



## Anti-Dermatophytic Activity of Titanium Dioxide Nanoparticle Synthesized using *Lawsonia Inermis*

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**Abstract:** The development of the green synthesis of nanoparticles using plant extract has received appreciable attention because of the easy preparation methods, less use of chemicals, and is also environment friendly. Hence green synthesis of Titanium dioxide nanoparticle using ethanolic leaf extract of *Lawsonia inermis* and to evaluate its antidermatophytic activity was attempted in the study. The formation of nanoparticles was indicated a change in color. The morphological image of the TiO<sub>2</sub> NPs was observed in a scanning electron microscope, which showed irregular structure with an average size of 71.125nm. The inhibitory activity of synthesized TiO<sub>2</sub> NP on the dermatophytes *T. mentagrophytes* and *M.gypseum* at concentrations from 2000µg/ml to 31.25µg/ml was evaluated by Disc diffusion method. The ethanolic extract derived TiO<sub>2</sub> NP could inhibit *T. mentagrophytes* significantly with a zone of 30 mm diameter at a MIC of 1000µg/ml, 28mm at 500µg/ml, and 25mm at 250µg/ml. Amphotericin B at a concentration of 1000µg/ml produced an inhibitory zone of 43mm dia. The results hence demonstrate potential anti - dermatophytic activity of ethanolic extract of *L. Inermis*

**Keywords:** *Lawsonia inermis*, Titanium dioxide nanoparticles, antidermatophytic activity, Disc diffusion method

### Introduction

In the synthetic and traditional systems of medicines, natural compounds are the most important and the most widely used. For over years *Lawsonia inermis*, commonly called henna has been used in cosmetics and medicines. Henna is readily available in tropical and subtropical countries like India. Historically in India Mehendi is used for decorating hands and feet. The leaf of henna has an orange-red dye which is used as a hair colour and used for treating skin diseases[1]. There

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are only a few pharmacological studies reported on its anti diabetic activity, immunomodulatory effect, hepatoprotective activity, antioxidant effect, antimicrobial activity.

Dermatophytes are assuming high significance in developing countries like India[2]. Dermatophytes use keratin of human skin and spread very easily and are responsible for various outbreaks. Due to the extensive use of broad-spectrum antibiotics and immunosuppressive drugs for these ailment resistant strains are also reported[2]. Hence new antifungals of plant origin could be beneficial with less risk of side effects and cost-effectiveness. Green synthesis of nanoparticles from plant extracts and microbes has received the attention of researchers in recent years. This method is economical and eco-friendly in nature due to the biological process which makes them. Titanium dioxide is a non-toxic and inexpensive material and is not classified as hazardous according to the United Nations Globally Harmonized System (GHS) of classification and labelling chemicals [3].

The increasing resistance of fungi against antibiotics, human sensitivity towards antibiotics, and the occurrence of resistant pathogens in the human body are the problems of using antibiotics as an antimicrobial agent. Hence the present study was aimed to synthesize and characterize Titanium dioxide nanoparticle using ethanolic leaf extract of *Lawsonia inermis* and to evaluate its antidermatophytic activity and toxicity analysis.

## **Methodology**

### ***Preparation of Plant Extract***

The healthy plant leaves of *L.inermis* were collected from Chennai. The authenticity of the plant part was done at Department of Plant Biology and Biotechnology Quaid-e-millath government college, Chennai. *L.inermis* leaves were surface sterilized with running tap water. The leaves were ground into a fine paste. 20 gms of the leaf paste was soaked with 100 ml of 95% Ethanol and stored overnight at 4°C. After overnight storage, the extract was centrifuged and collected. The centrifuged extract was filtered through a Millipore membrane filter of a 0.45 µm pore size. [4], [5].

### ***Synthesis of Titanium Dioxide Nanoparticle using ethanolic extract of L. inermis***

For the synthesis of TiO<sub>2</sub> nanoparticle 80ml of TiO(OH)<sub>2</sub> was added to 20ml of ethanolic extract of *L.inermis* and stirred for 24hrs at room temperature and was observed for change in colour. The synthesized nanoparticle was frozen in a deep freezer at -80°C. The frozen extract was loaded to a lyophilizer [6].

