



## ANTIMICROBIAL EVALUATION OF A CHITOSAN FILM INCORPORATED WITH EXTRACT OF *Piper solmsianum* (PIPERACEAE) LEAVES

Ana Paula Paterno Pacheco<sup>1\*</sup>, Clóvis Antônio Rodrigues<sup>1</sup>, Alexandre Bella Cruz<sup>1</sup> Rosi Zandoni da Silva<sup>2</sup>

<sup>1</sup> Master's Degree in Pharmaceutical Sciences, Universidade do Vale do Itajaí, Brazil.

<sup>2</sup> Department of Chemistry, Universidade Estadual de Ponta Grossa, Brazil.

\*E-mail: anappacheco@outlook.com

### INTRODUCTION

Nature provides us with several compounds with pronounced antimicrobial activity. The species *Piper solmsianum*, known in Brazil as pariparoba, has antibacterial and antifungal properties. From the exoskeleton of marine crustaceans, chitin can be obtained and subsequently converted into chitosan (CTS). CTS can be presented in different forms (solution, gel, film), exhibiting antimicrobial activity among others. The aim of this study was to prepare a film combining CTS and *P. solmsianum* extract and to evaluate the extract release from the film, cytotoxicity, physicochemical characteristics and antimicrobial activity.

### MATERIALS AND METHODS

The crude methanolic extract (CME) was obtained from the maceration of *Piper solmsianum* leaves at room temperature and subsequent filtering, solvent removal and extract concentration under reduced pressure at 50 °C. A 2.4% (m/v) chitosan solution was prepared by dissolving the material in 4.0% (m/v) CH<sub>3</sub>COOH. The release of CME from the QTPS film was evaluated by measuring the absorbance of an aqueous solution containing discs of the film. The physicochemical characterization of the QTPS film included analysis of thickness, opacity, moisture content, swelling ratio, water solubility and water vapor permeability. Cytotoxicity was assessed using L929 cells and the tetrazolium salt assay (MTT) to evaluate cell viability. Antimicrobial activity was investigated through the determination of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal/Fungicidal Concentration (MBC/MFC), compound interaction by checkerboard method, as well as disk diffusion and contact inhibition tests using the QTPS film.

### RESULTS

The extract exhibited antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*, while the chitosan dispersion demonstrated activity against *S. aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The combination assay between CME and chitosan indicated an additive effect against *S. aureus* and indifferent effect for *E. coli*, *P. aeruginosa* and *C. albicans*. Quantification of the CME release from QTPS film discs indicated a release of 6.9% over 24 h. Physicochemical characterization tests showed no significant differences in opacity, moisture content and water vapor permeability. However, an increasing trend in thickness and decreasing trends in swelling ratio and water solubility were observed when compared to the QT film (produced using just 2.4%(w/v) chitosan dispersion). The cytotoxicity assay revealed that the QTPS film maintained 80% cell viability. Microbiological analysis of the QTPS film demonstrated an inhibition zone of 11 mm ± 0.5 for *S. aureus*, with subculture confirming its bactericidal activity. In contrast, for *E. coli*, *P. aeruginosa* and *C. albicans* the activity was bacteriostatic/fungistatic.

### CONCLUSIONS

The tests indicated that the incorporation of *P. solmsianum* CME enhanced the antimicrobial spectrum of the chitosan film. Moreover, considering the evaluated physicochemical characteristics and cytotoxicity, the QTPS film demonstrates potential for various biomedical applications.

### ACKNOWLEDGMENTS

UNIEDU/FUMDES, CAPES/CNPQ and UNIVAL