



Antituberculosis Effect of *Polyclinum indicum* Ascidian Against Three *Mycobacterium* Strains

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KEYWORDS

Polyclinum indicum, tuberculosis, antimycobacterial activity, ascidian, resazurin.

ABSTRACT:

Introduction: *Polyclinum indicum*, a marine ascidian, has gained attention for its potential anti-tuberculosis (TB) properties. Tuberculosis, caused by *Mycobacterium tuberculosis* and related strains, remains a global health challenge due to rising drug resistance. Ascidians are known to produce bioactive compounds with antimicrobial properties, but their role in TB treatment remains underexplored.

Objectives: This study evaluates the anti-tuberculosis activity of methanolic extracts of *Polyclinum indicum* against *M. tuberculosis* H37Rv, SIT777, and SIT26 strains using the resazurin microtiter assay (REMA).

Methods: *Polyclinum indicum* samples were collected from the Rameshwaram Gulf Biosphere Reserve, extracted with methanol, and tested for antimycobacterial activity. REMA was used to determine the minimum inhibitory concentration (MIC). Rifampicin was used as a positive control. Extract dilutions (0.78–100 µg/mL) were tested against the mycobacterial strains, and MIC values were established based on colorimetric changes in resazurin.

Results: The methanolic extract exhibited moderate antitubercular activity with MIC values of 11.39 µg/mL for H37Rv, 16.14 µg/mL for SIT777, and 15.2 µg/mL for SIT26. These values were higher than rifampicin (2.4–4.8 µg/mL), indicating that while the extract demonstrated antimycobacterial potential, its efficacy was lower than the standard drug.

Conclusions: *Polyclinum indicum* exhibits moderate anti-TB effects, warranting further investigation into its bioactive compounds. Future studies should focus on compound isolation, mechanistic pathways, toxicity assessment, and potential synergistic effects with existing TB therapies.

1. Introduction

Polyclinum indicum ascidian, or colonial sea squirt, is a marine invertebrate belonging to the Phylum Chordata. [1] This organism has gained attention recently due to its potential anti-tuberculosis effects. [2] Tuberculosis (TB) is a contagious bacterial infection caused by various strains of the genus *Mycobacterium*, particularly *Mycobacterium tuberculosis*, *Mycobacterium bovis*, and *Mycobacterium africanum*. [3–5] These strains can cause respiratory symptoms, such as coughing, chest pain, and difficulty breathing, and can also affect other

parts of the body, including the bones, joints, and nervous system. [6–8]

Ascidians serve as a significant source of bioactive compounds with diverse medical applications, particularly in cancer research, though their potential extends to other therapeutic areas. [9] These marine organisms also host rich microbial communities—including bacteria, actinobacteria, cyanobacteria, and fungi, synthesizing potent natural products. Many of these compounds exhibit cytotoxic and antimicrobial properties, alongside antioxidant and anti-inflammatory



effects. [9,10]. Notably, extracts from the ascidian *Polycladium indicum* have demonstrated inhibitory activity against *Mycobacterium* species such as *tuberculosis*, *bovis*, and *africanum*. Laboratory and preclinical studies highlight their ability to suppress bacterial growth and replication, underscoring their promise as antitubercular agents. [11,12]

The exact mechanisms by which *Polycladium indicum* ascidian exerts its anti-TB effects are not yet fully understood. [13] However, it is believed that the bioactive compounds present in the ascidian possess antimicrobial properties that target the *Mycobacterium* cells. [14] These compounds may interfere with essential metabolic pathways of the bacteria, inhibit cell wall synthesis, disrupt protein synthesis, or induce apoptosis in the bacterial cells. [15]

Further studies are necessary to identify and isolate the specific active compounds responsible for the antimycobacterial activity of *Polycladium indicum* ascidian. Additionally, research is needed to investigate the potential toxicity, pharmacokinetics, and clinical efficacy of these compounds to determine their suitability as anti-TB drugs.

2. Objectives

The present study aims to focus on the antituberculosis effect of the methanolic extract of Ascidian *Polycladium indicum* against the mycobacterium strains H37Ra, SIT777, and SIT26.

3. Methods

3.1 Sample collection and extraction:

Ascidian specimens (*Polycladium indicum*) were collected from the coastal region of the Rameshwaram Gulf Biosphere Reserve (coordinates: 9.2876°N, 79.3129°E). After thorough rinsing with fresh water to remove debris, the samples underwent methanol extraction using a weight-to-volume ratio. The resulting extract was desiccated and pulverized for subsequent bioactivity assessments.

