

IN VITRO EVALUATION OF ORAL  
HYGIENE COMMERCIAL PRODUCTS FOR  
ANTIMICROBIAL SUSCEPTIBILITY AND IN  
COMBINATION WITH THE EXTRACTS  
FROM FOUR SPICES

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Doctor of Philosophy

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## **SUPERVISOR'S DECLARATION**

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy in Biotechnology

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## **STUDENT'S DECLARATION**

I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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## **DEDICATION**

*Dedicated to*

*My dearest family (father and mother)*

*For their extraordinary love and their endless care*

*My brothers and my sisters for their prayer*

*My husband for his support, encouragement, and patience*

*My son and daughter with love*

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## LIST OF ABBREVIATIONS

µL	Microliter
A	Amoxicillin
ADA	American Dental Association
An	Antagonism
AP	Ampicillin
Bac	Bactidol
Bg	BiG
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
Bn	Billion
BRICS	Brazil, Russia, India, China and South Africa
C	Chloramphenicol
CA	Colgate
Ca	cardamom
CAGR	Compound Annual Growth Rate
CFU	Colony Forming Units
Cg	Colgate
Ci	cinnamon
CI	Confidence Intervals
Ci/N	cinnamon /black cumin
CP	Colgate Plax
DC	Darlie Cleaning
DE	Darlie Enamel
ETM	Plant extract– Toothpaste – Mouthwash combination
FC	First Choice
FMCG	Fast-Moving Consumer Goods
FMI	Future market insights
FW	Fresh and white
GC-MS	Gas Chromatography –Mass spectrometry
GM	Gentamicin
Gu	Guardian

HCV	Hepatitis C virus
Hg	Halagel
HH	Himalayas Herbals
HIV	Human Immunodeficiency Virus
HN	Herbal Neem
IC <sub>50</sub>	Half maximal inhibitory concentration
LCM	Listerine Mint
LTC	Listerine Total Care
MBC	Minimum Bactericidal Concentrations
MDA	Malonaldehyde
MIC	Minimum Inhibitory Concentration
MP	Medicinal plant
mg	Milligram
MK	Morning Kiss
ml	Milliliter
mm	Millimeter
MO	Mu'min
MRSA	Methicillin-Resistant <i>Staphylococcus aureus</i>
MS	Mumin
MW	Mouthwash
N	black cumin
NE	Neomycin
OB	Oral-B Gum Care
OB1	Oral-B Spearmint
Ox	Oradex
OxG	Oradex Periodonatal
P	black pepper
P/N/Ca	black pepper / black cumin / cardamom
P/N/Ca/Ci	black pepper / black cumin / cardamom / cinnamon
PC-G	Penicillin G
R <sub>f</sub>	Retention Factor
RM	Raiya
RP	Rifampicin

SAC	Safi Complete
SAF	Safi Fresh
SAH	Safi Healthy
Sp	Sparkle
SS	Sensodyne
STM	Streptomycin
SY	Synergism
T	Tetracycline
TLC	Thin Layer Chromatography
TM	Toothpaste-Mouthwash
TMB	Toothpaste-Mouthwash with plant extracts combination B
TMBC	Toothpaste-Mouthwash with plant extract combination B and C
TMC	Toothpaste-Mouthwash with plant extract combination C
TP	Toothpaste
Wat	Watsons Mint
Wat1	Watsons Herbal
WHO	World Health Organization



