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Antifungal Activity of Henna Leaves *(Lawsonia inermis L.)* against *Trichophyton rubrum* and *Trichophyton interdigitale*

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ABSTRACT

Onychomycosis is a term used to describe fungal infection of the nails caused by some dermatophytes. The present study was designed to assess the antifungal activity of henna leaves *(Lawsonia inermis L)* extracts (n-hexane and petroleum ether) against two species of dermatophytes: *Trichophyton rubrum* and *Trichophyton interdigitale* using the Agar Radial Diffusion and Minimum Inhibitory Concentration techniques respectively. Results obtained revealed that n-hexane extract have no antifungal effect against the test organisms. However, crude extract of petroleum ether inhibited growth of both dermatophytes at concentrations of 12.5mg/ml - 200mg/ml with the largest inhibition zone of 81±1.4mm against *T. rubrum.* MIC values of both extracts were 50mg/ml against *T. rubrum.* The antifungal drug Itraconazole exhibited the largest inhibition zone of 71±1.8mm against *T. interdigitale* at 25mg/ml concentration. Phytochemical screening of the extracts revealed the presence of carbohydrates, saponins and alkaloids. This study concludes that, henna extract possesses antifungal activity and thus can be used to combat the problem of drug resistance.

Keywords: *Lawsonia inermis, Antifungal, Trichophyton rubrum, Trichophyton interdigitale, Itraconazole*.

INTRODUCTION

Onychomycosis is a term used to describe fungal infections of the fingernails or toenails (Ameen et al., 2014). Despite the geographical and racial variation in the aetiological agents of onychomycosis, an estimated 85–90% of nail infections are reported to be due to dermatophytes (Tchernev et al., 2012; Kenneth et al., 2016). Among dermatophytes, the most often isolated causative pathogens of onychomycosis are Trichophyton rubrum, Trichophyton interdigitale (formerly T. mentagrophytes), Epidermophyton floccosum and Trychophyton tonsurans (Tchernev et al., 2012; Piraccini and Alessandrini, 2015).

The condition is worldwide in distribution and problems associated with onychomycosis include difficulty in wearing footwear and walking, cosmetic embarrassment and lowered self-esteem (Shenoy and Shenoy, 2014). Some of the contributing factors causing this disease are occlusive footwear, genetic predisposition and concurrent diseases, such as diabetes and HIV infection, as well as other forms of immunosuppression (Ameen et al., 2014). It is a common misconception among physicians that as onychomycosis is a cosmetic problem it need not be treated (Kenneth et al., 2016).

Onychomycosis can result in disruption of integrity of the skin, providing an entry point for bacteria leading to the development of foot ulcers, osteomyelitis, cellulitis and gangrene in diabetic patients (Ameen et al., 2014; Kenneth et al., 2016). Furthermore, fungal diseases are contagious and may spread to other family members if not treated (Tchernev et al., 2012). However, the rate of treatment failure with standard antifungal drugs is on the rise due to poor patient compliance, low

bioavailability, lack of drug penetration into the nail, drug resistance and drug interactions (Ameen et al., 2014; Piraccini and Alessandrini, 2015).

According to World Health Organisation (WHO), medicinal plants could be important resources for obtaining new antimicrobial drugs (Khan et al., 2016). Today, it is estimated that about 80% of people in developing countries rely on traditional medicines for their primary health care (Sharma and Goel, 2018). Traditional medicines are becoming popular, due to high toxicity and adverse effects of orthodox medicament (Osman et al., 2012; Otang and Afolayan, 2016).

The name henna, refers to the dye prepared from the leaves of Lawsonia inermis L. (family Lythraceae) (Wagini et al., 2014; Rathi et al., 2017). This species has been used as medicinal plant since ancient times to cure numerous diseases but mainly as dye material (Rao et al., 2016). Recent studies showed that the leaves of this plant have anti-inflammatory, antipyretic, analgesic, antifungal and antibacterial activity (Nawasrah et al., 2016).