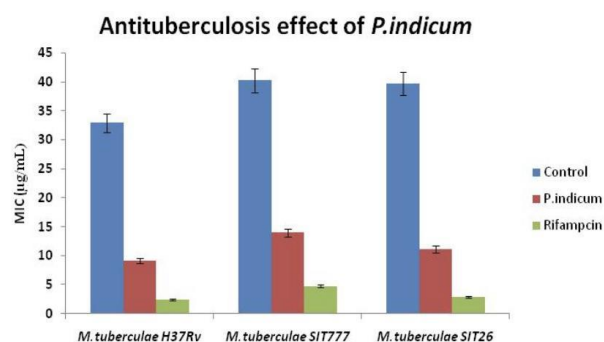
3.2 Anti-tuberculosis assay:

The antimycobacterial potential of the 80% methanolic extract from *P. indicum* was evaluated against *Mycobacterium tuberculosis* strains (H37Rv, SIT777, SIT26) using the resazurin microtiter assay (REMA). In

96-well microplates, Middle Brook 7H9 broth supplemented with OADC was combined with serially diluted extracts (0.78–100 µg/mL) and bacterial inoculum. Rifampicin (dissolved in DMSO) served as the positive control, while broth without extract acted as the negative control. Following 5–7 days of incubation at 37°C, 25 µL of 0.02% resazurin dye was added to each well. The minimum inhibitory concentration (MIC) was determined as the lowest extract concentration preventing resazurin's color shift from blue (no growth) to pink (bacterial viability).

3. Results

Polycladium indicum extract showed moderate antitubercular effect against *M.tuberculae* strains of H37Rv, SIT777, SIT26, at minimum inhibitory concentrations of 11.39, 16.14, and 15.2 µg/mL as IC50 values when compared to that of standard Rifampicin drug which showed MIC value at 2.4, 4.8 and 2.9 µg/mL. When compared to standard Rifampicin drug *Polycladium indicum* exhibited moderate antitubercular effect.



4. Discussion

In a prior investigation examining the anti-tuberculosis properties of various medicinal plants against multidrug-resistant *Mycobacterium tuberculosis* isolates it was found that extracts from five plants—*Acalypha indica*, *Adhatoda vasica*, *Allium cepa*, *Allium sativum*, and *Aloe vera*—demonstrated anti-tuberculosis activity in Lowenstein-Jensen (L-J) medium against MDR strains of *M. tuberculosis*. While the efficacy of *A. vera*, *A. vasica*, and *A. sativum* against these MDR isolates corroborated previous findings, the activities of



A. indica and *A. cepa* were reported for the first time. No inhibitory effects were observed against the rapidly growing strain *M. fortuitum* (TMC-1529). Additionally, in the BacT/ALERT system, these plant extracts exhibited significant inhibition against *M. tuberculosis*. The activity in L-J medium was quantified by calculating the percentage inhibition based on the mean reduction in colony counts in extract-treated wells compared to controls without extracts. [16,17]

In another study focused on the in vitro evaluation of anti-mycobacterial activity from selected medicinal plants against strains of *Mycobacterium tuberculosis* and *Mycobacterium bovis*, researchers found that crude extracts (80% methanol) from the roots of *C. aurea*, seeds of *O. basilicum*, and leaves of *A. abyssinica*, *C. macrostachyus*, and *E. camaldulensis* exhibited anti-mycobacterial effects, with minimum inhibitory concentrations (MIC) ranging from 6.25 to 100 µg/mL. These findings support the traditional use of these plants in tuberculosis treatment, suggesting their potential therapeutic value in combating TB. [18,19]

This study involved an in vitro assessment of the antituberculosis properties of methanolic extracts of *Polyclinum indicum* through in-vitro biochemical methods against mycobacterium strains H37Ra, SIT777, SIT26. It showed significant anti-tubercular activity at the MIC of

11.39, 16.14, and 15.2 µg/mL as IC₅₀ values against the respective strains. To our knowledge, there is currently no comprehensive review in the literature that analyzes the genus *Polyclinum*. This review aims to enhance understanding of its current research landscape and justify the need for thorough exploration and application of this genus. We will examine the botanical characteristics, ethnopharmacological uses, phytochemical composition, and pharmacological activities of *Polyclinum*, along with its distribution, potential mechanisms of action, and toxicological profiles.

5. CONCLUSION:

The ascidian *P. indicum* was evaluated for anti-tubercular effect against three mycobacterium strains through REMA in vitro methods. *P. indicum* has a moderate anti-tubercular effect when compared to the standard drug Rifampicin which is validated through

preliminary experimental results. Especially further research on *P. indicum* will lead to the isolation of drug candidates that can be used as an adjuvant for multidrug therapy in TB treatment. It is also significant because the inhibitory activity against many strains of Mycobacterium is observed; this indicates a potentially broad-spectrum efficiency in the fight against different types of tuberculosis. This adaptability is essential in light of the growing number of drug-resistant Mycobacterium strains, which present a major obstacle in the treatment of tuberculosis. The study also emphasizes how crucial it is to look into natural sources while looking for new drugs. Being a marine organism, *Polyclinum indicum* contributes to the increasing amount of data demonstrating the potential of chemicals produced from marine environments in pharmaceutical research. Although the results are promising, more research is necessary to identify the precise bioactive substances causing the reported antituberculosis effects. Furthermore, in vivo investigations and clinical trials are necessary to confirm the safety and effectiveness of treatments based on *Polyclinum indicum* in human beings. To sum up, this study's results add to the continuing attempts to find novel and efficient tuberculosis treatments. *Polyclinum indicum* has the potential to be an antituberculosis agent, which emphasizes the value of biodiversity in drug discovery and motivates more research into the therapeutic potential of marine species in the fight against infectious diseases.

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