

**UNIVERSITI TEKNOLOGI MARA**

**EFFICACY OF MALAYSIAN  
PROPOLIS AS AN INTRACANAL  
MEDICAMENT AGAINST  
*ENTEROCOCCUS FAECALIS*  
BIOFILM: AN EX-VIVO STUDY**

**OSAMAH HASAN HAMOOD AL-MASOODI**

Thesis submitted in fulfillment  
of the requirements for the degree of  
**Master of Dental Science**

**Faculty of Dentistry**

**October 2022**

## ABSTRACT

This study aimed to compare the different selected extraction methods of propolis extracts against *Enterococcus faecalis* (*E. faecalis*) and to assess and compare the antibacterial activity of calcium hydroxide and an ethanol extract of propolis used as intracanal medicaments on root canals inoculated with *Enterococcus faecalis*. Extraction of Malaysian propolis (MP) was carried out using 70 ethanol by using different selected extraction methods, such as centrifugation-assisted extraction (CAE), vacuum-assisted extraction (VAE), and shaking-assisted extraction (SAE) methods were used. Antimicrobial activity against *E. faecalis* was assessed using the antibacterial susceptibility test (AST), followed by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Moreover, eighty-four intact human single-rooted teeth were extracted, decoronated and the root canals were chemomechanically prepared. The samples were sterilized before being inoculated with a pure culture of *E. faecalis* and cultured for 21 days. Prior to medication, colony counts were taken following incubation. Following that, samples were randomly divided into three groups (n = 28). Each group was subsequently subjected to a variety of intracanal medicaments, including Malaysian propolis (group 1), calcium hydroxide [Ca(OH)<sub>2</sub>] (group 2), and 5% DMSO as a control (group 3). The antibacterial efficacy of each intracanal medicament was determined by calculating the percentage reduction in colony counts (%RCC) on days 1 and 3. The data were analyzed statistically using a one-way analysis of variance and the post hoc Tukey Honestly Significant Difference (HSD) test. The result showed that SAE, followed by CAE, had better antimicrobial properties as compared to those obtained by VAE. The inhibition zones including the diameter of the well for SAE, CAE, and VAE were 11.3 mm, 10.67 mm, and 10.16 mm respectively. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for extracted MP by SAE were 12.5 and 25 mg/mL, respectively. Furthermore, Ca(OH)<sub>2</sub>'s MIC and MBC were determined to be 50 and 100 mg/mL, respectively. The % of RCC was greatest for Malaysian propolis (SAE), which demonstrated a 100% reduction on day 3, followed by calcium hydroxide, which showed a gradual increase in antibacterial activity, reaching 76.5% on day 3. As a result, the raw MP extracted using the SAE method was more effective than Ca(OH)<sub>2</sub> against *E. faecalis*.

## ACKNOWLEDGEMENT

In the name of Allah; praise is to Allah, and blessings and peace upon our Nabi Muhammad (S.A.W.). Thanks to God for providing me with the strength and patience to complete this thesis.

With immense gratitude, I would like to acknowledge the support and help of my supervisors, Associate Prof. Col. (R) Dr. Ikmal Hisham Ismail, and Dr. Hasnah Begum Said Gulam Khan, for their continuous guidance and patience towards my research development progress. Special thanks to Prof. Dato' Dr. Mohamed Ibrahim Abu Hassan, Former Dean of Faculty of Dentistry, UiTM, for continuous support in completing this research.

In addition, I would like to express my sincere gratitude to my family for their continuous support and help, especially my parents, brothers, and sisters. They always cared for my study and tried to be patient in my absence; thanks to them for understanding and giving me this chance to complete my MDS research.

Last but not least, I would like to thank Ms. Amiyah Bismelah and Ms. Suhaidah Kamaruddin for their assistance with these experiments in a research laboratory and Mr. Amin Asyraf Tamizi from MARDI Malaysia for providing Malaysian propolis from Raub, Pahang, Malaysia.

Thank you

# TABLE OF CONTENTS

	<b>Page</b>
<b>CONFIRMATION BY PANEL OF EXAMINERS</b>	<b>ii</b>
<b>AUTHOR'S DECLARATION</b>	<b>iii</b>
<b>ABSTRACT</b>	<b>iv</b>
<b>ACKNOWLEDGEMENT</b>	<b>v</b>
<b>TABLE OF CONTENTS</b>	<b>vi</b>
<b>LIST OF TABLES</b>	<b>viii</b>
<b>LIST OF FIGURES</b>	<b>ix</b>
<b>LIST OF ABBREVIATIONS</b>	<b>xi</b>
<b>CHAPTER ONE INTRODUCTION</b>	<b>1</b>
1.1 Research Background	1
1.2 Motivation	5
1.3 Problem Statement	5
1.4 Research Objectives	6
1.5 Alternative Hypothesis	7
<b>CHAPTER TWO LITERATURE REVIEW</b>	<b>8</b>
2.1 Introduction	8
2.2 <i>Enterococcus faecalis</i>	9
2.3 Intracanal Medicaments	15
2.4 Propolis	20
2.5 Propolis as Intracanal Medicament	28
<b>CHAPTER THREE RESEARCH METHODOLOGY</b>	<b>29</b>
3.1 Introduction	29
3.2 Materials	29
3.3 Samples Collections and Preparations	31
3.4 Preparation of Bacterial Suspension	33

3.5	Antibacterial Assay	35
3.6	Scanning Electron Microscopy (SEM) Analysis	37
3.7	Ethical Approval	37
3.8	Calculation of Samples	37
3.9	Collection and Preparation of Teeth	37
3.10	Inoculation of Samples	40
3.11	Root Canal Medicaments	41
3.12	Obtaining the Intracanal Medicaments	41
3.13	Placement of Intracanal Medicaments	41
3.14	Antimicrobial Efficacy	41
3.15	Culture Study	42
3.16	Data Analysis	42
3.17	Anticipated Research Outcome	43
	<b>CHAPTER FOUR RESULTS AND DISCUSSION</b>	<b>44</b>
4.1	Introduction	44
4.2	Determination of Bacterial Cell Morphology	44
4.3	Antibacterial Activities of Propolis Using Selected Extraction Methods	45
4.4	Scanning Electron Microscope Analysis (SEM Analysis)	51
4.5	Efficacy of MP (from SAE) and Ca(OH) <sub>2</sub> on <i>E. faecalis</i> -Inoculated Human Dentin Blocks	53
4.6	Discussion	55
	<b>CHAPTER FIVE CONCLUSION AND FUTURE RECOMMENDATION</b>	<b>60</b>
5.1	Conclusion	60
5.2	Limitation and Future Recommendation	60
	<b>REFERENCES</b>	<b>62</b>
	<b>AUTHOR'S PROFILE</b>	<b>85</b>