Hence, this study involved antifungal testing of henna plant extracts against Trichophyton rubrum and Trichophyton interdigitale in comparison with conventional antifungal drug (Itraconazole).

2. MATERIALS AND METHODS

2.1 Collection of plant material and authentication: L. inermis Linn. plant leaves were obtained from Adaya village under Fune local Government Area of Yobe State and authenticated by a botanist at the department of Biological Sciences, Yobe State University. The leaves were washed thoroughly with tap water, shade dried and grinded using mortar and pistil. The powder was then stored in an air tight container until further use.

2.2 Fungal strains: Trichophyton rubrum and Trichophyton. interdigitale (formerly Trichophyton. mentagrophytes) derived from ATCC 28188 catalog number SKU: 0444K and ATCC 9533 catalog number SKU: 0442K respectively were purchased from Microbiologics Inc. through 108616 VSR INT LTD Distributor. The organisms were cultured on Sabouraud Dextrose Emmons Agar supplemented with lactic acid and incubated aerobically for 6 days at 25°C.

2.3 Inoculum Preparation: a loop full of the test organisms were inoculated in Sabouraud Dextrose Emmons Broth supplemented with lactic acid and incubated for 48hours at 25°C.

2.4 Extraction method (Maceration): Sterile bottles were labelled with the name of each solvent to which 100grams of henna powder was added. 500mls of n-hexane and petroleum ether were added to respective bottles to make 1:5% w/v of the extract. All the bottles were then kept on a universal shaker (model HS-501 digital Werke Company) for 24hours. Both extracts were then filtered using Whatman no. 1 (24cm) filter papers and solvents were recovered in rotary evaporator.

2.5 Preparation of extracts: 1g of each extract was weighed, transferred into respective containers and dissolved in 5ml of 1% DMSO in the solvent of extraction and 1000mg/ml of itraconazole was dissolved in 5ml distilled water. Thus we have 200mg/ml of all the stock solution. 7-folds serial dilutions were set up with the least concentration of 3.125mg/ml.

2.6 In vitro sensitivity tests: the antifungal activity of henna extracts was assessed in comparison with the antifungal drug itraconazole. All experiments were prepared in triplicates and the results shown represent the mean.

2.6.1 Agar Radial Diffusion: The well diffusion method was carried out as described by (Kannahi and Vinotha, 2013;

Rao et al., 2016) with slight modification as stated below:

Nutrient agar plates were prepared and allowed to solidify at room temperature. A loop full of the broth culture was picked and emulsified with distilled water. Using micropipettes, a drop of this was then aseptically transferred unto each nutrient agar plates and spread using a spreader. The plates were then allowed to sit for 30 minutes so as to suck up the inoculum. Afterwards, 4 wells were made in the plates using a sterile cork borer of 10mm diameter and labelled. The wells were filled with respective extracts using a micropipette and plates were then incubated at 25^0C for 48 hours and the diameter of the zones of inhibition measured in millimetre.

2.6.2 Minimum Inhibitory Concentration (MIC): The MIC values of n-hexane extract, petroleum ether extract and the reference drug (Itraconazole) were determined for the selected test organisms using tube dilution assay.

This was done by adding 2ml of TSB into seven test-tubes. 2ml of extract was added to the first test tube and mixed on a whirl mixer. 2ml of the contents from tube one were removed and aseptically added to tube two followed by mixing. The process was repeated up to tube six but no plant extract was added to tube seven as it was kept as a positive growth control. All seven test tubes were then inoculated with a drop of overnight culture diluted in distilled water. Another set of tubes were prepared in the same way but no culture added to them as negative control. The MICs were regarded as the lowest concentration that inhibited visible growth of the test organisms after incubation at 25^0 C for 48 hours in comparison with the controls.

2.7 Phytochemical screening: In order to determine the bioactive compounds of henna extracts, phytochemical screening was carried out as described by (Tiwari et al., 2011).

