Original Article

Evaluation Of Anti Dermatophyte Activity Of Crude Lawsone Isolated From Lawsonia Inermis Leaves

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Abstract

Dermatophytes are a group of filamentous fungi that cause infections of the skin .The various diseases caused by dermatophytes include athlete's foot, ringworm, jock itch, and nail infections (onychomycosis). Lawsonia inermis commonly called as henna and its leaves, flower, seeds, stem bark, roots have been found to exhibit antioxidant, antidiabetic, hepatoprotective, hypoglycemic, antimicrobial, anticancer and wound healing properties while Lawsone the naphtha quinone present in Lawsonia inermis leaves were reported to possess antifungal, antibacterial, bruises, skin disorders, amoebiasis, burns and bruises and leprosy. Lawsone was isolated from the aerial parts of L.inermis and quantified using HPTLC technique. The anti dermatophyte activity of the isolated lawsone was carried out by using Cup and Plate method and Poisoned plate method in dermatophytes viz. Trichophyton mentagraphyte and Trichophyton rubrum in the concentrations of 31.25, 62.5, 125, 250, 500 and 1000 µg/ml concentration. The minimum inhibitory concentration of lawsone was calculated by broth dilution assay method and found to be 500 µg/ml for both the dermatophytes and the percentage inhibition was found to be significant against T.mentagraphytes, 72.5% and 83.41% against T.rubrum when compared to fluconozole standard (96.74% and 98.42% respectively). From the results it was concluded that lawsone may be an effective phyto constituent responsible for anti dermatophyte activity.

keywords: Dermatophytes,, Lawsonia inermis , lawsone

1.Introduction :

Dermatophytosis is the most frequent superficial fungal infections in India. Clinical surveys have shown ringworm as one of the most common dermatomycoses caused by the species of Epidermophyton, Microsporum and Trichophyton. Allyl amines (e.g terbinafine) and orally active triazoles (e.g. itraconazole), has been considered as the most effective in dermatophytosis therapy[1]. Griseofulvin, the only antifungal drug which had been in use for many years for treatment of dermatophytes (Tinea capitis) is still the preferred drug, though it is reported that the fungus has already developed resistance to the said drug. Many secondary metabolites which plants produce show antifungal ability, include flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulphur compounds [2]

Fungal infection can occur in patients who are immunosuppressed due to organ transplantation, intensive care unit hospitalization, cancer, HIV, surgery, or leukemia as well as in patients who use antibiotics able to modify human microbiota.[3] Lawsone a naphtha quinone, a major constituent of L.inermis is responsible for colouring the skin is present in the aerial parts besides alkaloids, glycosides, gallic acid, glucose, mannitol, fat, resin and mucilage[4]. In the current study we have determined the anti dermatophytic activity of lawsone isolated from the aerial parts of L.inermis using cup plate and Poisoned plate method.

3.Materials and Methods

3.1. Collection and authentication of plant material.

Lawsonia inermis aerial parts were collected at the herbal garden, Sri Ramachandra Institute of Higher Education and Research, Chennai during September 2013. It was authenticated by the Pharmacognosy Department, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, Chennai.

3.2.Chemicals Used

The drugs and fine chemicals were purchased from Sigma-Aldrich, USA. All other chemicals and solvents were obtained from local firms (India and were of highest pure and analytical grade).

3.3.Preparation of Extract

3.3.1. Isolation of Lawsone from Lawsonia inermis

About 50 gms of crushed fresh leaves of Lawsonia inermis were extracted by agitation for 2 hours with 20% w/v sodium bicarbonate solution (200ml) and kept for overnight. The extract was filtered and marc was re extracted with 100 ml of same solution for 1 hr, filtered and the alkaline extracts were pooled together. The extract was acidified with dilute sulphuric acid and crude product obtained was re extracted with sufficient quantity of ammonium hydroxide and again acidified with dilute hydrochloric acid. The product was filtered extracted with two successive quantities of benzene (40ml) and filtered. The filtrate was distilled to yield yellowish brown coloured residue of lawsone and further confirmed by chemical test and HPTLC method. The percentage of lawsone was calculated[5].

