ANTIBIOFILM ACTIVITY OF THE HYDROALCOHOLIC EXTRACT OF PHYLLANTHUS NIRURI (STONE-BREAKER)

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RESUMO

Biofilms are surface attached microbial cell aggregates, embedded in a self-produced extracellular polymeric matrix. The majority of human infections are biofilm associated microbial infections. Biofilms show more resistant to routine antibiotic treatments. Therefore, it's essential to invent novel antimicrobial compounds for the treatment purposes. In this case, antimicrobial natural products play a significant role. Plant based antimicrobial therapeutics become the best alternative against infectious diseases because of the relatively low toxicity, wide availability, and high efficacy. Further, pathogens usually do not show or develop drug resistance to phytotherapeutic agents. *Phyllanthus niruri* Linn. (Phyllanthaceae) known as "Stone-Breaker" is one such medicinal plant which used for the treatment of various diseases, including renal and hepatic diseases and microbial infections. On the other hand, *Candida* species is one of the frequent fungal isolates associated with various oral and non-oral human infections ranging from mild superficial infections to life threatening systemic infections. The current study aimed to evaluate the antibiofilm activity of the hydroalcoholic extract of *P. niruri* (HE-Pn) against *Candida albicans* (ATCC MYA-2876) preformed and developing biofilms. HE-Pn was prepared using the whole crushed plant and maceration technique using 70% alcohol. The solvent was evaporated and the final residue was lyophilized to obtain the crude extract. HE-Pn was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) to identify its chemical composition. Inhibitory effect of HE-Pn on Candidal biofilm development was evaluated by quantifying the viability of *Candida* biofilms developed with the 24h exposure to HE-Pn using the semi-quantitative XTT viability assay. Effect on established biofilms was determined by treating the preformed *Candida* biofilms with different concentrations (0.25 to 16 mg/mL) of HE-Pn for 24h followed by XTT viability quantification. The ultrastructural features of developing and established biofilms with HE-Pn treatment were determined using Scanning Electron Microscopy (SEM). All experiments were performed in triplicates. The most abundant chemical component of HE-Pn was ethyl ester of linolenic acid (23.38%). HE-Pn demonstrated an inhibitory activity on *C. albicans* biofilm development. 32 mg/mL HE-Pn reduced the viability of developing biofilms by 69% compared to negative control. 39% viability reduction was observed with 32 mg/mL HE-Pn treatment on established 24h biofilms. Both developing and preformed *Candida* biofilms treated with HE-Pn for 24 hours showed ruptured cells and leakage of intracellular content that indicate cell death. HE-Pn demonstrated promising antibiofilm activity against *C. albicans* biofilms. It is more effective in preventing *C. albicans* (ATCC MYA-2876) biofilm progression at early stages. The antibiofilm activity of HE-Pn was dose dependent. HE-Pn demonstrates membrane active antifungal properties.

PALAVRAS-CHAVE: Antibiofilm, Candida albicans, Hydroalcoholic extract, Natural products, Phyllanthus niruri

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