2.8 Statistical analysis

The results are expressed as mean ± standard deviation (SD) for the measurements of inhibition zones in antifungal activity tests.

3. RESULTS

Petroleum ether henna extract exhibited antifungal activity against T. rubrum (table 1). Zones of inhibition were observed around wells containing 12.5mg/ml that increased with increasing concentration.

Table 1: Agar radial diffusion results of henna leaves extracts against Trichophyton rubrum after 48 hours of incubation at 25^0C.

Organism	Concentrations	Extracts/ Zones of inhibition in mm ±SD	
		n-hexane	Petroleum ether
T. rubrum			
	3.125mg/ml	N.I	N.I
	6.25mg/ml	N.I	N.I
	12.5mg/ml	N.I	41±0.9
	25mg/ml	N.I	4.9±1.8
	50mg/ml	N.I	63±1.4
	100mg/ml	N.I	74±0.9
	200mg/ml	N.I	81±1.4

NI: no inhibition; n=3.

Both n-hexane and petroleum ether extract showed little or no antifungal activity against T. interdigitale (table 2).

Table 2: Agar radial diffusion results of henna leaves extracts against Trichophyton interdigitale after 48 hours of incubation at 25^0C.

Organism	Concentrations	Extracts/ Zones of inhibition in mm ±SD	
		n-hexane	Petroleum ether
T. interdigitale			
	3.125mg/ml	N.I	N.I
	6.25mg/ml	N.I	N.I
	12.5mg/ml	N.I	N.I
	25mg/ml	N.I	N.I
	50mg/ml	N.I	N.I
	100mg/ml	N.I	51±1.4
	200mg/ml	N.I	64±1.2

NI: no inhibition; n=3.

The reference drug itraconazole exhibited antifungal activity against both organisms at all concentrations (table 3). Interestingly, the largest zone of inhibition of 71±1.8mm was observed against T. interdigitale at the concentration of 25mg/ml.

Table 3: Agar radial diffusion results of itraconazole against Trichophyton rubrum and Trichophyton interdigitale after 48 hours of incubation at 25^{0}C.

Concentrations	Zones of inhibition in mm ±Standard Deviation	
	Trichophyton. Rubrum	Trichophyton. interdigitale
3.125mg/ml	32±0.9	38±0.9
6.25mg/ml	37±0.5	42±0.8
12.5mg/ml	44±0.9	47±0.9
25mg/ml	50±0.5	71±1.8
50mg/ml	58±1.2	54±0.8
100mg/ml	62±0.9	57±0.8
200mg/ml	67±0.5	67±2.1

N=3.

For petroleum ether extract, T. interdigitale seems to be more sensitive with MIC value of 12.5mg/ml. Petroleum ether and n-hexane have the same MIC values of 50mg/ml against T. rubrum. However, Itraconazole showed the lowest MIC value of less than 3.125mg/ml for both test organisms.

Table 4: Minimum Inhibitory Concentration (MIC) results of henna extracts and itraconazole against Trichophyton rubrum and Trichophyton interdigitale after 48 hours of incubation at 25^{0}C.

Organisms	Extracts/ MIC (mg/ml)		
	n-hexane	Petroleum ether	Itraconazole
Trichophyton rubrum	50	50	< 3.125
Trichophyton interdigitale	100	12.5	< 3.125

The results of phytochemical screening carried out on both n- hexane and petroleum ether henna extracts is shown in table 5.

Table 5: Phytochemical screening of n-hexane and petroleum ether henna extracts

Phytochemicals	n-hexane	Petroleum ether
Alkaloids	+	+
Carbohydrates	+	+
Glycosides	-	-
Saponins	+	+
Phytosterols	-	-
Phenols	-	-
Tannins	-	-
Flavonoids	-	-

Present (+); Absent (-).

