

TITLE: Activity of Eugenol and characterization by ATR-FTIR in *Mycobacterium tuberculosis*

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ABSTRACT:

Tuberculosis (TB) is an infectious disease caused mainly by the acid fast bacillus *Mycobacterium tuberculosis* (*Mtb*). The standard TB treatment uses a combination of four drugs, in which isoniazid (INH), rifampicin (RIF) and ethambutol (EMB) compose part of the scheme. The emergence of *Mtb* drugs resistant leads to seek natural compounds with biological activity, and the development of new drugs is critical for the future control of TB. In this context, Eugenol is an essential oil with antibacterial, antifungal and anti-trypanosome activities. Some techniques involving spectroscopy and non-destructive method such as Raman spectroscopy and Attenuated Total Reflectance Fourier transform Infrared spectroscopy (ATR-FTIR), have been used in organic compounds, fungi and gram-positive bacteria for characterization and differentiation. Thus, the objective of this study was to detect the eugenol activity in *Mtb* H37Rv and characterize *Mtb* H37Rv and Eugenol in ATR-FTIR. The reference strain *Mtb* H₃₇Rv (ATCC 27294) was grown in Middlebrook 7H9-OADC and the Minimal Inhibitory Concentration (MIC) of Eugenol (Sigma-Aldrich E51791 99 %) was determinate by Resazurin Microtiter Assay Plate (REMA). For ATR-FTIR characterization, the *Mtb* H37Rv cells were washed with Milli-Q water 3 times, 5000 rpm 2 min, and heat inactivated at 80 °C for 30 min and the Eugenol was deposited directly in the sample compartment. Samples spectra were performed using a FTIR spectrometer equipped with ATR accessory, in the spectral range between 4000 a 400 cm⁻¹ with a resolution of 4 cm⁻¹ and 128 scans. Eugenol showed antimycobacterial activity, with MIC of 125 µg/mL and a promising compound for future studies. In *Mtb* H37Rv spectral, were observed some regions comprising mycolic acids (2800-3000 cm⁻¹), region of the carbonyl ester groups in lipids (1735-1745 cm⁻¹), organic phosphate groups (1224 cm⁻¹). The Eugenol spectral signal can be attributed to the presence of vinyl C=CH₂ (912, 995 cm⁻¹) vibration, aromatic C=C (1745, 1606, 1837 cm⁻¹) and methylene (1463 cm⁻¹) band in mid-infrared, a characteristic of lipophilic compounds. Therefore, Eugenol demonstrates a potential use, since the mycobacteria cell wall is highly lipophilic due to the presence of mycolic acids.

Keywords: ATR-FTIR Spectroscopy, *Mycobacterium tuberculosis*, Eugenol, tuberculosis