ANTIMICROBIAL ACTION OF FLAXSEED AND *NIGELLA SATIVA* OIL ON SELECTED ORAL PATHOGENS IN INTRACANAL NICHE

BY

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ABSTRACT

This research sought to identify the ability of flaxseed and *Nigella sativa* extract act as an alternative medicine for intracanal medicament which much safer for pregnant women and small children. The currently available conventional method of root canal therapy involves the use of chemical or synthetic antibiotic-based root canal dressing that are costly and had to be replaced. This will give the caustic side effects seen in the chemical or synthetic antibiotic dressing such as postoperative pain and etcetera. The potential use of intracanal dressing based herbal will provide better safety regards to lesser side effects. Flaxseed (*Linum usitatissimum*) and *Nigella sativa* have been shown to demonstrate some antimicrobial properties. However, its effects on oral pathogens are still limited. Hence, the present study aimed to investigate the antimicrobial effect of the flaxseed, *Nigella sativa* and the combination between those two against the selected oral pathogen (*Streptococcus pyogenes*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterococcus faecalis*). The efficacy of flaxseed, *Nigella sativa* and their combination was tested using disc diffusion test, minimum inhibition concentration (MIC) and minimum bacteria concentration (MBC) on the selected oral pathogen. Then all the extracts were determined efficacy within the controlled environment of the intracanal model. All the tests were done in triplicate.

خلاصة البحث

يسعى هذا البحث إلى تحديد قدرة بذور الكتان ومستخلص حبة البركة كدواء بديل للعلاج داخل القحف والذي يكون أكثر أمانًا للنساء الحوامل والأطفال الصغار. تتضمن الطريقة التقليدية المتاحة حاليًا لعلاج قناة الجذر استخدام الضمادات الكيميائية أو الاصطناعية التي تعتمد على قناة الجذر والتي تكون مكلفة ويجب استبدالها. هذا سيعطي الآثار الجانبية الحارقة التي تظهر في الضمادة الكيميائية أو الاصطناعية للمضادات الحيوية مثل آلام ما بعد الجراحة وما إلى ذلك .سيوفر الاستخدام المحتمل للأعشاب القائمة على الضمادة داخل الجلد سلامة أفضل فيما يتعلق بالآثار الجانبية الأقل .بذور الكتان و حبة البركة أظهرت بعض الخصائص المضادة للميكروبات .ومع ذلك، لا تزال آثاره على مسببات الأمراض عن طريق الفم محدودة .ومن ثم، هدفت الدراسة الحالية إلى بحث التأثير المضاد للميكروبات لبذور الكتان و حبة البركة والجمع بين هذين المسببين للممرض الفموي المختار. (المكورات العقدية المقيحة، المكورات العقدية، الزائفة الزنجارية، الكلبسية الرئوية، المكورات المعوية البرازية). تم اختبار فعالية بذور الكتان وحبة البركة ومزيجها باستخدام اختبار انتشار القرص، والحد الأدنى من تركيز التثبيط والحد الأدنى من تركيز البكتيريا على الممرض الفموي المختار. ثم تم تحديد فعالية جميع المستخلصات في البيئة الخاضعة للرقابة للنموذج داخل لقناة الجذر .تم إجراء جميع الاختبارات في ثلاث نسخ.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science (Biosciences)

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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LIST OF ABBREVIATION

SDG	Secoisolaricisinol diglucose
ALA	Alpha-linolenic acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
LA	Linoleic acid
SMG	Secoisolariciresinol monoglucoside
SECO	Secoisolariciresinol
TLC	Thin layer chromatography
DPPH	2.2'-diphenyl-p-picrylhdrazyl
TQ	Thymoquinone
GAS	Group A Streptococcus
rpm	Rotor per minute
NA	Nutrient agar
NB	Nutrient broth
DMSO	Dimethyl sulfoxide
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
CEJ	Cementoenamel junction
S.pyogenes	*Streptococcus pyogenes*
S.mutans	*Streptococcus mutans*
P.aeruginosa	*Pseudomonas aeruginosa*
K.pneumoniae	*Klebsiella pneumoniae*
E.faecalis	*Enterococcus faecalis*

CHAPTER ONE

INTRODUCTION

1.1 GENERAL INTRODUCTION

Nowadays the people are more concerned, conscious and are more inclined to use natural products and preference change to herbal instead of synthetic medication is because of severe side effects of some materials or facing the potential risk of bacterial resistance, in addition to that the cost of the synthetic material.