4. DISCUSSION

Henna, has been used anciently as a medicinal plant (Rao et al., 2016; Rathi et al., 2017). It is safe to use with no health effect (Nawasrah et al.,2016) except for some few reported cases of allergic reactions mostly in children (Namiranian et al., 2019). Ointment and oil from henna flowers has been prepared by ancient Egyptians for making the limbs supple (Dinesh Babu and Subhasree 2009). The main chemical components of this plant are Lawsone (2-hydroxy-1,4-naphthoquinone) which is responsible for the reddish brown coloration, tannic acid, gallic acid, mannite and mucillage (Al-Rubiay et al., 2008; Gull et al., 2013; Al-Snafi et al., 2019).

The n-hexane henna extract used in this study exhibited no antifungal activity against both dermatophytes. This may be due to the polarity of this solvent resulting in the precipitation of the active components of the plant during the extraction process. According to (Arun, 2010), hexane henna extract did not possess antibacterial effect against Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Proteus and Pseudomonas aeruginosa even at concentration of 1000 μg/ml. This is contrary to the findings of (Padron-Marquez et al., 2012) who observed the best antifungal activity with n-hexane which inhibited the growth of all the tested dermatophytes.

Furthermore, petroleum ether henna extract inhibited the growth of both T. rubrum and T. interdigitale at a varying degree. At the highest concentration of 200mg/ml, Petroleum ether showed inhibition zone of 81±1.4mm against T. rubrum and 64±1.2mm against T. interdigitale. According to the study carried out by (Kavitha and Vidyasagar, 2013), petroleum ether extract inhibited 100% growth of T. rubrum at 40000 μg/ml. Furthermore, the antifungal drug itraconazole exhibited inhibition zone of 67±0.5 and 67±2.1 against T. rubrum and T. interdigitale respectively. Surprisingly, at 25mg/ml concentration, itraconazole possess the highest zone of inhibition of 71±1.8 against T. interdigitale. This could be due to the structure of the fungus and also the mode of action of the drug.

In another study, the antifungal drugs fluconazole, ketoconazole and

nystatin showed little or no antifungal effect against Candida albicans and Microsporum spp. at all concentrations. However, mixing antifungal drug and henna plant extracts showed increased antifungal activity (Elmanama et al., 2011).

Both petroleum ether and n-hexane henna extracts have the same MIC values of 50mg/ml against T. rubrum. Petroleum ether have MIC value of 12.5mg/ml against T. interdigitale while that of n-hexane henna extract is 100mg/ml. The reference drug itraconazole has the lowest MIC value of < 3.125 against both dermatophytes.

Phytochemical screening technique was carried out to test for the presence of eight (8) bioactive components. In both extracts only alkaloids, carbohydrates and saponins were present. Among the three (3) components, only alkaloid (Tiwari et al., 2011) have been reported to have antimicrobial activity hence the little or no antifungal activity observed from the extracts. A similar study carried out by (El Massoudi et al., 2019) showed the presence of phenols, proteins, lipids, flavonoids and tannins. Variation in bioactive compounds in henna leaves can be related to factors like leaf maturity, climatic conditions, extraction method, solvents used in extraction as well as season of harvest and storage conditions (Tiwari et al., 2011; El Massoudi et al., 2019; Neeraj, et al., 2019).

5. CONCLUSION

This study showed that extracts of Lawsonia inermis L. leaves exhibit promising antifungal activity against Trichophyton rubrum and Trichophyton interdigitale. The response of the test organisms against the extracts in this study seems to be dose dependent as larger zones of inhibition were observed with increasing concentration. This suggests that extracts of Lawsonia inermis can be used to combat the problem of drug resistance. However, there is the need for toxicity tests and also synergistic activities of henna should be tested in combination with other plant extracts or antifungal drugs. This is because certain phytochemicals exhibit their antimicrobial action only with other phytoconstituents in a synergistic way. Furthermore, the susceptibility of T. interdigitale at 25mg/ml concentration of itraconazole instead of higher concentration needs to be investigated.

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