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## ABSTRAK

Evolusi mikroba, termasuk pembangunan jenis tahan dan penyesuaian, bergantung kepada pelbagai ahli penduduk mikroba yang boleh hidup di dalam keadaan yang baru. Oleh itu, mikroba membentangkan kebolehan yang luar biasa untuk berkembang lebih cepat daripada mereka lakukan. Karies gigi adalah salah satu penyakit berjangkit biasa yang menjejaskan manusia hari ini, pembubaran struktur gigi oleh asid yang dihasilkan akibat daripada penapaian karbohidrat makanan oleh bakteria oral. Kepekaan antimikrob tumbuhan ubatan pada ubat gigi dan pencuci mulut terhadap pencilan lisan untuk penambahbaikan kesihatan. Pencilan lisan berbeza daripada 50 individu terpilih berumur 3 hingga 60 tahun telah diperolehi daripada kedua-dua jantina, dan sebanyak 59 pencilan bakteria dan yis telah dikumpulkan, dan diuji terhadap empat tumbuhan ubatan yang berbeza komersial ekstrak untuk profil sensitiviti antimikrobial. Sebanyak 20 jenis ubat gigi komersial yang berbeza dan 10 jenis kumur telah dibeli, dan tindakan gabungan tumbuhan perubatan dengan ubat gigi / ubat telah dikaji terhadap pencilan oral. Kami mendapati populasi bakteria yang lebih tinggi adalah dalam kumpulan umur 3-40 tahun daripada dua kumpulan lain, dengan kira-kira 44%. Ujian kepekaan antibiotik terhadap pencilan menunjukkan bahawa kloramfenikol mempunyai kesan sensitiviti yang tertinggi dengan 83.2%. Di samping itu, hemolysin mempunyai keupayaan yang paling tinggi untuk menghasilkan faktor kebisaan (72.88%). Tindakan gabungan ekstrak tumbuhan aseton (bersendirian) terhadap pencilan oral menunjukkan profil kesan sinergi, dengan 61.02%, untuk kombinasi A (Ci/N). Sebaliknya, tindakan gabungan tumbuhan perubatan dengan ubat gigi bertambah baik sensitiviti antimikrob sebanyak 90% untuk kombinasi B. Sementara itu, tindakan gabungan tumbuhan perubatan dengan ubat kumur telah dipertingkatkan dengan 100% untuk gabungan C. Aktiviti antimikrob gabungan ubat gigi - kumur dengan perubatan kombinasi tumbuhan B (TMB) adalah lebih tinggi daripada kumpulan gabungan pada kepekatan  $10^{-2}$  dengan 95% selang keyakinan, keberkesanan ubat gigi dan pencuci mulut tidak berkaitan dengan kumpulan harga dan zaman. Sebatian konstituen utama ekstrak tumbuhan telah dikenal pasti menggunakan GC-MS dan ditunjukkan oleh analisis TLC yang asid stearik dan palmitik dan dipamerkan sensitiviti antimikrob yang tinggi terhadap pencilan lisan di bawah kajian. Kami menyimpulkan bahawa ekstrak acetone gabungan B (P/N/Ca) dengan ubat gigi menunjukkan aktiviti tinggi terhadap pencilan oral. Selain itu, gabungan C (P/N/Ca/Ci) dengan ubat kumur menunjukkan aktiviti tinggi, oleh itu; TMB adalah yang terbaik ejen calon antimikrob terhadap pencilan oral.

