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Anti-oxidant, anti-bacterial and anti-mycobacterial activity of the methanolic extract of *Dendrophthoe falcata* leaves

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Abstract
Emergence of virulent multidrug resistant strains of the tubercle bacilli has led to a serious search for new molecules for developing a new drug. Plants serve as resources for developing new scaffold of drugs. Dendropthoe falcata (DF), an arboreal, hemiparasitic plant is being used by indigenous medical practitioners for healing diseases such as asthma, psychic disorders, & tuberculosis. Here in this study we aim to determine the antioxidant, antimicrobial and antimycobacterial activity of Dendrophthoe falcata leaf extract. DPPH free radical scavenging activity of the extract through the absorbance measurements at 515 nm was done in a spectrophotometer. Disc diffusion method in the bacterial culture plates with discs of various concentrations ranging from 40mg/100µl, 60mg/100µl, and 80mg/100µl was used to assess the antibacterial activity. Antimycobacterial activity was assessed against H37RV, all sensitive MTB and MDR-TB. Luciferase reporter page (LRP) assay was done with concentrations 100 & 500 µg/ml of the plant extract with Rifampicin as assay control. IC_{50} of the DF extract and the control Quercetin determined were of 37.75 µg/ml and 7.79 µg/ml respectively. Antimicrobial activity was significantly greater in against the *Bacillus subtilis*, and *E. coli*. Test drug showed inhibition of H37RV and all sensitive M.tuberculosis and no inhibition with MDR tuberculosis. Dendropthoe falcata leaf extracts showed antioxidant, antibacterial and antimycobacterial activity. However further studies are required to elucidate the antimycobacterial compound present in the extract.

Keywords: *Dendropthoe falcata*, antimycobacterial activity, *Mycobacterium tuberculosis, herbal medicine*

1. Introduction
Robert Koch, the celebrated German physician and microbiologist discovered the causative agent of Tuberculosis (TB), *Mycobacterium tuberculosis* in the year 1882 [1]. Since then there is a severe competition among the drug discoverers in developing a drug that could hunt down this bacteria. The race is still on with the emergence of more complex and virulent Multidrug resistant (MDR) and extensively drug resistant (XDR) strains of the tubercle bacilli. Annual Tuberculosis report (2015) of WHO reports TB as one of the major health problem in the South East Asian region of the World with an estimated incidence of 3.4 million new cases of TB occurring each year [2]. India stands first among the 22 high TB burden countries in the World with 24% of the estimated global incidence and 20% of global TB related deaths. In this existing critical situation, it becomes necessary to look out for the solution from natural resources like medicinal herbs. An herbal medicine from plant sources saves 80% of the world population from various diseases. It is a well proven fact that plants and other natural products are the template for developing new scaffold of drugs [3, 4]. This is because of their safety factor over the side effects of the synthetic derivatives, profound therapeutic benefits and above all the affordable treatment. India has its unique wealth of medicinal herbs and a vast traditional knowledge on the use of herbal medicines for the treatment of various diseases. Forty percent of Indians are infected with tuberculosis. With the rapid emergence of MDR and XDR strains of *Mycobacterium tuberculosis* all over the world, medicinal plants are of great hope to develop a drug that could cure this disease. *Dendrophthoe falcata* has been used traditionally in the treatment of pulmonary tuberculosis since ages [5, 6, 7-15]. Use of this plant by indigenous healers to treat pulmonary tuberculosis is well documented in scientific literature; however there is less or no scientific study on its potential to act against mycobacterium. *Dendrophthoe falcata* commonly known as "Banda" is a hemi-parasitic shrub that is indigenous to India, Srilanka, Thailand, Indochina and Australia [16, 17]. Medicinal use of these plants has been proved in conditions like wound healing, menstrual disorders, asthma, psychic disorders etc. In regions of South India traditional healers use it as a medicine to cure pulmonary tuberculosis [18, 19]. Review of literature showed us the documented evidences that *Dendrophthoe falcata* is

a useful medicine in treating tuberculosis. However there is no single antimycobacterial work documented to prove the ability of this plant as having antimycobacterial activity. Hence we aim to explore the scientific basis for the traditional use and belief on this plant of having antituberculosis property.