In the pathogenesis of polio-periapic disease, bacteria and their products play an important part. The aerobic and facultative anaerobic species such as Streptococci, Enterococci, Staphylococci, Lactobacilli, Pseudomonas, Neisseria and Veillona are the predominant microbial groups frequently isolated from contaminated root canals. *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Bacteroides fragilis* are commonly seen (Ercan et al., 2006; Sawsan and Somaia, 2008). These types of organisms are often more than needed to isolate anaerobic species during the standard culture technique in formed periapical lesions (Ercan et al., 2006).

Flaxseed and flaxseed oil (also called linseed oil), come from the flax plant (*Linum usitatissimum*), which has been cultivated for domestic use since prehistoric times. Its use as a dietary supplement becomes more popular nowadays. However, there are still a lot of ongoing studies on means to optimize the beneficial effect of this so-called magic plant (Pan et al., 2009). Flaxseed extraction could be the source of new and better antimicrobial compounds mainly due to the effectiveness of its presence constituent against Gram-positive and negative bacteria (Amber et al., 2015). Extraction

of flaxseed protein showed antibacterial activity against the most test microorganism especially Gram-negative bacteria (Tehrani et al., 2014). Flaxseed oil has been shown to have antibacterial potential against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* K-12 (Kaithwas et al., 2011).

Nigella sativa goes by many different names, for example in old Latin it is called as `Panacea' meaning `cure all' while in Arabic it is termed as `Habbah Sawda' or `Habbat el Baraka' translated as `Seeds of blessing'. The *Nigella sativa* based-oils are claimed to have potent anti-inflammatory, anticancer, antidiabetic, antimicrobial, antihistamic and antihypotensive effects (Al-Rowais, 2002; Ali and Bluden, 2003; Salem, 2005).

1.2 PROBLEM STATEMENTS

The major reason of failure seen in endodontic treatment (root canal therapy) is primarily due to the bacterial contamination and the inherent difficulty to remove these pathogens. A non-vital dental intracanal space is essentially a dead space within the human body, virtually inaccessible to the host immune system thus making it a perfect breeding ground hostile micro bacterium. The currently available conventional method of root canal therapy involves the use chemical or synthetic antibiotic-based root canal dressing that are costly and had to be repeated numerous times to achieve a pathogen free canal to allow the root canal filling to be placed. This multiple attempt is essentially exposing the tooth and its supporting structure namely the alveolar bone and gingiva to the caustic side effects seen in the chemical or synthetic antibiotic dressing such as postoperative pain, mucosal necrosis, periapical inflammatory response, internal resorption as well as others. The potential use of herbal based intracanal dressing will not only be cost effective but also provide better safety with regards to the lesser side

effects as seen with the conventional chemical based intracanal medications. The intracanal medicament need to be use with precaution during pregnancy because it may cause side effect to pregnant women. Thus, the use of natural product with no or less side effect to pregnant women and small children is preferable.

1.3 SIGNIFICANCE OF THE STUDY

Finding new natural compounds with good antimicrobial activities have given promises from proteinaceous materials isolated from plant sources (Flores et al., 2002; Houshdar et al., 2002; Tavares et al., 2008). It is expected to see the bacteriostatic and bactericidal effect of flaxseed and *Nigella sativa* against dental pathogens.

1.4 OBJECTIVE

1.4.1 General Objective

The main objective of this study was to evaluate the antibacterial effect of flaxseed and *Nigella sativa* against the selected endodontic pathogen.

1.4.2 Specific Objectives

The specific objectives of this study are as follows

i. To assess the antimicrobial potency of each extract and their combination on selected oral pathogens.
ii. To determine the efficacy of flaxseed and *Nigella sativa* on selected oral pathogen within the intracanal model.
iii. To determine the efficacy of these extracts combination of the selected oral pathogen within the controlled environment of the intracanal model.

1.5 HYPOTHESIS

The flaxseed and *Nigella sativa* extracts oil show effective antibacterial activity to against endodontic pathogens.