## ABSTRACT

The evolution of microbes, including the development of resistant strains and adaptation, depend on diverse members of the microbial population that can thrive in new condition. Therefore, microbes present remarkable abilities to evolve faster than their hosts do. Dental caries considered as one of the most commonly infectious diseases affecting mankind today, the dissolution of tooth structure by acid produced as a result of the fermentation of dietary carbohydrates by oral bacteria. The antimicrobial susceptibility of medicinal plants including black pepper, black cumin, cinnamon, cardamom on toothpaste and mouthwash against oral isolates for healthcare improvement was studied. Different oral isolates from 50 selected individuals aged 3 to 60 years were obtained from both gender, and a total of 59 bacterial and yeast isolates were collected, purified, and tested against four different commercial medicinal plants extracts for antimicrobial susceptibility profile. A total of 20 different commercial toothpastes and 10 mouthwashes were purchased, and the combined action of the medicinal plants with toothpaste/ mouthwash were studied against oral isolates. We found a higher bacterial population was in the age group of 3–40 years than other two groups, with approximately 44%. Antibiotic susceptibility test against the isolates showed that chloramphenicol had the highest susceptibility effect with 83.2%. In addition, Hemolysin had the highest ability to produce virulence factors (72.88%). The combined action of acetone plant extracts (alone) against oral isolates showed a synergistic effect profile up to 61.02% when combination A (Ci/N) was added. Also, we found the combined action of plant extracts with toothpaste improved the antimicrobial susceptibility up to 90% in case of combination B. While, the combined action of medicinal plants with mouthwash was improved 100% with combination C. The antimicrobial susceptibility of combination toothpaste + mouthwash with plant extracts combination B (TMB) (3 in 1) was higher than combination group at concentration of  $10^{-2}$  with 95% confidence interval, the effectiveness of toothpaste and mouthwash are not related to the price and age group. The major constituent compounds of plant extracts were identified using GC-MS and demonstrated by TLC analysis which was stearic and palmitic acids exhibited high antimicrobial susceptibility against oral isolates under study. We conclude that the acetonic extract of the combination B (P/N/Ca) with toothpaste showed high susceptibility against oral isolates. Moreover, the combination C (P/N/Ca/Ci) with mouthwash showed high susceptibility, therefore, TMB was the best antimicrobial agent candidate against oral isolates.

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## APPENDIX H LIST OF PUBLICATIONS

### Published papers

1. Muna Jalal Ali, Essam A. Makky, and Mashitah M. Yusoff (2016): Microbiological Study against Bacterial Isolates from Different Patients. Australian Journal of Basic and Applied Sciences (AJBAS). (SCOPUS Index) **Accepted**
2. Muna Jalal, Essam A. Makky, Mashitah M. Yusoff (2016): “Impact of Antimicrobial Agents on Bacterial Isolates from Dental Decay” ARPN Journal of Engineering and Applied Sciences. Vol. 11, No. 16, pp 9689-9693 (SCOPUS Index).
3. Muna Jalal Ali, Essam A. Makky., Mashitah M. Yusoff (2016). Oral Health Care Improvement using Combined Toothpaste with Traditional Medicinal Plants. Advances in Environmental Biology (IF:0.21) ***Accepted***.
4. Essam A. Makky, Muna Jalal Ali and Mashitah M. Yusoff (2015): Impact of antibiotic and heavy metals susceptibility on oral isolate: A case study. Journal of Chemical and Pharmaceutical Research (JOCPR),7(7):1102-1108. (IF:0.32).
5. Muna Jalal Ali, Essam A. Makky, and Mashitah M. Yusoff (2015): Susceptibility of oral bacteria to antimicrobial agents and virulence factors. Journal of Chemical and Pharmaceutical Research (JOCPR), 7(3):1822-1829. (IF:0.32).
6. Muna Jalal Ali, Essam A. Makky, and Mashitah M. Yusoff (2015): Oral bacteria: Antimicrobial and virulence. Journal of Chemical and Pharmaceutical Research (JOCPR), 7(3):1816-1821 (IF:0.32).
7. Muna Jalal Ali, Essam A. Makky, Mashitah M. Yusoff (2015). Bacteriological study on dental caries and test their susceptibility to virulence factors and heavy metals. Advances in Environmental Biology,9(27); pp 301-306(IF:0.21).
8. Essam A. Makky, Muna Jalal Ali, Mashitah M. Yusoff (2015) Toothpaste Novelty and Reality Proceeding Book of Recent Advances on Environmental and Life. Korea: pp:164-170.
9. Tahira Batool, Essam A. Makky, Muna Jalal, Mashitah M. Yusoff. (2016) A Comprehensive Review on L- Asparaginase and Its Applications. Applied Biochemistry and Biotechnology.178(5):900-23.
10. Muna Jalal Ali, Essam A. Makky, Mashitah M. Yusoff (2015) Impact of Antimicrobial Agents against Oral Isolates. Proceeding Book of International Conference on Agricultural, Ecological and Medical Sciences (AEMS-2015): pp:19-22.

