

UNIVERSITI TEKNOLOGI MARA

**THE *IN-VITRO* ANTIBACTERIAL
ACTIVITY OF *PLECTRANTHUS
SCUTELLARIOIDES* (L.) R.BR.
ETHANOLIC LEAF EXTRACT
AGAINST BACTERIA ASSOCIATED
WITH PERI-IMPLANTITIS**

NOR AMIYAH BINTI BISMELAH

Thesis submitted in fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Dentistry)

Faculty of Dentistry

March 2022

ABSTRACT

Chlorhexidine is often used commercially in oral rinses for adjunctive treatment in peri-implantitis. However, it is not recommended for daily regime due to common side effects such as taste alteration and staining. Hence, the need for an alternative remedy with reduced side effects and suitable for long term use. The present study investigates *Plectranthus scutellarioides* (L.) R.Br. ethanolic leaf extract as potential antibacterial oral rinse against peri-implantitis that involve both early bacteria colonizers as well as the late bacteria colonizers. The study involved phytochemical screening of the ethanolic crystallized extract of *Plectranthus scutellarioides* (L.) R.Br. using mass-based identification to identify the presence of chemical constituent. The ethanolic extract was then screened for its antibacterial activity against the early colonizer's bacteria namely *Staphylococcus aureus*, *Streptococcus mitis*, *Streptococcus sanguinis*, *Streptococcus salivarius*, *Streptococcus oralis* and *Actinomyces viscosus* as well the late colonizer bacteria namely, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Treponema denticola*, *Prevotella intermedia*, and *Tannerella forsythia* using disc diffusion method. The ethanolic extract was subsequently tested for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) as well as for its cytotoxicity effects on human gingival fibroblast cell (HnGF). The ultrastructural bacteria morphological changes were examined by scanning electron microscopy (SEM). Four flavonoid compounds were identified from *Plectranthus scutellarioides* (L.) R.Br. ethanolic extract, namely quercetin-3-glucoside, quercitrin, quercetin 3-(6"-acetylglucoside) and quercetin 3-O-acetyl-rhamnoside. The sensitivity test revealed that *Plectranthus scutellarioides* (L.) R.Br. ethanolic extract was effective against all the bacteria tested. MIC concentrations for the early colonizers were in the range of 1.56-12.50 mg/ml, and the MBC concentrations were within 3.13-12.5 mg/ml. For the late colonizers, the MIC concentration were within 3.13-12.50 mg/ml and MBC within 6.25-200 mg/ml. The ethanolic extract did not have any cytotoxic effect on HnGF cells at the tested concentrations within all three time periods (24, 48 and 72 hours). SEM images showed bacterial cell wall disruption for all the bacteria tested. The results showed that *Plectranthus scutellarioides* (L.) R.Br. ethanolic extract exerts its antibacterial property by disrupting the cell wall of all the bacteria tested. In conclusion, *Plectranthus scutellarioides* (L.) R.Br. ethanolic extract exerted its antibacterial activity property by disrupting the cell wall of the early and late bacteria colonizers of peri-implantitis. Hence, *Plectranthus scutellarioides* (L.) R.Br. ethanolic extract may be recommended for use as oral rinse for adjunctive treatment of peri-implantitis subjected to further study of its safety.

ACKNOWLEDGEMENT

Firstly, I wish to thank Allah His merciful grace for giving me the strength and capability to embark on my PhD journey and giving me the health and patience to keep on going. My deepest gratitude goes to my supervisor, Professor Dr. Rohana Ahmad, who expertly guided and provided grants through my six years of PhD journey. Without her guidance and persistent help, this study would not have been possible. I am also grateful to my co-supervisors, Dr Zethy Hanum Mohamed Kassim and Professor Dr. Norhadiani Ismail for their utmost support in helping me with their respective expertise throughout my study.

