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In vitro Antidermatophytic activity of *Lawsonia inermis L.*(Heena) leaves extract against ringworm

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Abstract : Dermatophytes are the group of fungal genera which can invade the keratinized tissue and invade the skin. Some of the skin infections are known as "Ringworm" or "Tinea" restricted to the non-living cornified layer of skin confirmed by Cheesbrough [1].Plant *Lawsonia inermis* L. exhibit a lot of medicinal properties including antifungal properties by Iqbal et al [2] .This study was carried out to elucidate the antifungal activity of leaf extract of *Lawsonia inermis* L.against selected species of dermatophytes i.e. *Trichophyton rubrum* and *Microsporum gypseum* Different concentration of Heena leaf extract varing from 12.5mg to 100mg was tested against the dermatophytes.With the media (SDA) sabouraud dextrose agar, the leaf extract is treated against the dermatophytes and efficacy is recorded. The result shows that these leaf extract of Henna shows high inhibitory impacts in higher concentration. The findings of the study confirmed the usefulness of leaf extract of *Lawsonia. inermis* L.in the treatment of ringworm and can be used in the drug industry.

Keywords :- SDA, Dermatophytes, Extract, *Trichophyton, Microsporum, Lawsonia.*

INTRODUCTION

Heena (*Lawsonia inermis L.*) belongs to class Lythraceae is a tall flowering shrub or tree, about 5m in height, native to tropical and subtropical region by Singh et al [3]. Heena leaves, stem bark, roots have been found to exhibit antioxidant, antimicrobial and wound healing properties by Shahitha et al [4].

It is a shrub plant with small green leaves and frequently found in Ranchi district. Plant contains different Asteroids, Alkaloids etc. It is evident from the survey i.e. with the help of knowledgeable peoples, local healers, Vaidyas etc.

The extract of Henna leaf has been used for treatment of fungal infection in the area.According to the report of WHO 80% of the world's population depend mainly on traditional therapies which involve the use of plant extract and their various active substances by Daljit S.et al.[5].

Ringworm is a common contagious diseases caused by fungi known as dermatophytes which belong to a group of organisms that are able to break down the keratin in tissue such as epidermis, hair, nails in legs, hands etc by Mukhtar et al[6].

The aim of this work was to evaluate the potential antifungal activity of Henna leaf extract in vitro and to identify the effect of different concentration of leaf extract to prevent the growth of fungal species.

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MATERIALS AND METHODS

Identification, Collection &Preparation of Plant materials:-

Heena plant was identified and leaf was collected from various blocks in Ranchi district. Identification is done by the help of local knowledgeable people i.e. local healers, vaidyas etc.

Preparation of Ethanol Extract of Heena Leaf (*Lawsonia inermis L)*:- Henna leaf was thoroughly washed, dried under shade and grind to make powder. 100g of Henna leaf powder was suspended into 500 ml of ethanol mixed thoroughly. This solution was filtered with whatsmann filter paper no.1 in another beaker. This beaker with filtrate was covered with aluminum foil. The beaker was kept under room temperature until a semisolid substance is left in the beaker after evaporation of solvent. This dried extract was weighed and kept in freezer for further analysis by Kawo and Kwa [7].

To prepare ethanol soluble fractions of the plant, 100mg of extract was dissolved in 1ml of dimethyl sulfoxide ($DMSO_4$) to make a concentration of 100mg/ml. This was referred to as solution (a).

0.5 ml of solution (a) dissolved in 0.5 m $DMSO_4$ to make solution (b)

0.5 ml of solution (b) dissolved in 0.5 m $DMSO_4$ to make solution (c)

0.5 ml of solution (c) dissolved in 0.5 m $DMSO_4$ to make solution (d)

The concentrations of standard solution a, b, c, d were 100mg/ml, 50mg/ml, 25mg/ml & 12.5mg/ml respectively. However by incorporating 1.0ml of solution (a), (b), (c), (d) into 9.0ml of Sabourraud Dextrose Agar, a final concentration 5000 µg/ml. 2500µg/ml. 1250µg/ml and 625µg/ml. were prepared by Nasir et al [8].

The aforementioned concentrations of leaf extract with SDA (Sabouraud dextrose agar) aseptically and poured in petridishes of 150mm×30mm.These are allowed to solidify as described by Diwedi and Dubey [9]

Use of Fungal Species

Two species of fungus was procured from MTCC Chandigarh which are responsible for causing ringworm, i.e dermatophytes.

These are:-

Trichophyton rubrum (3272);

Microsporum gypseum (2830)

After solidifying the inocula of fungus was transferred and incubated in the middle of petriplates at 28^0C to 30^0C in the dark with the help of sterile iron loop known as inoculum transfer. For all the concentrations test was performed in triplicates. The growth of the dermatophytes on each culture plate was measured linearly (growth diameter) by the use of transparent millimeter rule daily for 7 days.

RESULTS

It was found that the leaf extract inhibits the growth of dermatophytic fungi in vitro condition, which is studied to varying extents. Table and Graph shows the measure of the diameter of mycelial growth for fungi *Trichophyton rubrum* and *Microsporum gypseum* respectively.

