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***In-vitro* MABA anti-tuberculosis assay of *Eclipta Alba* (L.) Hassk whole plant**

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Abstract

Tribal community are using traditional knowledge system to cure various diseases. They use natural source of drugs through trial and error method and the process is experienced over hundreds of years, this says that the medicinal plant have been in the focus as lifesaving drugs right from the beginning of the human civilization. *Eclipta Alba* (L.) Hassk one such plant which has a strong research support of its use in biological activities such as antihepatotoxic, ophthalmic, digestive, carminative, aphrodisiac, anti-inflammatory and antimycobacterial actions etc. *E. Alba* has shown a wide variety of medicinal importance, we have here tested *in-vitro* anti-tubercular activity by using Micro-plate Alamar Blue Assay (MABA) method. Ethanolic and aqueous extracts were prepared for evaluation of anti-tubercular activity against *Mycobacterium tuberculosis*. The result of the *in-vitro* anti-tubercular activity of the crude extracts at concentrations of 1 mg/mL, 25 mg/mL, 50mg/mL and 100mg/mL revealed that both the extracts (aqueous and ethanolic) inhibited the growth of the bacterium at MIC of 1 mg/ml.

Keywords: Antitubercular, *Eclipta Alba*, MABA, ethanolic extract, mycobacterium

1. Introduction

Before the introduction of chemical medicines, man relied on the healing properties of medicinal plants. It is thought that about 80% of the 5.2 billion people of the world live in the less developed countries and the World Health Organization estimates that about 80% of these people rely almost exclusively on traditional medicine for their primary healthcare needs [1]. It has been estimated, that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as India and China, the contribution is as much as 80%. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage [2].

The family Asteraceae or Compositae currently has 32,913 accepted species names, in 1,911 genera and 13 subfamilies. Most members of Asteraceae are herbaceous, but a significant number are also shrubs, vines, or trees. Asteraceae is an economically important family, providing products such as cooking oils, lettuce, sunflower seeds, artichokes, sweetening agents, coffee substitutes and herbal teas [3].

Eclipta Alba is commonly known as Guntakalagara or Bhringaraja or Maka belonging to the family Asteraceae / Compositae. The herb contain wedelolactone and demethylwedelolactone which possessing potent antihepatotoxic property [4]. Other Prominent chemical constituents present are Ecliptal, Ecliptine, Ecliptalbine, α -Terthienylmethanol, β -amyrin, Sigmasterol, Polypeptides etc. The other pharmacological activities shown by plants are Antiviral, Antibacterial, Spasmogenic, Hypotensive, Analgesic, Antioxidant etc [5].

Since *E. Alba* has shown a wide variety of medicinal importance, here we tested the anti-tubercular activity of the methanolic and aqueous extract of the whole plant by using Micro plate Alamar Blue Assay (MABA) method.

2. Materials and Methods

2.1 Plant material

The plant material *E. Alba* was collected from the local regions of Guntur, A.P., India, in August 2016. The plant species was authenticated by Dr. Maddi Ramaiah, Pharmacognosist, Department of Pharmacognosy, Hindu College of Pharmacy, Amaravathi Road, Guntur, A.P., India.

2.2 Preparation of crude extract by Maceration

The dried powdered plant material (whole plant, 1500g) was allowed to contact with solvent ethanol in a closed vessel and then allowed to macerate with occasional shaking for 7 days. Strain the liquid, press the marc; mix the liquids and finally clarifying by filtration. The extract thus obtained was concentrated under vacuum (50° C) by using Rotary Evaporator, dried completely and weighed. The extract thus collected subjected to preliminary phytochemical studies [6-10] and Invitro anti-TB assay. The percentage yield was found to be 16.67% w/w. Ethanolic and Aqueous extracts were prepared and were then filtered with the help of Whatman filter paper No. 1. The Samples were prepared with concentrations of 250mg/ml, 500mg/ml and 1000mg/ml.

2.3 Inoculum Preparation

Mycobacterium tuberculosis, Reference strain H37Rv was sub cultured and incubated at 37°C using Middle brook 7H9 broth which is supplemented with 0.2% glycerol and 10% OADC (to prepare 500ml 7H9 broth 50ml OADC was added) enrichment for 21 days (until logarithmic phase). The standard inoculum was prepared in sterile 7H9-medium adjusted to a McFarland standard No.1 (Equivalent to a standard suspension of 107 CFU/ml). This concentration was further diluted to 1: 25 and then used as an inoculum, to make that the bacteria were at the start of the log phase when the test commenced and 100µl was added to the inoculums to make the final volume of 200µl.

3. In vitro Anti-Tubercular Assay By Maba Method [11]

Mycobacterium tuberculosis reference strain H37Rv was used to evaluate the preliminary screening of given extracts at concentrations of 250mg/mL, 500mg/mL and 1000mg/mL. Microplate Alamar Blue Assay (MABA) method was used for the study. Prior to the bioassay, stock solutions of Rifampicin with the concentration of 32 µg/mL was prepared and stored to be used for the positive control. Control wells without the tested extracts and sterility controls were assayed simultaneously. Rifampicin was prepared from the stock solution just prior to inoculation time to the concentration of 5 µg/mL in the total volume of 200µl.