A review publication (Raju *et al.* 2007) [20] identified 225 out 365 Indian medicinal plants to be a source of antimycobacterial agents. But this plant *Dendrophthoe falcata* was not included in the review as there was no scientific evidence upon its antimycobacterial property. Novelty of our study is that, though most of the scientific articles mentioned Dendrophthoe *falcata* as a traditionally used drug for tuberculosis treatment, we will be the first to our knowledge to test its antimycobacterial property in both the normal and drug resistant strains.

Therefore the present study is planned to check the antibacterial activity of methanolic extract of *Dendrophthoe falcata* on the isolates of MTB H37RV, all sensitive MTB and MDR MTB strains of *Mycobacterium tuberculosis*.

2. Objectives

1. To study the antioxidant activity of *Dendrophthoe falcata* leaves by DPPH radical scavenging activity.
2. To study the antimicrobial activity of *Dendrophthoe falcata* leaves against selected gram positive and gram negative bacteria by disc diffusion method.
3. To study the antimycobacterial activity of *Dendrophthoe falcata* leaves against the MTB H37RV, All sensitive MTB and MDR MTB strains of Mycobacterium tuberculosis by Luciferase reporter phage (LRP) assay method.

3. Materials and Methods

3.1 Sample collection and preparation

The leaves of *Dendrophthoe falcata* (Loranthaceae) a parasite on *Mangifera indica* (Anacardiaceae) were collected in the month of May 2016. The plant specimen was authenticated by Dr. Reginald Appavoo, Professor of Botany, Scott Christian College, Nagercoil, Kanniyakumari District. We chose the *Dendrophthoe falcata* from *Mangifera indica* as one of the finding reveals the fact that there is a massive transfer of C-glucosyl xanthone mangiferin from the mango tree which is a phenolic compound that has lot of antibacterial property [21].

3.2 Extraction procedure

The shade dried leaves of *Dendrophthoe falcata* will be pulverized and the powdered material will be extracted with methanol (80%) by Soxhlet extraction procedure. The extract will be concentrated on a rotary vacuum evaporator and thus collected crude methanolic extract will be used for the further studies.

3.3 Bioassay study

3.3.1 Preparation of the bacterial suspension.

The bacterial suspension will be prepared to match the McFarland standard and will be prepared by dissolving 0.5 gm of $BaCl_2$ in 50 ml of dissolved water to obtain a 1% solution of Barium chloride. This will be mixed with 99.5ml of 1% Sulphuric acid solution. Three to five identical colonies of each test bacteria will be taken from the stock and dropped in Mueller Hinton broth (Himedia, Mumbai). The broth culture was incubated at 37ºC for 2 to 6 hours until it reaches turbidity similar to the McFarland standards.

3.3.2 Preparation of the extract concentrations and antibiotic

Stock solutions for the extracts will be prepared by dissolving 100 mg in 1 ml of methanol. An antibiotic control was made by dissolving 10 µg of Chloramphenicol in 1ml of sterile water.

3.3.3 Determination of the bioactivity of the extract.

Test pathogenic bacteria such as Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, Bacillus subtilis and Klebsiella was used for invitro antibacterial activity. These selected pathogenic strains was obtained from microbiological division, Jayagen Biologics, Chennai.

3.3.4 *In vitro* antibacterial activity

The antibacterial activity will be determined by disc diffusion method [22] About 25 mL of molten nutrient agar will be poured into a sterile petri plate (Himedia, Mumbai, India). The plates will be allowed to solidify, after which 18 hrs grown (OD adjusted 0.6) 100 µl of above said pathogenic bacteria cultures will be transferred onto plate and made culture lawn by using sterile L-rod spreader. After five minutes setting of the bacteria, the test samples will be dissolved in sterile water and impregnated onto the sterile disc (40µL) with various concentrations such as 20mg/100µL, 40mg/100µL, 60mg/100µL and 80mg/100µL (Himedia, Mumbai, India). The drug loaded discs will be deposited onto the plate between 24 mm diameter distance. The solvent water loaded disc will serve as control. The plates will be incubated at 37 °C in a 40 W fluorescent light source (~ 400 nm) for 24 h. The antibacterial activity will be determined by measuring the diameter of the zone of inhibition around the well using antibiotic zone scale (Himedia, Mumbai, India).