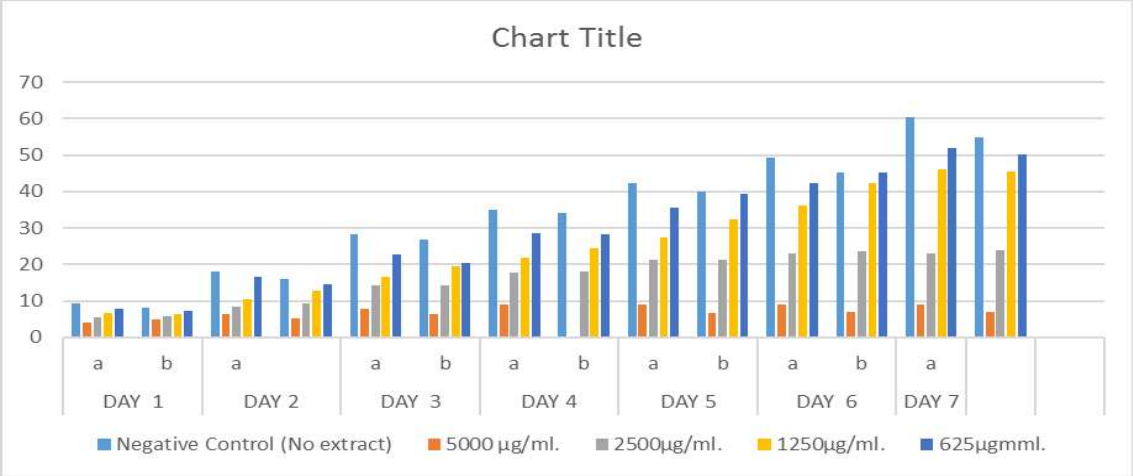
Microsporum species was more susceptible than *Trichophyton* species and so highly inhibited by the ethanolic leaf extract of *Lawsonia inermis L.*. It is observed that 5000 µg/ml. of shows highest inhibitory impact on the dermatophytes, and 625µg/ml. has lowest.

TABLE: -Measure of mycelial growth of *Trichophyton rubrum* and *Microsporum gypseum* on SDA incorporated with different concentration of *Lawsonia inermis* L.leaf extract.

Heena leaf Extract	DAY 1		DAY 2		DAY 3		DAY 4		DAY 5		DAY 6		DAY 7	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Negative Control (No extract)	9.2	8.2	18.1	16.1	28.4	26.8	35.1	34.1	42.2	40.1	49.4	45.3	60.4	55.0
5000 µg/ml.	4.1	4.9	6.3	5.3	7.9	6.5	8.9	6..8	9.1	6.8	9.1	7.0	9.1	7.0
2500µg/ml.	5.5	5.9	8.4	9.4	14.2	14.2	17.8	18.1	21.4	21.4	22.9	23.5	23.0	24.0
1250µg/ml.	6.6	6.5	10.6	12.9	16.5	19.5	21.8	24.6	27.4	32.5	36.2	42.2	46.1	45.5
625µgmml.	7.8	7.2	16.6	14.6	22.6	20.4	28.7	28.4	35.5	39.4	42.2	45.2	52.1	50.1

a - *Trichophyton rubrum* ; b - *Microsporum gypseum*

Graph :- Measure of mycelial growth of *Trichophyton rubrum* and *Microsporum gypseum* on SDA incorporated with different concentration of *Lawsonia inermis* L. leaf extract.



DISCUSSION

Various parts of *Lawsonia inermis* L. has been reported to be used in many countries for the treatment of various diseases. The result of present studies indicated that this plant has antifungal potentials against dermatophytes by Goyal, Nasreen and Mansour et al.[10, 11, 12].

Shahitha et al.[4] reported that total 6 species of dermatophytes *Trichophyton rubrum, T.verrsum, T. tonsurans, T. equinum, Microsporum canis, M. gypseum* were the suseptible dermatophytes i.e. Henna leaf extract is effective on these. In this study *Lawsonia inermis* L.(Henna) shoes antidermatophytic activity against both the fungus i.e. *Trichophyton rubrum* and *Microsporum gypseum*. The higher concentration of ethanol extract of Henna plant shows better fungicidal effect than lower concentration. Nasreen et. al. [11] reported that during screening of barks of 30 plant species against dermatophytes only *Lawsonia inermis L.* extract exhibited absolute toxicity. The extract showed toxic effect against 13 Ringworm fungi.

In the present report the dermatophytes, *Microsporum gypseum* and *Trichophyton rubrum* were used to test the antifungal potential of the leaf extract of *Lawsonia inermis* L. It is effective on both the dermatophytes but *Trichophyton rubrum* is more susceptible than *Microsporum gypseum.*

CONCLUSION

The result of present study show that the leaf extract of *Lawsonia inermis L.* is an effective antimycotic agent against dermatophytes in vitro. The chemical components are responsible for antifungal activity. The mechanism of action of drug is not known but these chemicals generally inhibits fungal growth by either disrupting fungal membrane permeability. It also inhibits the nucleic acid synthesis or protein synthesis by Betram [13]. This work gives indispensable approach to find out the antifungal potential in leaf extract of *Lawsonia inermis* L.

These findings shows that there is a "truth" in the claim of the traditional healers on the medicinal value of these two plants. Therefore the use of *Lowsonia inermis* L. leaf extract should be encouraged in the treatment of dermatophytes infections. This could be of significant interest to the expansion of new effective drugs.The Government shall pay more attention to our local medicinal plants and help in processing them. Further research will help in identifying and purification of active antifungal agents in Heena plant.This could be of significant interest to the expansion of new effective drugs.

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