IDENTIFICATION OF ORAL BACTERIA FROM SUBGINGIVAL PLAQUE AND THE EFFECT OF *Salvadora persica* EXTRACTS ON THE GROWTH

Nur Fatimah Zaharah binti Salehuddin

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AUTHOR DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

Date: 13th April 2023

Signature:

Name: Nur Fatimah Zaharah Binti Salehuddin

Matric No: 3201478

Address: No 10B, Lot 3085, Kampung Padang Balang, Sentul, 51100 Kuala Lumpur

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ABSTRAK

Penyakit mulut biasanya ditemui pada populasi manusia disebabkan oleh pengumpulan plak bakteria. Kajian mengenai jenis bakteria mulut dalam populasi Malaysia masih berkurangan. *Streptococcus mutans* (*S. mutans*) adalah salah satu daripada streptokokus mulut yang memulakan penyakit periodontal sebagai penjajah awal. Lama-kelamaan, anaerob Gram-negatif menjadi lebih mantap, terutamanya *Porphyromonas gingivalis* (*P. gingivalis*). Walau bagaimanapun, bakteria boleh membina rintangan antibiotik terhadap ubat-ubatan dari semasa ke semasa. Jadi, *Salvadora persica* (*S. Persica*) atau dikenali sebagai miswak diakui kerana kehadiran sebatian bioaktif yang mengandungi aktiviti perencatan terhadap bakteria mulut. Objektif utama dalam kajian ini adalah untuk mengenal pasti bakteria mulut daripada plak subgingiva dan menentukan aktiviti antibakteria SPSE (ekstrak batang *S. persica*) terhadap bakteria mulut terpilih. Objektif khusus dalam kajian ini adalah untuk mengenalpasti jenis bakteria mulut dalam plak subgingiva menggunakan jujukan 16S rRNA, untuk menentukan aktiviti antibakteria SPSE terhadap bakteria mulut terpilih dan untuk menyiasat komposisi fitokimia SPSE menggunakan Spektrometri Jisim Kromatografi Gas (GCMS). Kajian ini telah berjaya menjumpai 176 bakteria mulut daripada 10 sampel plak subgingiva. Sebanyak 17 bakteria mulut telah dikenalpasti daripada semua sampel. 97 jenis bakteria Gram-negatif berjaya diasingkan daripada semua sampel. Majoriti adalah Gram-positif iaitu spesis Streptococcus dan lain-lain adalah bakteria Gram-negatif, termasuk *P. gingivalis*, *A. actinomycetemcomitans* dan *K. penumoniae S. persica* ekstrak telah dihasilkan dengan menggunakan pelarut *n*-heksana, diklorometana, aseton, etanol dan metanol. Aktiviti antibakteria SPSE-*n*-heksana, SPSE-DCM, SPSE-aseton, SPSE-etanol, dan SPSE-metanol ditentukan dengan melakukan tiga teknik berbeza, seperti ujian resapan cakera (DDA), kepekatan perencatan minimum (MIC) dan minimum kepekatan bakteria mati (MBC) terhadap *S. mutans* dan *P. gingivalis*. Spektrometri jisim kromatografi gas (GCMS) telah dianalisis untuk menyiasat komposisi fitokimia dalam SPSE yang boleh menghalang pertumbuhan *S. mutans* dan *P. gingivalis*. Daripada dapatan, terdapat perbezaan yang signifikan antara jenis pelarut yang digunakan dalam SPSE dan diameter zon perencatan bagi *S. mutans* dan *P. gingivalis* ($p < 0.001$). Untuk keputusan MIC, *S. mutans* telah dihalang dengan SPSE-*n*-hexane pada 3.125 mg/mL. Sementara itu, *S. mutans* dibunuh dengan 6.25 mg/mL SPSE-*n*-heksana Dalam analisis GCMS, fitokimia utama bagi semua ekstrak ialah asid benzoik, asid heksadeseniok dan asid oktadekanoik. Kesimpulannya, *S. persica* amat disyorkan sebagai salah satu alat kebersihan mulut untuk merawat penyakit mulut kerana keberkesanannya menghalang pertumbuhan bakteria mulut.

