Evaluation of antimicrobial efficacy of four medicinal plants extract used as root canal irrigant on Enterococcus faecalis (An In-Vitro Study)

Thesis submitted to the Faculty of Oral and Dental Medicine, Cairo University, in partial fulfillment of the requirements for Master Degree in Endodontics.

By:-

Radwan Ikrimah Al-Nabulsi

B.D.Sc (2009)

(Misr University for Science and Technology)

Department of Endodontics

Faculty of Oral and Dental Medicine

Cairo University

2015

Supervisors

Prof. Dr. Randa El Boghdadi

Professor of Endodontics

Faculty of Oral and Dental Medicine

Cairo University

Assoc. Prof. Dr. Hend Abou El-Nasr

Associate Professor of Endodontics
Faculty of Oral and Dental Medicine
Cairo University

Prof. Dr. Camilia George

Professor of Pharmacognosy

Faculty of Pharmacy

Cairo University

Judgment committee

Prof. Dr. Randa El Boghdadi

Professor of Endodontics

Faculty of Oral and Dental Medicine

Cairo University

Associate Professor of Endodontics

Faculty of Oral and Dental Medicine

Cairo University

Prof. Dr. Camilia George

Professor of Pharmacognosy

Faculty of Pharmacy

Cairo University

Prof. Dr. Salma El Ashry

Professor of Endodontics

Faculty of Oral and Dental Medicine

Ain-Shams University

Prof. Dr. Manar Fouda

Professor of Endodontics

Faculty of Oral and Dental Medicine

Cairo University



صدق الله العظيم الأيله (32) سوره البيقره

List of contents

	Page No.
List of tables	V
List of figures	VI
Introduction	1
Review of literature	3
Aim of the study	22
Materials and method	23
Results	39
Discussion	54
Summary and conclusion	62
References	65
Arabic summary	

LIST OF TABLES

Table No.	Table Title	Page No.
(1)	Weight and percentage of the total yield extract of each plant.	27
(2)	Weight and percentage of dry extracts of each solvent for each plant.	28
(3)	Growth inhibition zone diameter in (mm) as detected by the agar well-diffusion test.	41
(4)	Pair wise statistical comparison between the most potent tested plants extracts along with the positive control.	45
(5)	MIC of the tested plants extracts and the positive control (5.25% NaOCl).	47
(6)	Results of the direct contact test of the tested plants extracts and control groups on <i>E. faecalis</i> .	50
(7)	Statistical comparison of the direct contact test results between the tested plants extracts along with the positive control (5.25 NaOCl).	51

LIST OF FIGURES

Figure No.	Figure title	Page
		No.
(1)	Samples of the tested medicinal plants.	26
(2)	Filtration funnel.	26
(3)	Rotatory evaporator device.	27
(4)	Separation funnel.	28
(5)	Samples of Muller Hinton agar plate showing the zone of inhibition measured in (mm).	32
(6)	Sample of MIC serial dilution test.	34
(7)	Flow chart of the root specimen grouping in	36
	the direct contact test.	
(8)	Bar chart of the antimicrobial effects of	42
	tested plants extracts against (E. faecalis).	
(9)	Samples of Muller Hinton agar plates	43
	demonstrating growth inhibition zones.	
(10)	Bar chart showing the effect of the tested	52
	plants extracts & the control groups on the	
	CFU/ml count.	
(11)	Muller-Hinton agar plates after sampling for	53
	the CFU.	

Acknowledgment

First of all I want to thank Allah who helped me and gave me the patience to complete this work.

I Would Like To Express My Sincere, Unlimited Appreciation And Gratitude To Prof. Dr. Randa El Boghdadi, Professor Of Endodontics, Faculty Of Oral And Dental Medicine, Cairo University, For Here Guidance, Valuable Advice, Support, Endless Help And Supervision During The Entire Course Of This Study.

I Would Like To Thank **Dr. Hend Abou El-Nasr**, Associate Professor Of Endodontics, Faculty Of Oral And Dental Medicine, Cairo University, For Her Unlimited Kindness, Care, Concern, Valuable Cooperation And Helpful Remarks.

I Would Like To Thank Prof. Dr. Camilia George, Professor Of, Pharmacognosi Faculty of Pharmacy, Cairo University, For Her Kindness, Concern and Valuable Cooperation. I Would also Like to Thank **Dr. Nabil Abo El-fetoh**, Associate Professor Of, Pharmacognosi Faculty of Pharmacy, Cairo University, and For His Concern, Valuable time and Cooperation.

I Would Like To Thank **Dr. Marwa Mostafa**, Lecturer Of, Microbiology, Al-Azhar University, For Her Concern and Valuable Cooperation

My deep appreciation goes to my colleagues Abdulrahman Al-Fadage, Ahmed Yaseen and Mohammad Sami for their good advice and valuable support that I needed during this thesis.

Dedication

To the Soul of my Mother, who taught me that even the largest task can be accomplished if it is done one step at a time, and gave me unbelievable love and support. (I hope that this achievement will complete her dream and make her proud).