My appreciation also goes to staff of research laboratory at Faculty of Dentistry, University Teknologi MARA, for their help and guidance during my work. Special acknowledgment to Atta-ur-Rahman Institute for Natural Product Discovery (Aurins), University Teknologi MARA and iMAGE Centre, Faculty of Pharmacy, Universiti Teknologi MARA for providing me with knowledge and instrumentation to carry out my research.

Finally, this thesis is dedicated to my loving family, my pillar of strength who constantly give me their moral support, time and financial aid during my study. This wonderful gift is for all of you. Thank You.

TABLE OF CONTENTS

	Page
CONFIRMATION BY PANEL OF EXAMINERS	ii
AUTHOR'S DECLARATIONS	iii
ABSTRACT	iv
ACKNOWLEDGEMENT	v
TABLE OF CONTENTS	vi
LIST OF TABLES	x
LIST OF FIGURES	xii
LIST OF PLATES	xv
LIST OF SYMBOLS	xvii
LIST OF ABBREVIATIONS	xx
CHAPTER ONE: INTRODUCTION	1
1.1 Background	1
1.2 Problem Statement	2
1.3 Research Questions	3
1.4 Aim and Objectives	3
1.4.1 Aim	3
1.4.2 Objectives	4
1.5 Significance of study	4
CHAPTER TWO: LITERATURE REVIEW	5
Literature Review Outline	5
2.1 Peri-implant Diseases: Peri-implant Mucositis and Peri-implantitis	6
2.2 Etiology and Pathogenesis of Peri-Implantitis	7
2.3 Peri-implantitis vs Periodontitis	11
2.4 Peri-implantitis Risk Factors	17
2.4.1 Poor Plaque Control	17
2.4.2 Previous History of Periodontal Disease	17
2.4.3 Smoking	18
2.4.4 Diabetes	19
2.4.5 Stagnation of Residual Cement Materials Following Implant Prosthesis Cementation	19
2.4.6 Soft Tissue Characteristics	20
2.5 Treatment of Peri-implantitis	23
2.5.1 Conservative Therapies	23
2.5.1.1 Manual Treatment (Scaling and Polishing)	23
2.5.1.2 Laser Therapy	24
2.5.1.3 Photodynamic Therapy	26
2.5.1.4 Drug Therapy	29
2.5.1.5 Oral rinse	31
2.5.2 Surgical Therapy	32
2.5.2.1 Osseous Resective Surgery	33
2.5.2.2 Regenerative Therapy	34

CHAPTER ONE

INTRODUCTION

1.1 Background

Traditionally, missing teeth and supporting oral tissues have been replaced with dentures or bridges that aid in permitting the restoration of masticatory function, speech, and aesthetics. However, for the past 30 years, following the introduction of dental implants as a treatment modality, numerous patients that lost a tooth or teeth due to caries, periodontal disease, an injury, or some other reasons may have benefited from having prostheses that are retained or supported by implants. These implants are surgically placed into the jaw and following a period of osseointegration, they are connected to the dental prostheses (Humagain *et al.*, 2008).

Plaque accumulation is a common problem in implant patients, especially in the elderly population (Mariotti, 1999). The plaque widely accumulates around the neck of the implant abutments and on the fitting surface of the overdenture. These plaques are difficult to remove and require dentist intervention. A study has shown that there is a cause effect relationship between bacterial plaque accumulation and the development of inflammatory changes in the soft tissues surrounding oral implants (peri-implantitis). Hence, prevention is important to prevent progressive destruction of the tissues supporting an implant, which may compromise its usage and ultimately lead to its failure (Wingrove, 2011).

Studies have also shown the huge potential of plant herbs in treating a wide range of infection by providing a safer alternative and fewer side effects compared to commercialized drugs. According to the World Health Organization (2005), as many as 80% of the world's population depend on traditional medicine for their primary health care needs and over 50% of all modern clinic drugs are of natural product origin. Herbs have also been studied for their potential use to treat dental diseases. The common mode of delivery is in the form of aqueous solution for oral rinse.