### ***Chemical Characterization***

#### ***U-V Visible Spectrophotometer Analysis***

U -V visible analysis was done to monitor the completion of bio reduction of titanium dioxide ions in ethanolic solution by using Shimadzu U-V visible spectrophotometer at 200-800nm range operated at a resolution of 1nm.

### ***Antidermatophytic Activity***

The synthesized titanium dioxide nano particle from the ethanolic extract of *L. Inermis* was tested for its antidermatophytic effect against clinical isolates of *Trichophyton rubrum* and *Microsporum gypseum* maintained in the department laboratory by standard disc diffusion method on Sabouraud Dextrose agar (SDA) medium. Mc Farland standard was used as a reference to adjust the turbidity of fungal suspensions so that the number of fungi in a specific volume of broth will be within a given range the turbidity was adjusted to 0.5 Mc Farland standards.

### ***Agar Disc Diffusion Method***

The antifungal activity of the extracts was determined by standard disc diffusion method on Sabouraud Dextrose agar (SDA) medium. A sterile swab moistened with the fungal suspension was spread on the solidified SDA plates. Samples were diluted for 1000 µg/ml. The sterile discs were soaked with a solution containing titanium dioxide nano particles synthesized from *L.inermis*. Amphotericin-B was used as a positive control. The plates were incubated at room temperature for 4 days. The diameter of the zone of inhibition was measured to determine the antifungal activity [7].

### ***Minimum Inhibitory Concentration***

The minimum inhibitory concentration of the ethanolic extract of synthesized titanium dioxide nanoparticle of *L. Inermis* was determined using the tube dilution technique. Serial two-fold dilution of the extract was carried out using Sabouraud Dextrose broth to obtain concentrations of 2000, 1000, 500, 250, 125, 62.5, 31.25 & 15.25 µg/ml. 0.1ml of standard suspension of the test organism (Mc Farland 1 to  $1.5 \times 10^8$  CFU / ml) was added to each of the test tubes and incubated for 30° C for 4 days. The tube with broth and extract, without inoculum, was used as a positive control. The tube with broth and inoculum was used as a negative control. Amphotericin- B served as drug control. The presence or absence of turbidity at the end of the incubation period recorded. The highest dilution of the extract (least concentration) showing no detectable growth was regarded as the minimum inhibitory concentration [7].

### ***Determination of Minimum Fungicidal Concentration***

The minimum fungicidal concentration of the ethanolic extracts was determined as follows. 0.1 ml of the inoculum from the last MIC test dilution that showed visible growth (turbidity) and all others in which there was no detectable growth was sub cultured on to a fresh extract free solid medium. The plates were incubated at 30° C for further 4 -7 days. The highest dilution that showed no fungal growth was considered as minimum fungicidal concentration [8].

## **Results**

### ***Synthesis of Titanium Dioxide Nanoparticles***

After overnight stirring reduction of titanium (IV) oxide to titanium dioxide after was observed by a gradual change in colour development from pale yellow to green colour. The nanoparticle was lyophilized (fig.1)



Fig. 1: Lyophilized Nanoparticle from ethanolic extract of *L.inermis*

**U-V Visible Spectrophotometer Analysis**

The formation and stability of the reduced titanium dioxide nanoparticle were monitored by UV-Vis spectrophotometer analysis. The UV absorbance range was from 350 nm- 360 nm (fig.2).