ABSTRACT

Oral diseases are commonly found in the human population caused by the accumulation of bacterial plaque. There is still lacking of study regarding types of oral bacteria in the Malaysian population. *Streptococcus mutans* (*S. mutans*) is one of oral streptococci which initiate periodontal disease as an early colonizer. Over time, Gram-negative anaerobes become more established, especially *Porphyromonas gingivalis* (*P. gingivalis*). Nevertheless, bacteria can develop antibiotic resistance to medicinal drugs over time. However, *Salvadora persica* (*S. persica*) or known as miswak is acknowledged significantly due to the presence of bioactive compounds that contain inhibitory activity against oral bacteria. The main objective in this study is to identify oral bacteria from subgingival plaque and determine the antibacterial activities of *Salvadora persica* Stem Extracts (SPSE) against selected oral bacteria. The specific objective of this study is to identify the type of oral bacteria in subgingival plaque using 16S rRNA sequencing, to determine the antibacterial activities of SPSE against selected oral bacteria and to investigate the phytochemistry compositions of SPSE using Gas Chromatography Mass Spectrometry (GCMS). This study successfully isolated 176 oral bacteria from 10 subgingival plaque samples. A total of 17 oral bacteria had been identified from all samples. About 97 Gram-negative bacteria were successfully isolated from all samples. The majority of the bacteria are Gram-positive which is Streptococcus species and others are Gram-negative bacteria, including *P. gingivalis*, *A. actinomycetemcomitans* and *K. pneumoniae*. SPSE were produced using *n*-hexane, DCM, acetone, ethanol, and methanol. Antibacterial activities of SPSE-*n*-hexane, SPSE-DCM, SPSE-acetone, SPSE-ethanol, and SPSE-methanol were determined by performing three different techniques, such as disc diffusion assay (DDA), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *S. mutans* and *P. gingivalis*. Gas chromatography mass spectrometry (GCMS) was analysed to investigate the phytochemistry composition in SPSE that can inhibit the growth of *S. mutans* and *P. gingivalis*. From the results, there is a significant difference between the type of solvents used in SPSE and the diameter of the inhibition zone for *S. mutans* and *P. gingivalis* ($p < 0.001$). For MIC results, *S. mutans* were inhibited with SPSE-*n*-hexane at 3.125 mg/mL. Meanwhile, *S. mutans* were killed with 6.25 mg/mL of SPSE-*n*-hexane. In GCMS analysis, the major phytochemical of all extracts was benzoic acid, hexadecanoic acid and octadecanoic acid. In conclusion, *S. persica* is highly recommended as one of the oral hygiene tools to treat oral diseases due to its effectiveness in inhibiting the growth of oral bacteria.

ملخص

هي واحدة من المكورات العقدية الفموية التي تسبب أمراض اللثة (*Streptococcus mutans* (*S.mutans* كمستعمر في وقت مبكر. ومع مرور الوقت، تصبح اللاهوائيات سالبة الجرام أكثر رسوخًا ، خاصةً) أو المعروفة باسم *S. persica*) ومع ذلك ، فإن سلفادورا بيرسيكا (*P. gingivalis*البورفيروموناس اللثوية (المسواك معروفة بشكل كبير بسبب وجود المركبات النشطة بيولوجيا التي تحتوي على نشاط مثبط ضد الهدف الرئيسي من هذه الدراسة هو التعرف على بكتيريا الفم من ترسبات تحت البكتيريا عن طريق الفم. ضد بكتيريا الفم المنتقاة. هذه الدراسة *S. persica*اللثة وتحديد الأنشطة المضادة للبكتيريا لمستخلصات نجحت في عزل 176 بكتيريا فموية من 10 عينات من البلاك تحت اللثة. غالبية البكتيريا كانت موجبة الجرام وهي من أنواع المكورات العقدية وغيرها من البكتيريا سالبة الجرام ، بما في ذلك وحيدات *K.* و الكلبسيلة الرئوية (*A. actinomycetemcomitans*) و *P. gingivalis*البورفيروموناس اللثوية ( ). تم تحديد الأنشطة المضادة للبكتيريا لمستخلصات سلفادورا بيرسيكا في المذيبات *pneumoniae* (الهكسان العادي، كلوريد الميثيلين، الأسيتون، إيثانول، ميثانول) من خلال تنفيذ ثلاث تقنيات مختلفة ، مثل فحص انتشار القرص ، و تحديد الحد الأدنى للتركيز المثبط و الحد الأدنى لتركيز مبيد الجراثيم و البورفيروموناس *S. mutans*) وفحص الأغشية الحيوية ضد المكورات العقدية الفموية MBC\MIC( ) للتحقق من تركيبة GC-MS. تم تحليل مقياس الطيف الكتلي اللوني للغاز (*P. gingivalis*)اللثوية (*S.*) التي يمكن أن تمنع نمو المكورات العقدية الفموية (*S. persica*)الكيمياء النباتية في مستخلصات (. من خلال النتائج ، تبين أن هناك فرق كبير بين *P. gingivalis*)ة (*s* و البورفيروموناس اللثوي*mutans* وقطر منطقة تثبيط لكل من المكورات العقدية *S. persica*)نوع المذيبات المستخدمة في مستخلصات ( ). بالنسبة لنتيجة للحد $p < 0.001$ (*P. gingivalis*) و البورفيروموناس اللثوية (*S. mutans*)الفموية ( ) بمستخلصات الهكسان العادي *S. mutans*) ، تم تثبيط المكورات العقدية الفموية (MICالأدنى للتثبيط (أي تم)MBC) عند 3.125 مجم / مل. وفي الوقت نفسه، الحد الأدنى مبيد للجراثيم (*S. persica*من ( ) عند 6.25 ملغ / مل من مستخلصات الهكسان العادي. *S. mutans*قتل المكورات العقدية الفموية (، كانت المادة الكيميائية النباتية الرئيسية GCMSوبالنسبة لنتائج تحليل مقياس الطيف الكتلي اللوني للغاز لجميع المستخلصات هي حمض البنزويك وحمض هيكساديسينويك وحمض الأوكتاديكانويك. في الختام ، كأحد أدوات نظافة الفم لعلاج أمراض الفم نظرًا لفعاليتها في تثبيط *S. persica*يوصى بشدة باستخدام نمو بكتيريا الفم

