Effective association of tea tree essential oil with conventional antibiotics to control Pseudomonas aeruginosa biofilms

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Pseudomonas aeruginosa is an important cause of acute nosocomial and biofilm-associated infections that are often difficult to eradicate. The increase of antimicrobial resistance, especially in sessile microorganisms, has promoted the search for new alternatives, as natural products, to control biofilm infections. Essential oils are natural, volatile, and complex compounds produced by aromatic plants as secondary metabolites[1]. Tea tree essential oil (TTO) is obtained from Melaleuca alternifolia, and it has been reported that exhibits a broad-spectrum antimicrobial activity that can be explored as an effective alternative to control biofilms[2,3]. This study aimed to assess the antibacterial activity of TTO associated with ciprofloxacin (CIP), a conventional antibiotic, against pre-formed P. aeruginosa biofilms, using two different application strategies: aggression of biofilms simultaneously with TTO+CIP, and aggression of biofilms firstly with TTO and then with CIP. Biofilms were characterized, before and after treatments, by total biomass, through crystal violet, and number of cultivable bacterial cells (log CFU/cm²). The association of the two compounds emerged after inspection of its individual antimicrobial activities that showed that biomass of P. aeruginosa biofilms was increasingly reduced when biofilms were treated with TTO, though it not significantly affected the viability of cells. On the other hand, CIP showed no considerably interference in biofilm control, since the reduction of biofilm biomass and number of viable cells was almost nonexistent, even for the highest concentration tested (80 μg ml⁻¹). Results related with the combination of TTO and CIP showed that both strategies experienced were very efficient, as they impaired significantly biofilm formation. It was observed a considerable decrease of biofilm biomass (>70 %) and number of cells (>3 log of reduction) even for 1.25 μg ml⁻¹ of CIP, when biofilms were aggresed with the TTO followed by CIP. The synergistic effect of TTO combined with CIP also resulted in a high decrease of biomass and a completely eradication of viable cells for 10 μg ml⁻¹ of CIP. These data highlighted the relevance of using natural products, as essential oils, to enhance conventional antibiotics activity, reducing the necessary concentration to treat an infection, and consequently reducing its toxicity and side effects. Therefore, this data provides further evidence that TTO-ciprofloxacin combinations may be a useful therapeutic option in the treatment of P. aeruginosa biofilm associated infections.

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Keywords: Biofilms, Tea tree essential oil, Conventional antibiotics, Pseudomonas aeruginosa.
In healthy individuals, many species of Candida are endogenous commensals of the gastrointestinal and urogenital tracts [1]. However, the prevalence of opportunistic fungal infections (candidosis) has been increasing dramatically over the recent decades and this is particularly evident in immunocompromised individuals [2]. The importance of candidosis is the potential synergistic effect on virulence and subsequent difficulties encountered in treatment. Moreover, a major virulence factor of Candida is its ability to adapt to a variety of different habitats and the consequent formation of surface attached microbial communities known as biofilms [3-4]. The resistance of Candida biofilms to antifungal agents was first demonstrated by Hawser et al., 1995 [5]. Although most episodes of candidosis are attributed to C. albicans, nowadays non-Candida albicans Candida (NCAC), such as C. glabrata, C. tropicalis and C. parapsilosis have emerged as important pathogens [6-7] and tend to be inherently less susceptible to commonly used antifungal agents [8-9]. So, in the last years the interest in natural compounds has increased, specifically some phenolic extracts which have been known in folk medicine as antimicrobial agents [10-11]. Thus, this work aimed to perform a screening of the antifungal potential of phenolic extracts from Castanea sativa, Filipendula ulmaria and Rosa micrantha flowers against Candida biofilms. The extracts were exhaustively characterized by HPLC-DAD-ESI/MS. Hydrolysable tannins were the main group of phenolic compounds in C. sativa and F. ulmaria samples, while flavonoids were the most abundant group in R. micrantha (6090 ± 253 mg/Kg). The minimal inhibitory concentration (MIC) was determined according to the guidelines in NCCLS document M27-A2 [12] with some modifications. All extracts revealed promising antifungal effect, with MIC values ranging from concentrations under 0.05 to 0.625 mg/ml. R. micrantha extract showed a strong effect (MIC ≤ 0.155mg/ml) against all strains tested and, it is also possible to assume that was against C. glabrata and C. parapsilosis that the phenolic extracts showed the highest activity (MIC).


Keywords: Biofilms, Candida species, Natural extracts, Antifungal effect.