3.3.5 Free radical scavenging activity

The effect of methanolic extract of *Dendrophthoe falcata* leaves on DPPH radical will be estimated according to the procedure described by Von Gadow *et al.* (1997) [23]. Two mL of 6 ×10-5 M methanolic solution of DPPH will be added to 50 µl of a methonolic solution (5 mg/1 ml) of the sample. Absorbance measurements will be commenced immediately. The decrease of absorbance at 515 nm will be continuously recorded in a spectrophotometer for 16 min at room temperature. Methanolic solutions of pure compound [quercetin] will be tested at 1 mg/ml concentration. The scavenging effect (decrease of absorbance at 515 nm) will be noted against the time and the percentage of DPPH radical scavenging ability of the sample will be calculated from the absorbance value at the end of 16 min duration using the formula of Yen and Duh(1994) [24].

Percentage of inhibition (PI) = [(AC (0) – AA (t) / AC (0))] × 100

Where AC (0) is the absorbance of the control (DPPH) at t = 0 min and AA(t) is the absorbance of the plant extract at t = 16 min.

3.3.5 Antimycobacterial activity Testing

I. Microbial strain for anti-Mycobacterium tuberculosis Assays

Standard strain H37RV, one clinical sensitive strain and the other a clinical resistant strain will be used for the anti mycobacterial assays.

II. Susceptibility Testing of Mycobacterium tuberculosis

Luciferase reporter phage (LRP) assay

The method used by Molly Antony *et al.* (2012) [25] will be adopted for our study. MTB H37RV, All sensitive MTB and MDR MTB strains will be grown in Middle brook 7H9 complete medium with and without extracts of *Dendrophthoe falcata* for 3 days at 37^0C.

Luciferase Reporter Phage Assay will be done using concentrations of 100 and 500 µg/ml of the *Dendrophthoe falcata* extracts. Rifampicin will be included as an assay control and DMSO as the solvent control. LRP phage AETRC21 will be added and the samples will be incubated for 4 hours. Equal volume of the cell phage mixture will be mixed with 0.3Mm D-Luciferin in 0.05M sodium citrate buffer of pH 4.5 and light output will be immediately measured as RLU (Relative light units) in the luminometer at 10 seconds integration. Compounds exhibiting a reduction of 50% or more in RLU in the test vials compared to that of the control will be considered to have anti mycobacterial activity. These LRP assays offer an elegant means of detecting viable mycobacteria and provide a rapid tool for drug susceptibility screening.

4. Results

Our study results showed that the methanolic extract of *Dendropthoe falcata* leaves possessing a positive antioxidant, antibacterial and antimycobacterial activity. However this is a preliminary report that needs more extensive studies to explore the further possibilities of using *D. falcata* as a medicine.

