Comparative study between effect of *Lawsonia inermis, Punica granatum* and miconazol nitrate on *Trichophyton rubrum*

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ABSTRACT: Aims of this study to determine the effects of antifungal components from natural products on *Trichophyton rubrum*. *Lawsonia inermis, Punica granatum* extracts were evaluate and compare it's active with miconazole nitrate against *Trichophyton rubrum*. The results revealed by the method of diffusion in solid medium, the water extracts of *Punica granatum* and *Lawsonia inermis* were able to inhibit the growth of *Trichophyton rubrum* especially at concentration 10 mgl $^{-1}$ in same act of miconazole nitrate. *Punica granatum* and *Lawsonia inermis* extracts have antifungal activities against *Trichophyton rubrum* which referred to phenolic compounds. The chemical compound in *Punica granatum* and *Lawsonia inermis* play main role in inhibition growth of *Trichophyton rubrum*. So the aqueous extracts of two plants have same effect of miconazole nitrate, those plant can be consider as antifungal agent which will be useful in development of fungicidal medicine against dermatomycoses from natural and without any side effect.

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KEY WORD: *Lawsonia inermis* - *Punica granatum* – *Trichophyton rubrum*- miconazole nitrate - dermatophyte

INTRODUCTION

Dermatophytes are the most important microorganisms that cause superficial mycoses. Fungi such as Trichophyton rubrun reported as the most common type of human infection. Amphotericin B and nystatin and natamycin, miconazole and other azole drugs such as intraconazol were use for therapeutic for treatment superficial mycoses. (Kingsbury *et al.* 2012). However, people needs natural antifungal agents because the toxic side effects of these medicine, may interact with other drugs, and become ineffective as a result of development of microbial drug resistance.

(Shin and Lim 2004; Martunez-Rossi *et al*.2008;Welsh *et al.* 2010; Kingsbury *et al*. 2012. *Lawsonia inermis* (Henna) and *Punica granatum* (Pomegranate) were shown to have medicinal properties. *Lawsonia inermis* characterized by its multiple uses as apoultice against eczema antibacterial, antifungal, anti-inflammatory activities. (Hseini and Kahouadji 2007; Panda and Ray 2012). *Punica granatum* peel is traditionally used to treat genital infections, allergic dermatities, tympanitis, scalds and also as an antioxidant(Lansky and Newman 2007; Elmanama *et al.* 2011).

The objective of this study are to evaluate the antifungal activity of *Lawsonia inemis*, *Punica granatum* on *Trichophyton rubrum* which will be very much useful in the development of new fungicidal drugs against dermatomycoses on low cost with out producing any side effects in patients.

MATERIALS AND METHODS

Plant material and preparation of the Extract

The plants used were fresh leaves of *Lawsonia inemis* and peel of *Punica granatum* collected and dried in the oven at a temperature of 60^{o}C. The dried leaves and peel were ground to a powder. Each sample was weighed into 3 portions: 2, 5 and 10 g and were put into different 250 ml conical flasks. One hundred milliliters of sterile distilled water were used for extraction (2, 5 and 10 mgl^{-1}) and were shaken on a shaker for 3 h. The micronazole nitrate 20 mg /g is the medicine was also dissolved in water at the same concentration as above. (Adejumo and Bamidele 2009).

Microorganism and Media

The fungi of *Trichophyton rubrum* were obtain from microbiology laboratory of the government hospital. The fungi were maintained on Sabouraud Dextrose Agar (SDA) slants at 4^{o}C until required.

Antifungal test

This test was to determine the radial growth inhibition of *Trichophyton rubrum* by the plant extracts compare with miconazole nitrate medicine. Two milliliters of each extract was incorporated into 15 ml of molton SDA inside a petri dish.Three plates were used for each treatment. The media were swirted and allow to solidify. Two week old *Trichophyton rubrum* were inoculated at 30^{o}C for 15 days.The measurements were then taken again using linear measurement method.

RESULTS

The results of the effects of *Lawsonia inermis* and *Punica granatum* extracts against *Trichophyton rubrum* are presented in table (1 and 2). For extracts at 2 mgl^{-1} *Lawsonia inermis* and *P. granatum* were not effected on *Trichophyton rubrum.* At 5 mgl^{-1} there was inhibition on the growth of *T. rubrum* and its more active in strains no. 2 than strain no.1.