4. Anti dermatophytic activity of Lawsone isolated from L.inermis

The anti dermatophytic activity was carried out using Cup and Plate method and Poison plate method.

Trichophyton species used - Trichophyton mentagrophytes (ATCC-9533), Trichophyton rubrum (ATCC- 28188)

4.1. Cup and Plate method[6]

The preliminary screening of lawsone isolated of L.inermis was tested against two dermatophytes viz Trichophyton mentagrophytes and Trichophyton rubrum. Sabourauds dextrose agar medium was prepared and used for the study . The prepared concentration of standardized dermatophytes inoculums were seeded over the surface of sterile SDA plates, and allowed to dry. The 9 mm cork borer was used to bore holes at equidistant on the agar surface and lawsone (250, 500 and 1000 µg/ml) were dispensed onto the respective wells. The plates were allowed to stand for 5 mins so that the lawsone could diffuse into the media. The same amount of DMSO used in the preparation of lawsone was added to the control. The experiments were carried out in triplicates

The plates were incubated at room temperature for 7 days after which the mean zones of inhibition were measured and recorded in millimetre. The diameter of the zone of inhibition caused by lawsone in the plates inoculated with Trichophyton mentagrophytes and Trichophyton rubrum were measured.

4.2.Poisoned plate method [7]

The entire samples were dissolved separately in DMSO solvent for screening of dermatophyte activity. They were added into the sterile petri dishes at 1000 µg/ml using micropipette. Then 20 ml of the sterilized sabourauds agar medium was poured into each petri dish. After solidification of the medium, the dermatophyte mycelia of 9 mm dia wells were drilled from the fresh culture plate and inoculated into the center of the plate. Instead of the

compound the solvent DMSO was used as a standard for comparative study. The plate with the solvent alone and the, mycelia was kept as the control. The plates were incubated at room temperature and after 21 days, the growth of the mycelia was measured.

The dermatophyte activity results were expressed in terms of the diameter of zone of inhibition and diameter less than 9 mm zone was considered as inactive; 9-12 mm partially active; while 13-18 mm as active and .18mm as very active. The mean and standard deviation of the diameter of inhibition zones were calculated[8].

Percentage of inhibition (%) =

$$\frac{\text{Growth diameter in control (mm) – Growth of Diameter in poison medium (mm)}}{\text{Growth diameter in control}} \times 100$$

4.3. Determination of minimum inhibitory concentration of the isolated Lawsone from Lawsonia inermis by double dilution method[9]

4.3.1.Procedure

SD broth was prepared and autoclaved. Prepared 4mg/2ml of stock and transferred it further to series of test tubes dispensed with 1ml of broth in order to get the dilutions of 1000µg/ml, 500µg/ml, 250µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.62 µg/ml, 3.9 µg/ml of lawsone. The fungal cultures were suspended in saline broth and incubated for 3 days. The turbidity produced by the growth of fungus was adjusted corresponding to the Mc farland solution. Then added equal volume (1ml) of culture to all tubes and incubated for 72 hrs at room temperature. After 72 hrs checked the turbidity and then streaked on SDA plate. The plates were incubated at room temperature for 72 hrs and checked for growth. The concentration at which no growth of fungi observed was considered as minimum inhibitory concentration. The plates were observed for growth or no growth and shown in fig – 1

Results and Discussion :

The anti dermatophyte activity of the various extracts against the dermatophytes (T. mentagraphytes and T.rubrum) by cup and plate method showed that the lawsone isolated from L.inermis possessed significant antifungal activity at 1000 µg/ml concentration, whereas moderate activity at 500 µg/ml, when compared to the standard fluconazole. The results were further confirmed by the Poisoned plate method. At the tested concentration (1000 µg/ml) after 21st day of incubation, the lawsone isolated from the henna leaves significant activity against T.mentagraphytes, 72.5% and 83.41% against T.rubrum when compared to fluconozole standard (96.74% and 98.42% respectively). Lawsone, 2-hydroxy-1,4-naphthoquinone might have inhibited/prevented the growth of dermatophytes viz. Trichophyton mentagraphyte and Trichophyton rubrum. The anti dermatophyte activity carried out by Nazir et al., further confirms with our findings with lawsone isolated from Lawsonia inermis[10].

