Evaluation of the anti-tubercular activity of *Kielmeyera membranacea* constituents

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Tuberculosis remains the leading cause of mortality from a single infectious agent worldwide, with 1.25 million deaths and 13.4 million new cases in 2023. The emergence of multidrug-resistant and extensive drug-resistant strains underscores the urgent need for new therapeutic alternatives. Natural products represent a valuable source of bioactive compounds, historically providing a significant contribution to antimicrobial drug discovery.

Kielmeyera membranacea (*Calophyllaceae*) has previously demonstrated antitubercular activity. This study aimed to identify the compounds responsible for this activity and to evaluate the influence of climatic factors on their production. Plant material was collected from four restinga regions across all seasons. Antitubercular activity was assessed using the Resazurin Microtiter Assay against *Mycobacterium tuberculosis* H37Rv, revealing MIC_{90} the lowest value as 125 µg/mL, with variation across regions suggesting a influence of climatic factors on bioactive compound production.

Phytochemical analysis identified the biflavonoid podocarpusflavone A (PCFA) as one of the major constituents. PCFA exhibited synergistic activity with isoniazid, significantly inhibiting intracellular bacterial growth in THP-1 macrophages. *In silico* analysis suggested protein tyrosine phosphatase B (PtpB) as a potential target. To investigate this mechanism, *ptpB*-overexpressing strain was evaluated in infection assays. Treatment with PCFA (128 µM) and isoniazid (0.2 µM) resulted in a ~10-fold increase in CFU/mL compared to H37Rv strain suggesting that PtpB overexpression reduced susceptibility to the PCFA/isoniazid combination. Additionally, IL-6 and caspase-3 levels were significantly modulated, indicating that PCFA probably modulates host immune defense mechanism.

These findings highlight PCFA as a promising antitubercular candidate with immunomodulatory properties and potential for combination therapy.