Table 1 The effect of aqueous plant extracts on *Trichophyton rubrum* (strain 1)

	Trichophyton rubrum		
Concentration (mgl^{-1})	2	5	10
Lawsonia inermis	85	20	17
Punica granatum	85	15	10
Miconazole nitrate	0	0	0
Control	85	85	85

There was no growth of *T. rubrum* at concentration 10mgl^{-1} on plates of strain 2, but there was little growth on plates of strain. No. 1.

Table 2. The effect of aqueous plant extracts on *Trichophyton rubrum* (strain 2)

	Trichohyton rubrum		
Concentration (mgl^{-1})	2	5	10
Lawsonia inermis	85	5	0
Punica granatum	85	0	0
Miconazole nitrate	0	0	0
Control	85	85	85

Plant extracts showed considerable antifungal activity especially on concentration 10 mgl^{-1} of *P. granatum*. The growth of Trichophyton were inhibition by the *P. granatum* more than *L. inermis* extracts. Also miconzole nitrate showed inhibition the growth of *Trichophyton rubrum* at all concentration 2,5 and 10 mgl^{-1}.

DISCUSSION

The main objective of the present study was to evaluate the ability of extracts from two plants to inhibit the growth of *Trichophytor rubrum.* The aim is to explore possible future use of these extracts as alternatives to common antibiotics. The present study showed that henna extracts (*L. inermis*) were capable of inhibiting the growth of *Trichophyton rubrum* especially when concentration were increased. Phytochemical analysis of this plant revealed the predominance of phenoilic compounds (coumarins, flavonoids, and naphthalene derivative of gallic acid) (Siddiqui *et al*. 2003). Other compounds such as triterpenoids, steroids and aliphatic carbohydrates have also been isolated (siddiqui and kardar 2001). (Abulyazid *et al.* 2010) reported the richness of the plant by naphthoquinones which the main components are the Juglone, lawsone and plumbagone. Also, our results showed that the pomegranate extracts *Punica grunatum* were capable of inhibiting the growth of *Trichophyton rubrum* and its be more effective than *Lawsonia inermis* extracts, especially at 5 and 10 mgl^{-1}. The constituents of *P. grunatum* include gallocatechins, delphinidin, cyanidin, gallic acid, ellagic acid, Pelargonidin and sitosterol, which are very well known for their therapeutic properties (Sigh *et al.* 2002; El Manama *et al.* 2011).Furthermore,(Elfalleh *et al*.2012) reported that polyphenols are highest in peel than any part of pomegranate and ther was positive correlated with increasing concentration. Many reports cite the inhibitoy activity of *L. inermis* and *P. granatum* extracts against *Trichophyton* sp. (Shin and Lim2004; Muhammad and Muhammad 2005; Yasoubi *et al*.2007; Adejumo and Bamidele 2009; Hafidah *et al*.2010; Mansour-Djaalab *et al*.2012).

In this study, *P. granatum* and *L. inermis* extracts showed inhibitory activity on *T. rubrum*. (Rahmoun *et al*.2012,2013) who worked on the *L. inermis* compound reported that the prospective antimicrobial activity of the quinonoid compounds isolated from natural sources has mostly remained unexplored. The antimicrobial activities of *L. inermis* would indicate the probable involvement of lawsone, i.e.z.. hydroxynaphthoquinone, which is known to be the main bioactive constituent of this herb, in the antimicrobial activity. (Habbal *et al*. 2005) reported that the potential range of quinone anti microbial effects is great. Portable targets in the microbial cell are surface exposed adhesions, cell wall polypeptides and membrane bound enzymes Quinones may also render substrates unavailable to the microorganisms. Mode of action for flavonoids are: lysis and leakage of intracellular constituents, Perturbation of cell homeostasis, inhibition of enzymes, electron transport, oxidative phosphorylation, interaction with macro molecules and effects on macromolecular biosynthetic processes (Lambert 2008).

In conclusion *L. inermis* and *P. granatum* extracts have anti fungal activities against *T. rubrum* compare with active of dermatophyte medicine. The use of plants extracts in the treatment of dermatomycoses will help to reduce the dependence on the use of microbial or chemically synthesized antimicrobials, and thus overcome anti fungal chemical on various etiological agents of dermatophyte infections.

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