To my Father, who taught me that the best kind of knowledge to have is that learned for its own sake.

To my Wife, who pushed me ahead, offered me unconditional love, care and kindness.

To my Cousin Irfan, Brothers, Sister and my mother-in-law, who provided me with hearty worth advices and forceful support throughout my life.

Introduction

Success of endodontic therapy depends on many factors, starting from access cavity preparation, biomechanical preparation, and three dimensional obturation of the root canal system.

Eradication of the root canal infection is very important during endodontic treatment, since residual infection is one of the chief factors leading to post treatment failure. *Enterococcus faecalis* is one of the most frequently isolated microorganisms from endodontic infections. This is explained by its various survival and virulence factors; including its ability to compete with other microorganisms, invasion of dentinal tubules and resistance to nutritional deprivation.

Debridement of the root canals to remove all pulpal tissues and microbes contributes to a great extent to the success of root canal therapy.

The irrigants that are currently used during cleaning and shaping include sodium hypochlorite (NaOCl), chlorhexidine, hydrogen peroxide, ethylenediaminetetraacetic acid (EDTA) and a mixture of tetracycline, an acid and a detergent (MTAD). These chemical irrigants lead to many harmful accidents during treatment.

Because of the cytotoxic reactions of most of the commercial irrigants used and their inability to totally eliminate bacteria from root canals, trend of recent medicine attempts to use biologic irrigants extracted from natural plants.

Herbal products have been used since ancient times, involving both eastern and western medicinal traditions. Many plants with biological and antimicrobial properties have been studied since there has been a relevant increase in the incidence of antibiotic overuse and misuse. In dentistry phytomedicines have been used as anti-inflammatory, antibiotic, analgesic and sedative agents

Hence it seems beneficial to study and evaluate the antibacterial efficiency of some medicinal plants extracts when used as root canal irrigants on *Enterococcus faecalis*.

The medicinal plants used in this study were, Neem (Azadirachta indicia A.Juss) leaves, Ginger (Zingiber officinale Roscoe) rhizomes, Miswak (Salvadora Persica L.) sticks and Lemon (Citrus limonum Risso) fresh solution.

Review of Literature

There is a direct relationship between the presence of microorganisms before the root canal obturation and the failure of the endodontic therapy ⁽¹⁾. *Enterococcus faecalis* is commonly found in a high percentage of root canal failures and it is able to survive in the root canal as a single organism or as a major component of the flora ^(2,3).

Root canal irrigation plays an important role in the debridement and disinfection of the root canal system and is an integral part of root canal preparation procedures. The most frequently used irrigant is NaOCl (4). Its and disinfecting capability have been tissue dissolving demonstrated in several investigations (5-9). However its use may lead to many accidents during endodontic treatment as damage to dentist or patient clothing, and contact with the patient's or operator's eyes results in immediate pain, profuse watering, intense burning and erythema (10). Injection of sodium hypochlorite beyond the apical foramen may lead to immediate strong reactions with extreme pain, signs of hematoma, ecchymosis, profuse hemorrhage from the root canal and burning sensation felt by the patient (11), allergic reactions and hypersensitivity to sodium hypochlorite which may cause severe pain, burning sensation, lip and cheek swelling, ecchymosis, profuse hemorrhage from the root canal and breathing problems (12, 13).

Role of medicinal plants in endodontics:-

Herbal products have been used since ancient times in folk medicine, involving both eastern and western medicinal traditions. Many plants with biological and antimicrbiological properties have been studied since there has been a relevant increase in the incidence of antibiotic overuse and misuse. In dentistry Phytomedicines has been used as anti-inflammatory, antibiotic, analgesic and sedative agents ⁽¹⁴⁾.

1- Neem (*Azadirachta indica A.Juss*), Family Meliaceae. نبات النيم Chemical constituents of Neem leaf extract:-

Neem leaves contains as much as an oil rich in; Nimocinol, Nimocinolide, Isonimocinolide, Meldenin, Isomeldenin, Melianol, Zeeshanol, Zafaral, Meliacinol, Meliatetraone, Meliacinanhydride, Meliateraolenone, Salanin, Nimbin, Azadirone, Azadirachtins (15, 16).

Neem has been used as a medicinal agent for more than 2,000 years, about 75% of Ayurevedic remedies contains some form of neem, Indians call the sacred neem tree "the village pharmacy" (17). All parts of the neem tree are used; flowers, seeds, fruits, roots, bark, and in particular leaves which were used to treat more than 100 diseases (18). Even in Western medicine, the list of reported medicinal benefits in published studies (18, 19) is extensive, and includes, antibacterial, anticarcinogenic, anticlotting, antifungal, antihepatic, antihyperglycemic, anti-inflammatory, antileprosy, antimalarial, antimicrobial, antimutagenic, antioxidant, antiparasitic, antipurgative, antirheumatic, antiseptic, antituberculosis, antiulcer, antiviral, antiworm, for boils, blood detoxifier, diuretic, for eye diseases, immunomodulatory, pimples and for skin diseases (17-19).