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 shave 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 antihapatotoxic, 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 Makbelon belonging to the family Asteraceae / Compositae. The herb contain wedelolactone and demethylwedelolactone which possessing potent antihapatotoxic property [4]. Other Prominent chemical constituents present are Ecliptal, Ecliptine, Ecliptalblime, α-Therhiylnmethanol, 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.


*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 was 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 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.

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.

Columns1-6: 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, 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

5. Conclusion

By the above experiment we conclude that the both aqueous and ethanolic extracts of Eclipta Alba shows anti-tubercular activity at 1mg/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.

6. References
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