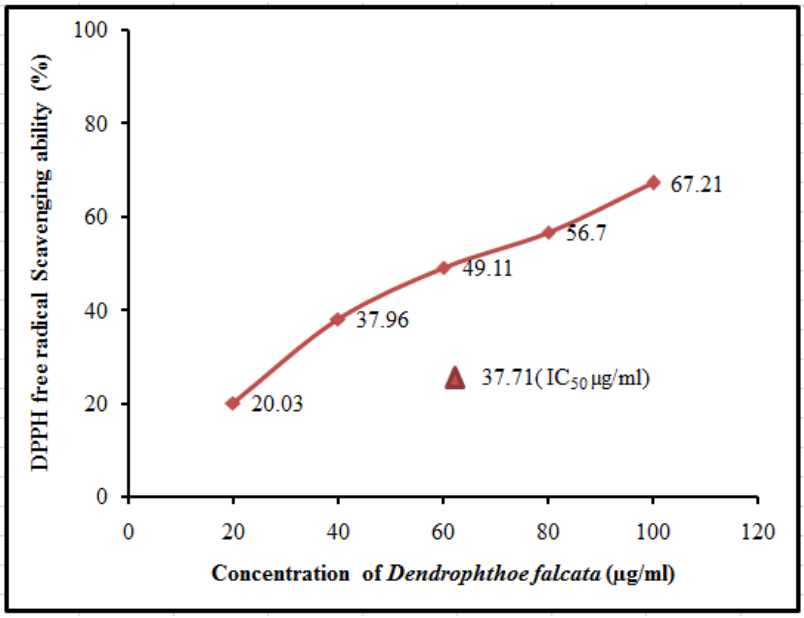

Fig 1: Antioxidant activity of the extract by DPPH radical scavenging activity

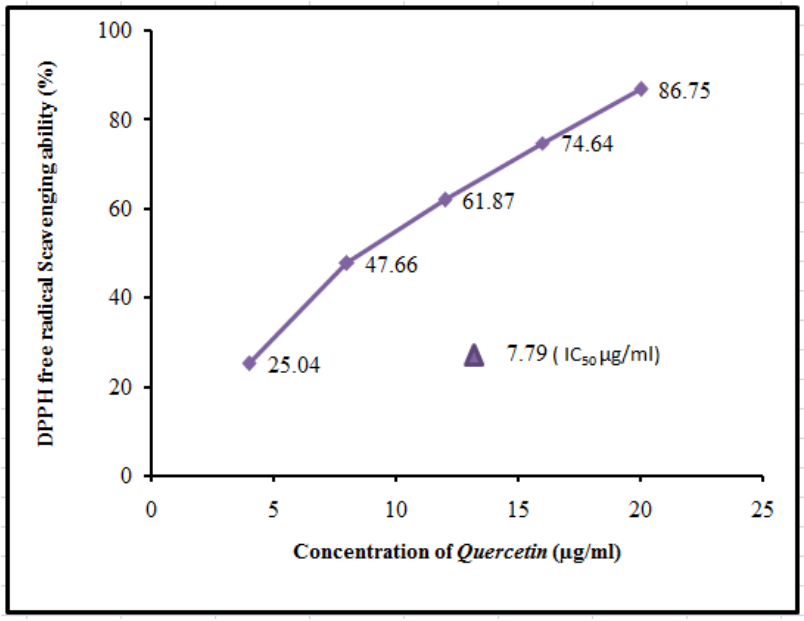

Fig 2: Antioxidant activity of the standard quercetin by DPPH radical scavenging activity

Table 1: Antimicrobial activity of the extract on selected species of bacteria.

Name of the organisms	ZOI(mm) Test Extract				ZOI (mm) Azithromycin
Concentration	**50 µg/ Well**	**100µg/ Well**	**150µg/ Well**	**200µg/ Well**	**30µg/ well**
Staphylococcus aureus	18.03±0.47	22.70±0.10	25.40±0.20	29.13±0.31	21.33±0.15
Klebsiella pneumoniae	19.07±0.42	21.33±0.12	23.40±0.20	26.33+0.50	22.00±0.20
Bacillus subtilis	22.90±0.26	24.53±0.31	26.53±0.23	28.20±0.35	24.40±0.20
Escherichia coli	20.13±0.50	21.07±0.23	23.27±0.12	25.40±0.20	21.40±0.20
Enterococcus feacalis	17.00±0.53	20.13±0.31	23.60±0.20	26.07±2.01	21.40±0.20
*Candida albicans**	18.40±0.20	19.60±0.20	21.27±0.12	22.47±0.31	20.70±0.44

