

**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:**

*E. faecalis* and *C. 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 biofilmes 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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