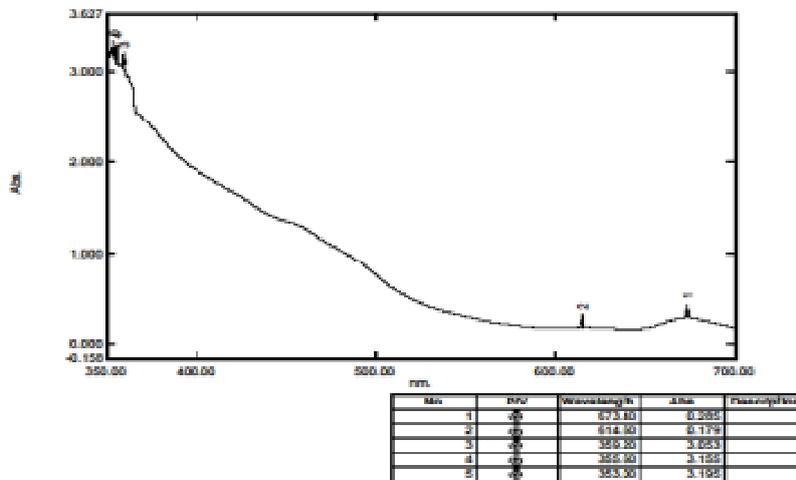
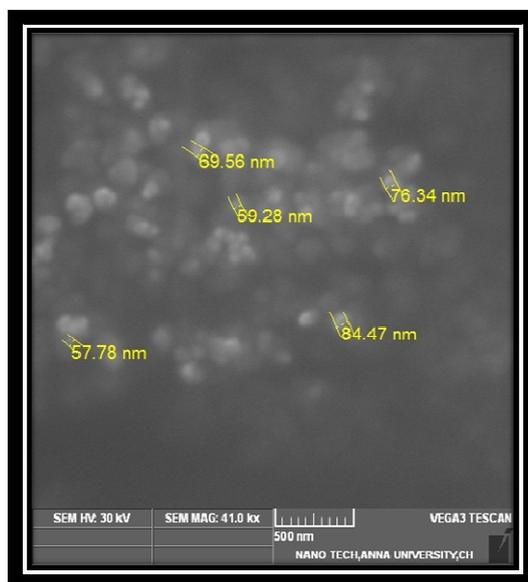


Fig. 2: UV-Visible spectrophotometer of Titanium dioxide nanoparticles

**SEM Image**

The morphological characters of synthesized TiO<sub>2</sub> NPs were analysed by VEGA3 TE SCAN (SEM) at the Department of Nano Technology, Anna University, Chennai which showed spherical shape particles and clusters with sizes was ranging from 57.78 to 84.47nm (fig.3).



**Fig. 3:** SEM image of biosynthesized titanium dioxide nanoparticle from ethanolic extract of *L. Inermis*

### Agar Disc Diffusion Method

The diameter of the zone of inhibition around each well with titanium dioxide nanoparticles is given in table 1, fig.4 Graph 1

**Table: 1** Disc diffusion Zone of inhibition

ORGANISM	ZONE OF INHIBITION						ANTIBIOTIC (1 mg/ml)
	CONCENTRATION ( $\mu\text{g/ml}$ )						
	1000	500	250	125	62.5	31.25	
<i>Trichophyton rubrum</i>	30	28	25	21	20	17	43
<i>Microsporium gypseum</i>	11	-	-	-	-	-	40

**Graph.1** Graphical representation of inhibition

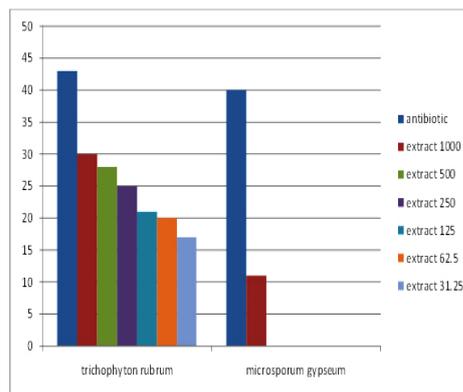




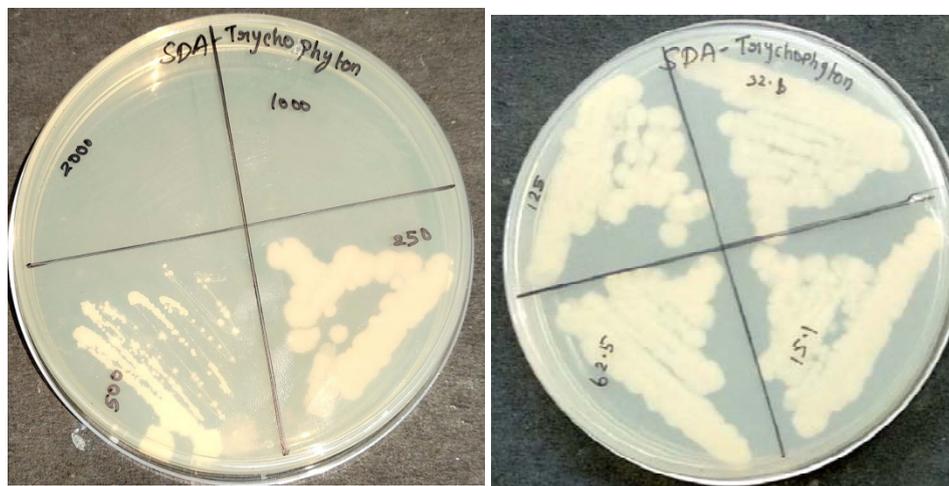
Fig 4: A and B *Trichophyton rubrum* (Disc diffusion test)

