TITLE: ANTIVIRULENCE ACTIVITY OF Copaifera spp. OLEORESINS AND PURE COMPOUNDS AGAINST Porphyromonas gingivalis

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ABSTRACT:
Periodontitis is a polymicrobial infection that affects the tooth supporting tissue. It is characterized by connective tissue and alveolar bone loss. This work aimed to determine the antivirulence activity of the Copaifera paupera, C. pubiflora, and C. reticulata oleoresins and their pure compounds polyalthic acid (1), kaurenoic acid (2), and hardwickic acid (3) against the oral pathogen Porphyromonas gingivalis, which is involved in periodontal diseases. Two P. gingivalis clinical isolates (PG 01 and PG 03) and P. gingivalis (ATCC 33277) were used. To analyze the antivirulence activity, the following techniques were employed: Gingipain inhibition assay and Hemagglutination assay. The gingipain activity was measured by using the fluorescent protease substrates N-(p-tosyl)-Gly-Pro-Lys-4-nitroanilide acetate salt and Nα-benzoyl-L-arginine-7-amido-4-methyl-coumarin hydrochloride for lys-gingipain (Lys-X) and arg-gingipain (Arg-X), respectively. One of the oleoresins or pure compounds at concentrations ranging between 0.79 to 3.12 µg/mL was added to a bacterial suspension; the negative control was the bacterial suspension without the oleoresins or pure compounds. Released 7-amido-4-methylcoumarin was measured with a fluorimeter, and the proteolytic activity was compared to the proteolytic activity of the untreated control. The results were analyzed by one-way ANOVA with Tukey’s post-test. The C. reticulata oleoresin inhibited the Arg-X and the Lys-X enzymatic activities by 78.81% and 72.09%, respectively. The C. paupera oleoresin inhibited the Arg-X and the Lys-X enzymatic activities by 63.71% and 54.24%, respectively. The C. pubiflora oleoresin inhibited the Arg-X and the Lys-X enzymatic activities by 51.47% and 62.66%, respectively. As for the pure compounds, kaurenoic acid inhibited the Arg-X and the Lys-X enzymatic activities by 80.04% and 74.66%, respectively, while polyalthic acid inhibited the Arg-X and the Lys-X enzymatic activities by 66.11% and 61.94%, respectively. In turn, hardwickic acid inhibited the Arg-X and the Lys-X enzymatic activities by 54.78% and 51.10%, respectively. In conclusion, we have presented potential inhibitors of P. gingivalis cysteine proteases. The evaluated oleoresins and pure compounds inhibited the hemagglutination activity at all the evaluated dilutions. The evaluated Copaifera spp. oleoresins and pure compounds can be employed in the search for new effective agents that can act against the virulence of P. gingivalis.

Keywords: antibacterial activity, diterpenes, virulence, oral bacteria