

TITLE: ANTIMICROBIAL ACTIVITY OF POMEGRANATE EXTRACT FOR THE PREPARATION OF ORAL HYGIENE PRODUCTS OF DOGS

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ABSTRACT:

Herbal therapies are used worldwide to treat a variety of health conditions, including dental conditions in veterinary medicine. In this context, the use of medicinal plant-based formulations as a therapeutic and prophylactic resource in veterinary dentistry is worth highlighting. In order to develop a pomegranate extract formulation that improves oral hygiene of dogs, this work aimed to evaluate the antibacterial activity of the pomegranate extract against gram-positive and gram-negative strains in order to find the most efficient concentration to be added in the formulation. The minimum inhibitory concentration (MIC) was performed by the microdilution method on 96-Well polystyrene plates and reading on Elisa reader (600nm filter). *S. aureus* ATCC 25.923 and *E. coli* ATCC 25.922 were employed and prepared in specific medium and standardized at a concentration of 1.5×10^7 CFU/ml. Hydroalcoholic extract of pomegranate peels was prepared by percolation, followed by rotary evaporation and freeze drying. Solutions of extracts at the concentration of 100, 75, 50, 25, 10, 5, 2.5 and 1.25 mg/ml dilutions were prepared in 5% dimethylsulfoxide (DMSO) and sterile Mueller-Hinton broth. Negative control of the inhibitory activity of DMSO diluent and chlorhexidine 0.12%, bacterial growth and culture medium was performed. Plates were incubated at 35°C for 24h and MIC was defined as the lowest extract concentration capable of inhibiting microbial growth. The tests were done in triplicate in the same microplate, and in two different microplates. Growth of *E. coli* isolate was inhibited by a concentration of 25 mg/ml of extract, while the *S. aureus* showed no growth in the lowest concentration tested (1.25 mg/ml). Both the strains used in this study are sensitive to chlorhexidine 0.12%. According to the literature, the MIC values of pomegranate extracts determined in different studies significantly vary due to the different extraction method, part of fruits used and variations in the season and region of growth. There is a need for new effective pharmaceutical dosage forms for the maintenance of oral health in dogs. Our data indicate that pomegranate extracts have an effective inhibitory effect on bacterial growth. These data are extreme interest for the use of natural bioactive compounds, replacing synthetic active ingredients for the preparation of hygiene products for animal use.

Keywords: Minimal inhibitory concentration, pomegranate peel extract, *Punica*

granatum L.

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