**Anti-Dermatophytic Activity**

The anti-dermatophytic activity of the synthesized titanium dioxide nanoparticles was carried out against *Trichophyton rubrum* and *Microsporum gypseum* using a disc diffusion method, and minimum inhibitory concentration (MIC) (fig.5) and minimum fungicidal concentration (MFC) (fig.6) was determined.



Fig 5 : MIC tubes of *T. rubrum*



**Fig 6: MFC *T. rubrum***

## Discussion

Biological products are an important source of lead drugs. Several modern drugs used have been developed from natural products. Natural products can contribute to the search for new drugs to combat drug resistance. The use of plant extract has many advantages such as they are available easily, safe to handle, and have many metabolites.

Nanoparticles synthesized by physical and chemical methods are much hazardous and were shown to be less stable. Hence green synthesis methods employing plants prove to be eco-friendly. Biosynthesis routes provide NPs a better-defined morphology and size than physicochemical methods of production.

Henna is widely applied in the skin and it's readily available in India, hence the plant was chosen in the study. Dermatophytosis is a common superficial fungal infection found in India[9]. Nanoparticles are better antifungal agents than the corresponding bulk material of the same composition, because of their greater surface area[10]. Hence the present study evaluated the antidermatophytic activity of TiO<sub>2</sub> Nanoparticles from leaf extracts of *Lawsonia inermis* as it has not been attempted earlier.

The plant extracts were mixed with Titanium IV isopropoxide for the synthesis of NPs. After overnight stirring, the colour changed to light green. There was an observable colour change in the ethanolic extract. The results conform with earlier study which reported colour change after 24 hrs of incubation from *Psidium guajava* aqueous leaf extract [11].

To confirm the bio reduction of Titanium dioxide ions in the resulting solution, UV-visible analysis was done. The absorption maximum depends on the size and shape of the nanoparticle. In the present study, the absorption of TiO<sub>2</sub> appeared at 350-360nm. This conformed with earlier

report[12]. From the SEM images, it was noted that most of the TiO<sub>2</sub> nanoparticles was ranging from 57.78 to 84.47nm, an average of 71.125nm in size.

The inhibitory activity of synthesized TiO<sub>2</sub> NP on the dermatophytes *T. mentagrophytes* and *M.gypseum* at varying concentrations from 2000µg/ml to 31.25µg/ml was evaluated by Disc diffusion method. The diameter of the inhibition zone (mm) around each well with TiO<sub>2</sub> nanoparticles is represented in the table (1).

The ethanolic extract derived TiO<sub>2</sub> NP could inhibit *T. mentagrophytes* significantly with a zone of 30 mm diameter at a MIC of 1000µg/ml (30mm), 28mm at 500µg/ml and 25mm at 250µg/ml. Amphotericin B at a concentration of 1000µg/ml produced an inhibitory zone of 43mm dia. However, the NP did not show significant activity to *M.gypseum* (11mm). The results hence demonstrate potential anti - dermatophytic activity of ethanolic extract of *L. inermis* on *T. mentagrophytes*.

The earlier study on anti - dermatophytic activity of *L. inermis* reported inhibition of *T.mentagrophytes* at 2500µg/ml [7]. The MFC was estimated by the recovery plate technique which showed the synthesized NP at the concentration of 1000µg/ml to be fungicidal. The obtained results prove the anti dermatophytic activity of the synthesized titanium dioxide nanoparticle from the ethanolic extract of *L. inermis*.

