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SUMMARY OF DOCTORAL THESIS

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Title: Biological constituents from Sudanese medicinal plants hinder the pathogenicity of *Porphyromonas gingivalis* TDC60

（*Porphyromonas gingivalis* TDC60の病原性を阻害するスーダン薬用植物の成分）

Oral illnesses are one of the very important chronic diseases. Of them, periodontal disease (periodontitis) is a major oral health problem, caused by bacterial inflammation of the tissues supporting the teeth. Numerous diverse bacteria exist simultaneously in the oral cavities of patients with periodontal disease. Particularly, *Porphyromonas gingivalis* a Gram-negative, asaccharolytic, obligate anaerobic rod bacterium appears to be the prime etiological agent associated with the progression of the inflammatory events underlying periodontal disease. *P. gingivalis* produces several virulence factors in the outer cell membrane. The primary stage of bacterial pathogenesis begins with the adhesion of bacterial cells to host tissues followed by colonization, which provides nutrients and allows bacteria to survive, grow, and produce further virulence factors. These virulence agents include numerous molecules associated with adherence and colonization such as fimbriae, lipopolysaccharides, proteases like gingipains, and hemagglutinins. The most important virulence factors that *P. gingivalis* produces are the hemagglutinins and gingipains, which play a vital role in the infection process through facilitating adhere of *P. gingivalis* to the surface of host cells following by acquisition of heme through erythrocyte binding and causes lysis and aggregation of erythrocytes via several proteolytic enzymes to release the heme moiety from the hemoglobin molecule as a nutrient.

Development of antiadhesive agents against *P. gingivalis* hemagglutinins and gingipains could be a promising cytoprotective strategy to prevent the harmful effects of long-term bacterial infection. Over the past few years, several plants derived natural products have been described for oral disease treatment and as adjuvant remedies that aim to reduce inflammation through interaction with the adhesion of *P. gingivalis* to host cells. In this study, we aimed to screen a group of Sudanese medicinal plants that are used traditionally in the Sudanese folkloric medicine against *P. gingivalis* TDC60 proliferation and virulence factors. Consequently, thirty-eight organ parts from twenty-five plant species were collected from Khartoum state, Sudan (The voucher specimens were prepared and identified at the faculty of agriculture and faculty of forest, university of Khartoum, Khartoum, Sudan) to investigate the potential inhibitory activities of their aqueous extracts. Thereafter, the research was aimed to isolate, purify and identify the biologically active compounds of the most active plants extract on *P. gingivalis* proliferation and specific virulence factor/s.

The thesis consists of five chapters, chapter one is a general introduction, research objectives, and thesis outlines. In chapter two, details of non-specific inhibitor isolated from *Origanum vulgare* leaves which restrains *P. gingivalis* TDC60 growth and virulence factors. Chapter three focuses on isolates from *Monechma ciliatum* seeds' extract that hampered *P. gingivalis* TDC60 hemagglutinins while chapter four demonstrated the influence of long-chain fatty acids using sub-MICs model against *P. gingivalis* TDC60 pathogenicity. Finally, chapter five shows: argeloside I, from *Solenostemma argel* leaves, a candidate hostile to the pathogenicity of *P. gingivalis* TDC60.

Predominantly, the crude aqueous extracts from 38 plant organs of 25 plant species were

screened to inhibit hemolysis, hemagglutination, gingipains and bacterial growth were illustrated in chapter two. Although several plants inactivated virulence factors (hemolysis, hemagglutination, and gingipains) independent of cell growth suppression, only three plants namely *O. vulgare* (leaves), *Glycyrrhiza glabra* (areal parts) and *Salvia offinalis* (areal parts) hindered cell growth and virulence agents of *P. gingivalis*. Among them, the aqueous extract of *O. vulgare* provided low MIC values for heme aggregation, erythrocyte lysis, and for gingipain. The inhibitory compound from *O. vulgare* leaves was isolated and characterized as a hydrolysable tannin-like compound that non-specifically suppressed *P. gingivalis* proliferation and virulence factors. Hydrolysable tannin-like compound was characterized against tannic acid using HPLC, Prussian blue staining and MALDI-TOF MS. The isolated compound inhibited the cell growth with a MIC of 0.05 $mg.mL^{-1}$ and gingipains (IC50 = 0.05 $mg.mL^{-1}$) as well as heme aggregation and erythrocyte lysis with a MIC of 0.0063 $mg.mL^{-1}$ for both virulence's.

