

**TITLE:** *IN VITRO* ACTIVITY OF EUPOMATENOID-5 IN NEUTRAL AND ACIDIC ENVIRONMENT AGAINST *Mycobacterium tuberculosis*

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**ABSTRACT:** The tuberculosis treatment (TB) has been exhibiting many problems since the beginning of chemotherapy due to long time and high toxicity. The ongoing worldwide problem has stimulated for searching new and more potent drugs. Eupomatenoid-5 (EUP-5) a natural compound, isolated from leaves of members of family *Piperaceae*, showed excellent *in vitro* antimycobacterial activity at neutral pH and excellent selectivity index, showing to be a promising compound to future new anti-TB drug. Pyrazinamide (PZA) is a prodrug, which is activated by the *Mycobacterium tuberculosis* pyrazinamidase inside of the macrophage (pH 5.5), contributing to short-term anti-TB therapy. As all studies with EUP-5 were carried out in neutral pH and the medium pH interferes with drug activity, the objective of the present study was to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of EUP-5 at acidic pH in *M. tuberculosis* H<sub>37</sub>Rv (ATCC 27294) strain. For the EUP-5 MIC determination, at pH 6.0, the colorimetric REMA (Resazurin microtiter assay plate) was performed in triplicate on different days. Initially for the MBC study, the antimicrobial agent-free growth control was determined by using the same previously standardized REMA inoculums (1.5 X 10<sup>6</sup> CFU/mL) followed by 1:500 dilution in sterile water and, 100 µL was seeded on Middlebrook 7H11 supplemented with OADC (oleic acid, albumin, dextrose and catalase) enrichment. For detecting the EUP-5 MBC, 100 µL of each compound dilution in REMA microplates, in which resazurin retained the original color (no growth observed) were seeded on Middlebrook 7H11 supplemented with OADC enrichment. The colony forming units (CFU) counting was visually performed for antimicrobial agent-free growth control and each EUP-5 dilution after 21 days at 37 °C. The numbers of colonies allowable for a 99.9 % EUP-5 MBC endpoint were determined. EUP-5 showed MIC 3.9 µg/mL in acidic culture medium as well as in neutral pH medium. Similar to MBC at neutral pH, previously determined with EUP-5, no bactericidal effect was observed at pH 6.0 in the tested concentrations. Although EUP-5 had no bactericidal effect in *M. tuberculosis* H<sub>37</sub>Rv in two different pH conditions, it remains a promising compound for the treatment of TB, since it showed excellent activity at acidic and neutral pH against the bacillus. Further studies are underway to clarify its activity in clinical isolates at acidic pH of this potential drug.

**Keywords:** Tuberculosis, acidic pH, minimal inhibitory concentration, minimal bactericidal concentration

**Development Agency:** Capes and CNPQ