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LIST OF UNITS OF MEASUREMENTS

Colony forming unit per millilitre	CFU/mL
Gram	G
Mass-to-charge ratio	m/z
Micro litre	µL
Milli gram	Mg
Milli gram per millilitre	mg/mL
Millilitre	mL
Milli molar	mM
Nanometre	Nm
Svedberg	S
Volt	V

LIST OF SYMBOLS

°C	Celsius
=	Equal
<	Less than
X	Multiply
%	Percentage
:	Ratio

LIST OF ABBREVIATIONS

A. actinomycetemcomitans	*Aggregatibacter actinomycetemcomitans*
ADA	american diabetes association
ANOVA	analysis of variance
BHI	brain heart infusion
BLAST	basic local alignment search tool
Bp	base pair
CHX	Chlorohexidine
C. rectus	*Camphylobacter rectus*
CXCL8	c-x-c motif chemokine ligand 8
DCM	dichloromethane
DDA	disc diffusion assay
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
E. clocae	*Enterobacter clocae*
E. corrodens	*Eikenella corrodens*
E. ludwigii	*Enterobacter ludwigii*
E. mori	*Enterobacter mori*
E. nodatum	*Eubacterium nodatum*
E. roggen kampii	*Enterobacter roggen kampii*
E. tabacii	*Enterobacter tabacii*
F	*F*-value
F. nucleatum	*Fusobacterium nucleatum*
Gbp	glucan binding protein
GCMS	gas chromatography mass spectrometry
Gtf	glycosyltransferase
IL	interleukin
Kgp	lyseine-specific
K. pneumoniae	*Klebsiella pneumoniae*
MBC	minimum bactericidal concentration
MIC	minimum inhibitory concentration
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NA	not available
NCBI	national centre for biotechnology information
OD	absorbance
P	*p*-value
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
P. gingivalis	*P. gingivalis*
pH	potential of hydrogen
P. intermedia	*Prevotella intermedia*
P. micros	*Peptostreptococcus micros*
QS	quorum sensing
Rgp	arginine-specific
Rpm	revolutions per minute

rRNA	ribosomal ribonucleic acid
S. agalactiae	*Streptococcus agalactiae*
S. anginosus	*Streptococcus anginosus*
SD	standard deviation
S. downei	*Streptococcus downei*
S. gordonii	*Streptococcus gordonii*
S. lutetiensis	*Streptococcus lutetiensis*
S. mutans	*Streptococcus mutans*
S. oralis	*Streptococcus oralis*
S. persica	*Salvadora persica*
spp.	several species
SPSE	*Salvadora persica* stem extract
SPSS	statistical package for the social sciences
S. salivarius	*Streptococcus salivarius*
S. sanguinis	*Streptococcus sanguinis*
TAE	tris-acetate-EDTA
T. denticola	*Treponema denticola*
T. forsythia	*Tannerella forsythia*
TGF-β	transforming growth factor beta
Tm	temperature
TNF-α	tumour necrosis factor alpha
TNTC	too-numerous-to-count
USIM	university sains islam malaysia
WCA	wilkins chalgren anerobic
WHO	world health organization