## Conclusion

The ethanolic extract of *L. inermis* reduced Titanium IV isopropoxide to TiO<sub>2</sub> nanoparticles. The synthesized TiO<sub>2</sub>nanoparticle exhibited significant inhibition of *T. mentagrophytes* than on *M. gypseum*. The results suggest future research on characterization of active constituents from this potential plant.

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**Conflict of interest:** None

## References

1. Gagandeep Chaudhary, Sandeep Goyal, Priyanka Poonia., 2010, *Lawsoniainermis* Linnaeus: A Phytopharmacological Review, Int. J. Curr. Pharm. Res., 2, pp. 91-98.
2. Murlidhar Rajagopalan, Arun Inamadar, Asit Mittal, Autar K. Miskeen, C. R. Srinivas, Kabir Sardana, Kiran Godse, Krina Patel, Madhu Rengasamy, Shivaprakash Rudramurthy and Sunil Dogra., 2018, "Expert Consensus on The Management of Dermatophytosis in India (ECTODERM India)". BMC Dermatology., 18.pp. 6-10.
3. Aadarsh Mishra., 2014, "Analysis of Titanium Dioxide and its application in industry", Int. J. Mech.Eng. & Rob. Res., 3, pp. 560-565.
4. Chidambaram, S., Swaminathan, R., 2013, "Determination of anti-tubercular activity of four Indian medicinal plants against *Mycobacterium tuberculosis* by broth micro dilution method", Int. J. Pharm. Sci. Res., 4, pp. 3932-3937.

5. Lutfana Banu, A., Soundhari, C. and Karkuzhali, S., 2018, "Green synthesis of titanium dioxide nanoparticles using *Lawsonia inermis* leaf extract and its antibacterial activity", World J. Pharm. Pharm. Sci., 7, pp. 180-190.
6. Renuka, M. and Soundhari, C., 2018, "Antibacterial And Anticancer Activity of Green Synthesised Titanium Dioxide Nanoparticle from *Terminalia chebula*", World J. Pharm. Pharm. Sci., 7, pp. 1164-1179
7. Maria Elisabete da Silva Barros, Daniel de Assis Santos, and Ju'nia Soares Hamdan., 2007, "Evaluation of susceptibility of Trichophyton mentagrophytes and Trichophyton rubrum clinical isolates to antifungal drugs using a modified CLSI microdilution method (M38-A)", J. Med. Microbiol., 56, pp. 514-518.
8. Rubini, B., Shanthi, G., Soundhari, C. and Rajarajan, S., 2013 Antifungal activity of *Terminalia chebula* and *Terminalia catappa* on two dermatophytes. J. Med.Arom. Plants.,4, pp.15-19.
9. KavithaSagar. and Vidyasagar G.M., 2013, "Anti-dermatophytic activity of some traditionally used medicinal plants of North Karnataka., Reg. J. Appl. Pharma. Sci., 3, pp. 077-083.
10. Sara George, A., Shailaja Raj, A., Diana Solomon, and Roselin., 2014, " A Comparative Study of the Anti-Fungal Activity of Zinc Oxide and Titanium Dioxide Nano and Bulk Particles with Anti-Fungal against Fungi Isolated from Infected Skin and Dandruff Flakes", Res. Rev. J. Micro. Biotech., 3, pp. 23-30.
11. Thirunavukkarasu Santhoshkumar., Abdul Rahuman., Chidambaram Jayaseelan., Govindaswamy Rajakumar., Sampath Marimuthu., Arivarasan Vishnu Kirthi., KanayairamVelayutham., John Thomas., JayachandranVenkatesan., Se-Kwon Kim., 2014, Green "Synthesis of Titanium Dioxide Nanoparticles using *Psidiumguajava* extract and its Antibacterial and Antioxidant properties", Asian Pac. J. Trop. Med., pp. 968-976.
12. Ganesan S, Ganesh Babu I, Mahendran D, IndraArulselvi P, Elangovan N, Geetha N, Venkatachalam P., 2016, "Green Engineering of Titanium Dioxide Nanoparticles using *Ageratinaaltissima*(L) King &H.E.Robines medicinal plant aqueous leaf extracts for enhanced photocatalytic activity",Ann. Phytomed., 5, pp. 69-75.