TITLE: *Punica granatum* HYDROALCOHOLIC LEAF EXTRACT ASSOCIATED WITH CALCIUM HYDROXIDE ARE EFFECTIVE AGAINST MONO- AND POLYMICROBIAL BIOFILMS OF *Enterococcus faecalis* AND *Candida albicans*


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ABSTRACT:
*Enterococcus faecalis* and *Candida albicans* are common and important microorganisms found in teeth with refractory endodontic infection. *Enterococcus faecalis*, a gram-positive facultative anaerobic microorganism capable of invading the dentinal tubules, is more likely to be found in persistent infections than in primary infections. *Candida albicans* is a commonly identified species of fungi and has higher prevalence in saliva and in the root canal. Bacteria and fungal in the root canal organize either as free-floating single cells or organize communities attached to each other or to the areas of inaccessible root canal walls to form a biofilm. One of the major challenges in endodontic therapy is to remove pulpal debris and bacterial populations from the root canal system. The *Punica granatum* hydroalcoholic extract can be an alternative as channel dressing. The objective of this study was to evaluate the antimicrobial activity of hydroalcoholic leaf extract of *Punica granatum* (HEPg) against *Enterococcus faecalis* and *Candida albicans*, isolated and in mono- and polymicrobial biofilms. The agar diffusion technique was carried out, following the microdilution test in broth and biofilm inhibition evaluations were carried out against *Enterococcus faecalis* and *Candida albicans*. A significant antimicrobial effect of the HEPg and its association with calcium hydroxide against planktonic cells and mono- and polymicrobial biofilms was evidenced. Evaluation of the antimicrobial effects of PgL showed a MIC of 62.50 µg/mL for *C. albicans* and 15.62 µg/mL for *E. faecalis*. For calcium hydroxide MIC values were 125 µg/mL for both microorganisms. The results showed that 31.25 µg/mL of PgL is able to inhibit significantly monomicrobial biofilms composed of *Candida albicans* or *Enterococcus faecalis*. Calcium hydroxide (62.50 µg/mL) is able impair formation of *C. albicans* biofilms, but not *E. faecalis* biofilm at concentrations that we tested. The association between calcium hydroxide and PgL inhibits *Candida* biofilm formation at all concentrations tested and *Enterococcus* biofilm formation from concentration of 500 µg/mL + 250 µg/mL. The *Punica granatum* hydroalcoholic leaf extract presented good antimicrobial effect against biofilms important pathogens associated with endodontic infections and should be further investigated, including in vivo, to introduce it into the sanification of root canal systems.

Keywords: *Candida albicans*, *Enterococcus faecalis*, *Punica granatum*, endodontic infections.

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