Table : 1. Growth Of Fungus in the petri plate inoculated with Test Concentrations, Positive And Negative Control

Test	**Concentration**	**Lawsone**	
		TM	**TR**
1	1000	-	-

2	500	-	-
3	250	+	+
4	125	+	+
5	62.5	+	+
6	31.25	+	+
NC	Broth only	-	-
PC	Broth with fungus	+	+

Note: " +" → growth, "-"→ no growth, NC – negative control, PC- positive control

Legends - TM – Trichophyton mentagraphyte, **TR** – Trichophyton rubrum

Table: 2.Minimum Inhibitory Concentration of Lawsone

S.no	**Name of Extract**	**T.mentagraphyte**	**T. rubrum**
1.	Isolated Lawsone	500µg/ml	500 µg/ml

Table 3- anti dermatophyte activity of Lawsone by Poisoned plate method

S. No.	Percentage Inhibition	
	Trychophyton mentagraphytes	Trychophyton rubrum
Lawsone	72.5± 0.23	83.41± 0.79
Fluconazole (standard)	96.74 ± 1.07	98.42 ± 1.43

Each value represents mean ± SD of triplicates

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Reference:

1. Kennedy T. Wariso, Jeremiah A. Igunma, Ibinabo L. Oboro, Pattern of Dermatophytes Isolated in the Medical Microbiology Laboratory of the University of Port Harcourt Teaching Hospital, Rivers State, Nigeria, Advances in Microbiology, Vol.5 (5), May 18,2015
2. Kavitha Sagar and Vidyasagar G.M. Anti-dermatophytic activity of some traditionally used medicinal plants of North Karnataka Region, Journal of Applied Pharmaceutical Science Vol. 3 (02), pp. 077-083, February, 2013
3. Ranu Singh, Aashaq Hussain allaie, aijaz Ahmad Ganaie, ravi Prakash Mishra, Manisha vyas, Hilal ahmad Ganaie, Shoket Ali. Anti fungal activity of medicinal plants with special reference to anti dermatophytic activity: a review, Pharma Innovation, 2017, 6(11), 251-259
4. Ali Esmail al-snafi. A Review on Lawsonia inermis: a potential medicinal plant, International Journal of current Pharmaceutical Research, 2019, 11, (5), 1 – 13
5. Ghufran Saeed, Seema Ashraf, shahina Naz, syed Asad Sayeed, Rahmanullah Siddiqui, Ahmed M Mesaik, Rashida Ali. A new method for the isolation and purification of lawsone from Lawsonia inermis and its ROS inhibitory activity, Pakistan Journal of Botany, 2013, 45(4), 1431-1436

6. Akinyemi K.O., Oluwa, O.K., Omomigbehin, E.O. Antimicrobial activity of crude extracts of three medicinal plants used in south-west nigerian folk medicine on some food borne bacterial pathogens. , African Journal of Traditional, complementary and Alternative Medicines. 2006, 3 (4), 13 – 22
7. Martin Muthee Gakuubi, Angeline W. Marina, John M Wagecha. Antifungal Activity of Essential Oil of Eucalyptus camaldulensis Dehnh. against Selected Fusarium spp., 2017, doi: 10.1155/2017/8761610.
8. Ahmad, I. and Beg, A.Z. Antimicrobial and Phytochemical Studies on 45 Indian Medicinal Plants against Multi-Drug and Resistant Human Pathogens. Journal of Ethnopharmacology, 2001, 74, 113-123
9. Ohikhena FU, Wintola OA, Afolayan AJ. Evaluation of the Antibacterial and Antifungal Properties of Phragmanthera capitata (Sprengel) Balle (Loranthaceae), a Mistletoe Growing on Rubber Tree, Using the Dilution Techniques, Scientific World Journal. 2017; 1-8, doi 10.1155/2017/9658598.
10. Nasir Hassan Wagini, Mohamed said Abbal, Amira soliman, Yasser Adel Hanafy. In Vitro and in Vivo Anti Dermatophytes Activity of Lawsonia Inermis L. (Henna) Leaves against Ringworm and Its Etiological Agents, American Journal of Clinical and Experimental Medicine, 2014, 2(3):51-58.