The third chapter works were focused on the potent hemagglutinin inhibition found from *M. cilliatum* aqueous extract. Hemagglutinins are vital molecules, allow *P. gingivalis* to uptake iron and heme by attaching, aggregating, and lysing erythrocytes. To isolate and identify the inhibitory compounds, the water extracted from dry powdered seeds of *M. ciliatum* was partitioned using ethyl acetate followed by reversed-phase chromatography, thin-layer chromatography, ESI-MS, and NMR analysis resulting in the isolation of four compounds identified as oleic acid, coumarin, 1,2-dioleoylglycerol, and 1,3-dioleoylglycerol with MICs of 15–100 $\mu g.mL^{-1}$ against hemagglutination. This evidence of inhibitory activity will encourage the application of *M. ciliatum* effectively either as a functional food or therapeutic agent. Subsequently, in chapter four we extended our study to compare the influence of long-chain fatty acids on hemagglutinins in correlation to *P. gingivais* growth and gingipains. Ten fatty acids with different saturation degrees and carbon chain length were selected for the first screening against *P. gingivalis* growth and hemagglutinins activity. Of them, unsaturated fatty acids showed lower MIC values compared to that of saturated fatty acids. Five unsaturated fatty acids were selected for further analysis using sub-minimum inhibitory concentration (sub-MIC) model against *P. gingivalis* growth, hemagglutination activity and gingipains. The results showed that the sub-MICs dosages of unsaturated fatty acids significantly affected *P. gingivalis* growth as well as hemagglutinins and gingipains secretion. The N-terminal analysis of partially purified protein revealed that six protein bands corresponding to gingipain R1 hemagglutinin, lysine gingipain, and hemagglutinin genes decreased inversely with the sub-MICs concentrations of unsaturated fatty acids. The results of this section might support and expand the uses of the fatty acids as safe additives to impede undesired microbes to prevent periodontal diseases in the early stages.

The study in chapter five was focused on the isolation, purification and identification of the most inhibitory compound in the aqueous extract of *S. argel* leaves that reveal the most powerful inhibition activity against *P. gingivalis* proliferation among all test plants. We isolated and identified the inhibitory compounds by extracting *S. argel* leaves with distilled water, followed by several purification steps, including liquid-liquid separation, column chromatography, ^{1}H- and ^{13}C-NMR, and HR-ESI-MS resulting in a known pregnane glycoside, named as argeloside I. Argeloside I hindered cell growth of *P. gingivalis* TDC60 at 15 $\mu g.mL^{-1}$ and inhibited the hemagglutinins with a MIC value of 60 $\mu g.mL^{-1}$, as well as Arg and Lys specific gingipains with IC50 of 400 and 500 $\mu g.mL^{-1}$, respectively. The results show a new function for the pregnane glycosides, and argeloside I that might be a candidate preventing agent to reduce the risk of *P. gingivalis* TDC60 and adhesion factors.

In conclusion, the current dissertation demonstrated the inhibitory activities of selected plants used traditionally in Sudanese folkloric medicine against *P. gingivalis* pathogenicity. Several compounds were introduced to the scientific community as candidates to hostile *P. gingivalis* and/or its virulence factors including oleic acid, coumarin, 1,2-dioleoylglycerol, and 1,3-dioleoylglycerol, and argeloside I.