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# Inhibitory activity of *Euphorbia hirta* (Tawa- tawa) extracts against *Mycobacterium tuberculosis* and other non mycobacterial pathogens

**Thirunavukkarasu Rajasekar, Sivaraj Anbarasu, Radhakrishnan Manikkam, Jerrine Joseph* and Vanaja Kumar**

*Centre for Drug Discovery and Development, Sathyabama University, Rajiv Gandhi Salai, Chennai*

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**ABSTRACT**

*Methanol, ethyl acetate and n-hexane extracts from the leaves of Euphorbia hirta were screened against non-mycobacterial pathogens and Mycobacterium tuberculosis $H_{37}Rv$ using agar well diffusion and Luciferase Reporter Phage (LRP) assay, respectively. Methanol extract of Euphorbia hirta showed 10-15 mm inhibition against B. subtilis, E. coli, and V. cholerae at 100µg/ml whereas no activity was observed against S. aureus and E. faecalis. The ethyl acetate extract showed activity only B. subtilis (12mm) and E. faecalis (10mm) while n- hexane extract showed no activity. In LRP assay, the ethyl acetate extract showed better activity with maximum of 64.73% reduction in RLU against M. tuberculosis $H_{37}Rv$ compared to methanol and n-hexane extracts. Further bioassay guided fractionation is in progress for the isolation, characterization and identification of active principle from Euphorbia hirta leaves.*

**Keywords:** Luciferase reporter phage assay, *Mycobacterium tuberculosis* $H_{37}Rv$, Tuberculosis, *Euphorbia hirta*

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## INTRODUCTION

Tuberculosis (TB), an infectious disease, is one of the major causes of mortality in the world. The burden of TB is highest in Asia and Africa, in which India and China together account for almost 40% of the world's TB cases [1]. According to WHO global tuberculosis report (2014) [2], over nine million people developed TB and 1.5 million died from the disease in 2013. The alarming increase in the rate of HIV-related TB, pediatric TB, latent TB, Multi Drug Resistant (MDR) and Extensively Drug Resistant (XDR) TB pose serious problem around the world. In addition, the side effects associated with most of the anti-TB drugs lead to patients' non-adherence to current anti TB therapy, resulting in the emergence of drug resistant tuberculosis. Hence, there is a need for new molecules to fight against dreadful diseases like TB. Natural, semi-synthetic or synthetic molecules from plant derived natural products have become a promising source for anti-infective therapy. Almost 85% of the world population was using the herbal medicines for treatment of various diseases and demand is increasing [3]. India has unique diversity of medicinal plants and vast traditional knowledge of their use as herbal medicine for curing various diseases [4].

Herbal drugs from plants have been reported to treat AIDS, cancer, malaria, chronic complaints, asthma, etc [5-7]. About 25% of the herbal drugs were obtained from higher plants [8]. Many natural compounds from plants are reported to be inhibitory to drug sensitive and drug resistant strains of *M. tuberculosis* [9]. Pure compounds have shown good inhibitory activity against *M. tuberculosis* $H_{37}Rv$, clinical isolates of MDR *M. tuberculosis* and non-

tuberculous mycobacterial [10]. Recent reports highlight the notable examples of natural compounds that may prove to be useful leads for TB drug discovery [11].

In this context, the *Euphorbia hirta* was selected for this study to determine the antimicrobial activity against *M. tuberculosis* and other non mycobacterial pathogens. *E. hirta* herb belonging to Euphorbiaceae family is found in many parts of the world. In India, this herb is distributed in Southern Western Ghats and Northern East Coast of Tamil Nadu [12]. The herbal plant is commonly known as Amman pachirisi in tamil and also called as Tawa-tawa, snake weed and asthma weed. Globally, the extracts of the plant are used for the treatment of asthma and respiratory tract infections, cough, chronic bronchitis and other pulmonary disorders, diarrhea, amoebic dysentery, boils, sore and wound healing [13]. The present study was undertaken with a view to test the potential of *E. hirta* leaf extracts for antimicrobial activity against *M. tuberculosis* and other non mycobacterial pathogens.