### Conference Proceeding

1. Muna Jalal, Essam A. Makky, Mashitah M. Yusoff: “Impact of Antimicrobial Agents against Oral Isolates” Proceeding Book of International Conference on Agricultural, Ecological and Medical Sciences (EAMS\_2015), 10–11 Feb. 2015, Penang, MALAYSIA. pp. 19 – 22.

2. Essam A. Makky, Muna Jalal Ali, Mashitah M. Yusoff: Toothpaste Novelty and Reality, Proceeding Book of Recent Advances on Environmental and Life Science.5-7 Sep.2015, Korea: pp164-170.
3. Muna Jalal, Essam A. Makky, Mashitah M. Yusoff: "IMPACT OF ANTIMICROBIAL AGENTS ON BACTERIAL ISOLATES FROM DENTAL DECAY" SCOPUS (9<sup>th</sup> MUCET 2015), JOHUR, Malaysia 11 – 13 Oct.2015.
4. Muna Jalal, Makky E.A., Mashitah M. Yusoff: "Microbiological Studies on Bacteria Associated with Dental Caries" Proceeding book of (8<sup>th</sup> MUCET 2014), Melaka, Malaysia 10 – 11 Nov. 2014.
5. Essam A Makky, Chan Cai Wen, Muna Jalal, Tahira Batool, Mashitah M. Yusoff : "Termite Control Biotechnology " Proceeding book of (GCEAS)GlobalConference on Engineering and Applied Science, Hong Kong, 6-7July .2015.

## Award

### Internal

- 1- Essam A. Makky, Muna Jalal Ali, and Mashitah M. Yusoff. 2017.3N1 ORAL CARE. Creation, Innovation, Technology and Research Exposition (CITREX), Universiti Malaysia Pahang, Gambang, Pahang, Malaysia (**Gold Medal**).
- 2- Essam A. Makky, Muna Jalal Ali, and Mashitah M. Yusoff. 2016. MOUTHWASH FACTS AND ORAL HYGIENE. Creation, Innovation, Technology and Research Exposition (CITREX), Universiti Malaysia Pahang, Gambang, Pahang, Malaysia (**Silver Medal**).
- 3- Essam A. Makky, Muna Jalal Ali., and Mashitah M. Yusoff. 2015. Do you trust in your toothpaste? *Creation, Innovation, Technology and Research Exposition (CITREX)*, Universiti Malaysia Pahang, Gambang, Pahang, Malaysia (**Silver Medal**).

### National

- 1- Essam A. Makky, Muna Jalal Ali, and Mashitah M. Yusoff. 2016. MOUTHWASH VS OP: REALITY and IMPROVEMENT. International Invention and Innovation Exhibition (ITEX\_2016), MALAYSIA. (**Gold Medal**).
- 2- Essam A. Makky, Muna Jalal Ali, and Mashitah M. Yusoff. 2017. MY-CARE: 3N1 ORAL HYGINE. International Invention and Innovation Exhibition (ITEX\_2017), MALAYSIA. (**Gold Medal**).

### International

- 1- Essam A. Makky, Muna Jalal Ali., and Mashitah M. Yusoff. 2016.EFFECTIVE HERBAL MOUTHWASH. Seoul International Invention Fair (SIIF-2016), Korea (**Silver Medal**).
- 2- Essam A. Makky, Muna Jalal Ali., and Mashitah M. Yusoff. 2016. NG- Toothpaste at International Festival Innovation on Green Technology (I-FINOG 2016), Malaysia (**Bronze Medal**).

### Patent

- UI2016701413: An oral care composition against oral pathogens,2016.  
 UI2017700692: A Composition of Oral Hygiene, 2017.