ZOI: Zone of inhibition * Clotrimazole 30µg/well

Table 2: Luciferase reporter phage (LRP) assay

Conc. tested	Test organism								
	H37RV			All sensitive M.tuberculosis			MDR Tuberculosis		
	Control drug INH 0.2 µg/ml	Control drug RIF 2 µg/ml	Test drug	Control drug INH 0.2 µg/ml	Control drug RIF 2 µg/ml	Test drug	Control drug INH 0.2 µg/ml	Control drug RIF 2 µg/ml	Test drug
500 µg/ml	S	S	I	S	S	I	R	R	No -I
100 µg/ml	S	S	No-I	S	S	No -I	R	R	No -I

INH – Isoniazid, RIF –Rifampicin, MDR – multidrug resistant; S- Sensitive, R-Resistant,
NA – Not Available, ND – test not done, I- inhibition, No-I – No inhibition

5. Discussion

Dendrophthoe falcata showed antioxidant, antimicrobial and antimycobacterial activity at definite doses of the methanolic extract.

5.1 Antimicrobial activity of the Leaf extract

Continous exposure to microbial organisms in the environment is always a threat for survival of any living beings. Antimicrobial products from plants are less harmful than the synthesized in laboratories. The antimicrobial activity of the extract revealed that the extract had extensive antimicrobial activity that includes both antibacterial and antifungal properties. Staphylococcus aureus responded to the extract by showing maximum inhibition as well as the fungi candida albicans.

S P Pattanayak and P Sunita (2008) in their study also confirmed the use of this plant extract as an external application on wounds preventing the microbial invasion through the wound [10].

Karthikeyan A *et al.* (2012) in their study have confirmed the antibiofilm activity of the methanolic extract of leaves of *D Falcata* among the 17 bacterial strains [26].

The study results of Patil SH *et al.* (2012) and our were concurrent to each other. The growing evidence on the antimicrobial activity of the methanolic extract of the leaves of *Dendropthoe falcata* in the recent findings in literature gives an indication that in the near future with further studies could be leading to a new antimicrobial drug [27].

5.2 Antioxidant activity of the leaf extract

The presence of Triterpenes, Vitamins and Sterol compounds in the extract of *Dendropthoe falcata* leaves were found responsible for its antioxidant property [28]. Antioxidant activity of the extract was estimated through the DPPH radical scavenging activity. Free radical scavenging activity was found to be present in comparison to that of the Quercetin compound however the strength of the dosage of the extract required to bring a radical scavenging activity was found to be greater.

Quercitrin was isolated as a major component from the leaves of this plant which is supposedly a derivative of the standard drug quercetin for comparing the antioxidant activity of any other herbal extract [29].

SP Pattanayak (2011) in their study have confirmed the role of phenolic compounds present in the leaves of the plant to be the source of antioxidant properties exhibited by it [30].

Another study as reported by Nipun Dishora (2011) attributes the antioxidant activity of the plant to its polyphenol, flavonoid and phytosterol constituents [31].

Our study results showed an IC50 value of 37.71µg/ml whereas the reports of Rajdoula Rafe (2017) showed an IC50 of 43.49 through the same DPPH assay method of estimation of antioxidant property [32].

Our results were in concurrence with the findings of earlier studies reported.

5.3 Antimycobacterial activity of the leaf extract

Mycobacterium tuberculosis, the causative agent of disease tuberculosis is an ever evolving pathogen of all times. It is high time to research on this particular bacteria that has already evolved into drug resistant strains to a level of multidrug resistant (MDR) to extensive drug resistant (XDR) type bacteria.

Antimycobacterial activity through the Luciferase reporter page assay has given a promising result with antimycobacterial activity noted in a concentration of about 500µg/ml against both the basic H37RV and the all sensitive strains. Though there was no inhibition in the levels of 100µg/ml, the results with 500µg/ml was promising. MDR strain showed no inhibition for both the concentrations.

6. Conclusion

Use of the crude extract of this plant in traditional medicine in India for the cure of tuberculosis is well documented in the scientific literature however there is no single scientific evidence for the ability of this plant or its extracts activity against *Mycobacterium tuberculosis*.[33-36] Though the preliminary evidence against the bacteria is documented in this study, it requires further exploration of the plants different parts against the microbe that's taking toll of human lives everywhere across the globe.

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