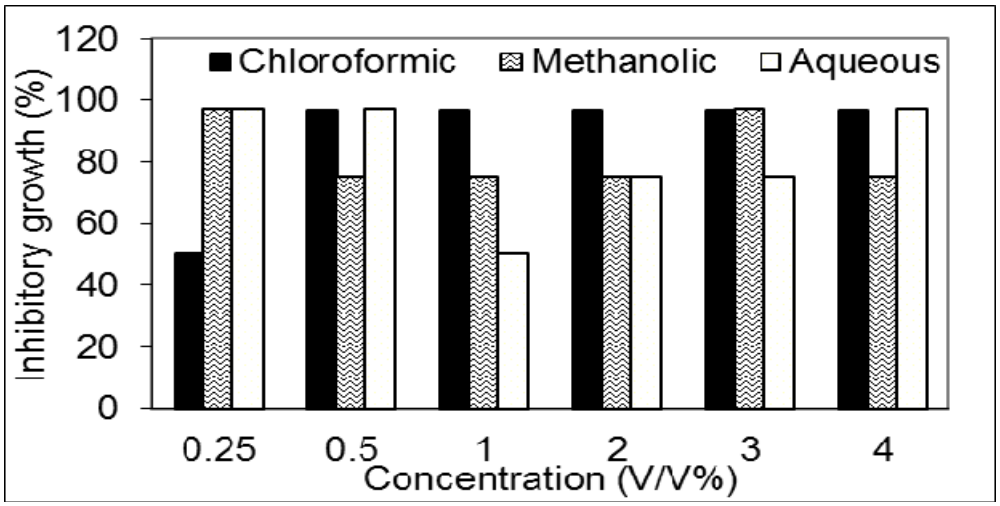

Fig. 1: Antifungal (*Malassezia*) effects of different concentrations of chloroformic, methanolic and aqueous extract of henna

The results in this study revealed that henna extracts may possess some compounds that have antifungal activity on *Malassezia*. However this current study does not evaluate clinical activity of these extracts. In clinical settings, many other factors are involved, and controlled comparative clinical studies would have to be done to

show that these herbal extracts are indeed effective. As we develop into a more scientific world, we must use natural healers and products and replace these with scientific and synthetic medicines. The authors believe that many chemical drugs can be replaced with natural healers and herbal products.

Table 1: Antifungal effects of different concentrations of chloroformic, methanolic, aqueous extract of henna on *Malassezia*

Inhibitory growth rate henna extracts %V/V	0.25	0.5	1	2	3	4
Chloroformic	++	++++	++++	++++	++++	++++
Methanolic	++++	+++	+++	+++	++++	+++
Aqueous	++++	++++	++	+++	+++	++++

Table 2: Antifungal effects of different concentrations of miconazole nitrate

Miconazole Nitrate %V/V	1	2	3	6	8
Inhibitory growth rate	++++	++++	++++	++++	+++

Conclusion

The results demonstrated that henna has antifungal activity against *Malassezia* and aqueous extract is more effective on *Malassezia* than methanolic and chloroformic extracts.

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