

Antibacterial Activity of *Calendula officinalis* Extracts with Potential Use in
Biotechnology

Carrijo, L.C.^{1,2}, Tessarolo, N.G.^{1,2}, Pereira, T.H.A.^{1,2}, Romeiro, R.S.³, Baracat-
Pereira, M.C.^{1,2}

¹Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Viçosa (UFV), Brazil; ²BIOAGRO, UFV; ³Departamento de Fitopatologia, UFV, Viçosa, Brazil

Antimicrobial peptides (AMPs) have important role in protecting plants against various pathogens such as fungi, bacteria, viruses and nematodes. Ten families of AMPs from plants have been identified. *Calendula* (*Calendula officinalis* L.) is a medicinal plant, known for its healing and antiinflammatory actions and AMPs could be involved in these mechanisms. The objective of this study was to extract, purify and evaluate the action of antimicrobial peptides in *calêndula* flowers. Dried *Calêndula* flowers were macerated (Tris-HCl added of protease inhibitor) and centrifuged, and the soluble extract (SE) was recovered in the supernatant. The precipitate was extracted with LiCl in presence of protease inhibitors for obtainment of the cell-wall extract (CWE). SE and CWE were fractionated by ultrafiltration (cut-off 30kDa, 10kDa and 1kDa). Three concentrated fractions were obtained from each extract: SE1-10, SE10-30, SE greater than 30kDa, CWE1-10, CWE10-30, and CWE greater than 30kDa. All six fractions were precipitated with ammonium sulfate (35-75% sat.) and then desalted by ultrafiltration (1kDa), following anion-exchange chromatography(AEC). The obtained bands were according to the molecular masses of the ultrafiltrated extracts. For 12 hours of culture, SE promoted inhibition of *Raltonia solanacearum* (10-32%) and *Clavibacter michiganensis* subsp. *michiganensis* (up to 47%), and CWE inhibited the growth of these bacteria up to 31%. The antimicrobial fractions obtained after the AEC are being submitted to mass spectrometry analysis (after reversed phase-HPLC), for identification of AMPs for biotechnological applications. Other tests are in progress for evaluation and characterization of the peptides obtained to use in plant defense, as a healing ointment, and other biotechnological applications.

Palavras chave: *Calendula*, Antimicrobial peptides, Antibacterial Activity
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