## MATERIALS AND METHODS

### Plant collection

*Euphorbia hirta* leaves were collected in September, 2014 from five regional localities in Mammadur village, Thiruvannamalai District of Tamil Nadu, India. The identity of the plant was authenticated by botanist from Loyola College, Chennai.

### Preparation of plant extracts

The leaves of the plant were gently removed, washed three times in tap water and finally with distilled water. After drying at room temperature for 24 hours, the leaves were ground in to fine powder by using mixie (Prestige). Crude extracts of the leaf powder were obtained using methanol, n-hexane and ethyl acetate. Briefly, 5 gm of leaf powder was soaked in 25 ml of methanol in 100 ml sterile conical flask and mixed thoroughly. Mixture was covered with cotton wool, plugged and wrapped with aluminum foil. Flasks containing the mixtures were kept at 100 RPM in orbital shaker for 24 hours at room temperature. Each mixture was then filtered using Whatman No.1 filter paper and the filtrate was dried at room temperature overnight. Extracts were divided into aliquots of 5.0 mg and dissolved in 5.0 ml of 1% DMSO (Dimethyl sulphoxide) to make a 5000 µg/ml solution (stock-1). Further each was diluted to achieve the concentration of 2500 µg/ml (stock-2), 500 µg/ml (stock-3), 250 µg/ml (stock-4) and 100 µg/ml (stock-5). The same procedure was adopted for the extraction with n-hexane and ethyl acetate. All the extracts were stored at 4$^{o}$C until further use.

### In vitro screening for antimicrobial activity

***Preliminary screening against non-mycobacterial pathogens:***

The different solvent extracts of *E. hirta* leaves were tested for antimicrobial activity against non-mycobacterial pathogens such as *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Vibrio cholerae,* and *Enterococcus faecalis* at 100µg/ml concentration by agar well diffusion method. Briefly, 7 mm well were made in Muller Hinton agar plates previously seeded with test organisms (1.5 ×10$^{8}$ CFU/ml equal to 0.5 McFarland). One hundred microlitre of different solvent extracts were added in to the wells. The zone of inhibition was measured after 24 hours of incubation at 37$^{0}$C and expressed in millimeter in diameter [14].

***Antimycobacterial screening by LRP Assay***

Antimycobacterial activity of different solvent extracts of *E. hirta* was tested against *M. tuberculosis* H37Rv at 250 and 500 µg/ml concentrations by adopting LRP assay [15]. About 350 $\mu$L of G7H9 broth supplemented with 10% albumin dextrose complex and 0.5% glycerol was taken in cryovials and added with 50 $\mu$L of stock-1 of crude extract in order to get the final concentration of 500 $\mu$g/mL. Stock-2 extract was used to make 250 $\mu$g/mL concentrations as mentioned above. Hundred $\mu$L of *M. tuberculosis* cell suspension was added to each vial. DMSO (1%) was also included in the assay as solvent control. All the vials were incubated at 37$^{o}$C for 72 h. After incubation, 50 $\mu$L of high titre phage phAE129 and 40 $\mu$L of 0.1 M CaCl2 solution were added to the test and control vials. Isoniazid and Rifampicin was also included as standard drug control. All the vials were incubated at 37$^{o}$C for 4 h. After incubation, 100 $\mu$L from each vial was transferred to luminometer cuvette. About 100 $\mu$L of D-Luciferin was added and relative light unit (RLU) was measured in luminometer (Berthold). Extracts showing RLU reduction by 50% or more when compared to control were considered as having antitubercular activity.

$$\text{RLU reduction (\%)} = \frac{\text{Control RLU} - \text{sample RLU}}{\text{Control RLU}} \times 100$$