The growth inhibition result was explained by Microplate Alamar Blue Assay (MABA) using 1% resazurin. The reagent allows the detection of microbial growth in microtiter plates without the use of spectrophotometer. The susceptibility test conducted by the Microplate Alamar Blue Assay was using 96 well microtiter plate to evaluate the susceptibility of H37Rv MTB reference strain to the extract. The inhibitory concentration of all extracts was evaluated with concentrations of 250 mg/ml, 500 mg/ml and 1000 mg/ml in the total volume of 200µl. Prior to inoculation the crude extracts of 50mg, 100mg and 200mg were measured to make the proposed concentration in 200µl. The measured extracts were mixed with the bacterial suspension and the diluent media (7H9) in the well. One hundred micro liters of Middle Brook 7H9 broth and the test inoculum were added to all testing wells, as well as to the drug/extract-free control wells. Then, one hundred micro liters working extract solutions were poured into the first well of each row from which two-fold dilution series were made through the micro plate column. Each extract concentration was assayed in triplicate. Each micro plate was then sealed with parafilm and incubated for 5–7 days at 37°C in normal atmosphere. After the incubation period, 25 µL of resazurin 0.02% w/v was added to each wells and re-incubated at 37°C for 24 h for color development. The

Alamar Blue oxidation-reduction dye is a general indicator of cellular growth and/or viability; the blue, non-fluorescent, oxidized form becomes pink and fluorescent upon reduction. Growth was therefore determined by a visual color change.

Minimum inhibitory concentration (MIC) was determined for those extracts showing inhibitory effect. The MIC was conducted at various concentrations of 1, 6, 12.5, 25, 50, 100 mg/ml of the extracts. The visual MIC was defined as the lowest drug/extract concentration that prevented the color change of resazurin reagent from blue to pink. Blue color in the well was interpreted as there is no mycobacterial growth and pink color was scored as growth occurrence (Figure 1&2).

4. Results and Discussion

Herbal medicines are free from side effects, adverse effects and they are economical and easily available will be beneficial for the mankind over the centuries [12]. Phytochemical studies on the selected plant revealed the presence of flavonoids, alkaloids, tannins, triterpenoids, steroids and carbohydrates. The presence of above constituents in selected plant extract alone or in combination might be responsible for the observed activity.

Due to lack of its pathophysiology, the challenging task at this moment is to identify the novel methods which can identify and develop molecules. Even though *in vivo* models provide more predictable results, the National Institute for Research in Tuberculosis (NIRT) has planned an *in vitro* screening prior to *in vivo* testing of a therapeutic agent for reducing the consumption, usage and death of animals and for testing a large number of compounds in small quantities. In this study, ethanolic and aqueous extract of *E. Alba* were used to treat Tuberculosis. The result of the *in-vitro* anti-tubercular activity of the crude extracts at concentrations of 1 mg/mL, 25 mg/mL, 50mg/mL and 100mg/mL revealed that both the extracts inhibited the growth of the bacterium at MIC of 1 mg/ml. We conclude that the plant *E. Alba* could be used as adjuvant therapy for TB at different concentrations.



Fig 1: Minimum Inhibitory Concentration (mg/ml) of ethanolic extract of *E. Alba* showing Anti-Tubercular activity.

Columns 1-6: Concentrations of 100, 50, 25, 12.5, 6.125, 1mg/ml, respectively (Triplicates).

Rows: 7th Row -40µg/ml of Positive Control Rifampin,

8th Row-100µl of pure isolate of H37Rv strain of MTB.

Interpretation: Blue Color Change-Inhibition of Organism

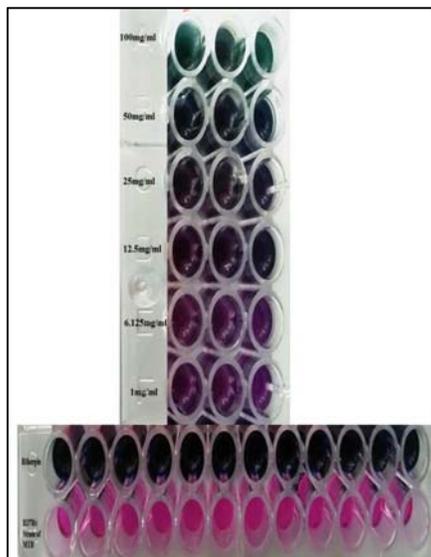


Fig 2: Minimum Inhibitory Concentration (mg/ml) of aqueous extract of *E. Alba* showing Anti-Tubercular activity.

Columns 1-6: Concentrations of 100, 50, 25, 12.5, 6.125, 1 mg/ml, respectively (Triplicates).

Rows: 7th Row -40 µg/ml of Positive Control Rifampin,

8th Row-100 µl of pure isolate of H37Rv strain of MTB.

Interpretation: Blue Color Change-Inhibition of Organism

5. Conclusion

By the above experiment we conclude that the both aqueous and ethanolic extracts of *Eclipta Alba* shows anti-tubercular activity at 1 mg/ml and it may be used for further investigations. Presence of flavonoids, alkaloids, tannins, triterpenoids, steroids and carbohydrates in selected plant extract alone or in combination might be responsible for the observed activity. Further studies need to be carried out to isolate the individual compounds from the crude ethanolic extract, their purification, characterization and pharmacological screening will be an informative tool in revolutionizing the plant based medicine for treatment of TB.

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