## RESULTS AND DISCUSSION

The preliminary screening results of *Euphorbia hirta* leaves extracts against non-mycobacterial pathogens are summarized in Table 1. Among the three solvent extracts tested, methanol extract of *E. hirta* showed 10-15 mm inhibition against *B. subtilis*, *E. coli*, and *V. cholerae* whereas no activity was observed against *S. aureus* and *E. faecalis* at 100µg/ml. The ethyl acetate extract showed activity only against. *B.subtilis* (12mm) and *E. faecalis* (10mm), while n- hexane extract showed no activity. Mapatac et al. [16] reported that the methanolic extract of *E. hirta* showed good antimicrobial activity against *B. subtilis, S. aureus, P. aeruginosa* and *E. coli.* Shanmugapriya *et al*., [17] have shown that ethyl acetate extract of *Euphorbia hirta* displayed mild activity against *E. coli*, *S. aureus* and *B. subtilis* and hexane extract did not show activity against *E. coli*, *S. aureus* and *B. subtilis*. The plant, *Euphorbia hirta* is believed to possess strong antibacterial activity than *Euphorbia thymifolia* due to presence of tannin, alkaloids and flavonoids [18]. In our study, the leaves were collected in September month which showed good antimicrobial activity. It is observed that *E. hirta* plant leaves contain higher amount of antimicrobial constituents during mid August to December end [19].

There is a need for promising molecules to fight against drug resistant *M. tuberculosis.* For thousands of years, natural products from plants, animals and microorganisms have played an important role throughout the world in treating and preventing human diseases [20]. There are many antimycobacterial compounds belongs to the classes of flavonoids, terpenoids, chalcones and coumarins are previously isolated various plants around the world [21]. The detailed literature survey revealed that there are no reports on antimycobacterial activity of *E. hirta*. The present study reports the antimycobacterial activity of ethyl acetate extracts of *E. hirta* leaves. The ethyl acetate extracts at concentration of 500 µg/ml showed maximum reduction in RLU about 64.73% when compared to other extracts. In addition, the LRP assay is a high throughput method for anti TB screening. Previously many authors reported the anti TB activity of extracts from plants [22] and microbes [23] and synthetic compounds [24] by adopting LRP assay. The phytoconstituents present in the leaves of *E. hirta* includes saponin, sterol, terpene, alkaloids, polyphenols, tannins and flavonoids. Hence further bioassay guided fractionation along with chemical screening of the ethyl acetate extract of *E. hirta* leaves will result in the detection of chemical class responsible for anti TB activity.

**Table 1: Antimicrobial activity of different solvent extracts of *Euphorbia hirta leaves against* non-mycobacterial pathogens**
***Zone of inhibition expressed in millimeter in diameter**

| | Non-mycobacterial pathogens* | | | | |
|---|---|---|---|---|---|
| **Solvent extracts** | ***B. subtilis*** | ***S. aureus*** | ***E. coli*** | ***V. cholerae*** | ***E. faecalis*** |
| Methanol extract | 15 | - | 20 | 10 | - |
| n-Hexane extract | - | - | - | - | - |
| Ethyl acetate extract | 12 | - | - | - | 10 |

**Table 2: Activity of extracts of *Euphorbia hirta* against *M. tuberculosis* H37Rv by LRP assay**

| Compound | Concentration (µg/ml) | Percentage reduction in RLU |
|---|---|---|
| **Standard drugs** | | |
| Isoniazid (H) | 2 | 96.76 |
| Rifampicin (R) | 0.2 | 99.81 |
| ***E. hirta* extracts** | | |
| Methanol extract | 500 | 48.92 |
| | 250 | 32.02 |
| n-Hexane extract | 500 | 44.51 |
| | 250 | 10.57 |
| Ethyl acetate extract | 500 | 64.73 |
| | 250 | 42.24 |

## CONCLUSION

*Euphorbia hirta* exhibits significant antimycobacterial property. There are no reports on the effect of *Euphorbia hirta* against *M. tuberculosis*. Further detailed purification and identification of active compound will be carried out to develop the lead compound responsible for the anti TB activity.

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