1.6 EXPECTED OUTCOMES

This study aims to achieve new findings regarding the effectiveness of flaxseed as well as *Nigella sativa* essential oil in limiting or reducing the microbial count within the intracanal infection. This is especially, as infection within the dental intracanal space is difficult to control as it essentially is a dead space within the human body that is shielded against the immunological response of the body. The antimicrobial effectiveness by of these essential oils taken from these herbs will lay the fundamental groundwork knowledge to expand into the application aspect of flaxseed and *Nigella sativa* extract use in dental intracanal dressing, filling and tooth salvages in the clinical dentistry field. Endodontic infections have a polymicrobial nature, with obligate anaerobic bacteria conspicuously dominating the microbiota in primary infections and no known medicament is effective against all the bacteria found in infected root canals (José, 2001; Narayanan and Vaishnavi, 2010). An important limitation of this study is that the only selected oral pathogen was chosen to be treated with flaxseed and *Nigella sativa* extract. Probably the effectiveness of antimicrobial action of flaxseed and *Nigella sativa* oil can be doubted. Calcium hydroxide becomes a choice of intracanal dressing in contemporary endodontic practice nowadays also root canal filling using Gutta-percha (Larz and Markus, 2002). The second limitation of this study is it flaxseed and *Nigella sativa* were able to act as intracanal dressing and root canal filling, this is needed to prove by several of treatment.

CHAPTER TWO

LITERATURE REVIEW

2.1 FLAXSEED (*LINUM USITATISSIMUM*)

2.1.1 Flaxseed as Ancient Medicine

Flaxseed also is known as linseed (*Linum usitatissimum*) has many benefits for human health (Rubilar et al., 2010 and Anjum et al., 2013). Flaxseed is believed to have originated in Egypt and has been grown worldwide in the past for many years because of its oil and fibre (Kaithwas and Majumdar, 2013).

Flaxseed (*Linum usitatissimum*) is becoming an increasing demand as a functional food ingredient and has been integrated into many nutraceutical products, such as juices, dairy products, baked goods, dry pasta and meat (Goyal et al . , 2014). Its use as a dietary supplement becomes more popular nowadays, due to a series of researches on its multitudinous effect on human health. But still have a lot of undergoing studies to optimize the beneficial effect of this so-called magic plant (Flaxseed and Flaxseed Oil, 2016; Touré and Xueming, 2010; Kris-Etherton et al., 2002; Ilhan et al., 2005)

Many researchers have given particular attention to flaxseed in recent years since previous studies have shown such a prominent health benefit including anti-cancer and antibacterial properties (Adolphe et al., 2010). In addition, flaxseed oil and fibres have also been found to help manage many illnesses such as atherosclerosis, cardiovascular disease, cancer, diabetes, arthritis, osteoporosis, autoimmune and neurological disorders (Goyal et al., 2014).

Figure 2.1 Flaxseed (*Linum usitatissimum*) Seed

2.1.2 Bioactive Components of Flaxseed

Flaxseed derivatives such as defatted flaxseed meal or flax hulls have higher secoisolariciresinol diglucose (SDG) concentration and were found to contain about 2.3% to 4% of SDG (Alaa et al., 2013). The flaxseed contains the largest amount of lignin, SDG among all the grains and it is the richest dietary source of the plant-based SDG (Zhang and Xu, 2007; Liggins et al., 2000). Whole flaxseed (ground meal, powder, or intact seed) contains 28% dietary fibre (7-10% soluble fibre and 11-18% insoluble fibre), 40% fat (73% polyunsaturated fatty acids), and 21% protein. Other nutrients in flaxseed include vitamins E and B, sterols and mineral nutrients like calcium, iron and potassium. In flaxseed, more than 50 percent of fat is an essential fatty acid called omega-3 fatty acid (alpha-linolenic acid, ALA), which makes flaxseed the richest plant source of omega-3 fatty acid (Bloedon et al., 2008). Nevertheless, due to several factors such as genetics, climate, seed processing and analytical procedure, the composition of flaxseed metabolites can differ accordingly (Daun et al., 2003).

Alpha-linolenic acid (ALA) is a major unsaturated fatty acid that plays a critical function for the human body as it acts as a fundamental component of the extended unsaturated omega-3 fatty acids, docosapentaenoic acid, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) synthesis (Baker et al . , 2016). Unfortunately, omega-3 and omega-6 fatty acid, linoleic acid (LA) cannot be synthesised by a human from any dietary precursor, so proper dietary intake of ALA and LA foods is very important for maintaining healthy lifestyle. (Ribeiro et al., 2013).

Flaxseed (*Linum usitatissimum*) is a dietary source from a plant rich in different forms of phenolic compounds, including phenolic acids, lignans, flavonoids, tannins and phenylpropanoids (Kasote, 2013). Due to the seasonal effect, the substance of phenolic compound in flaxseed differs mainly, and the the location also significantly affected the substances (Laurine et al., 2018). Lignan is secondary metabolites of plants which are present abundantly in edible plants. Flaxseed (*Linum usitatissimum*) is extraordinarily rich in lignan SDG and has been found to be the richest SDG sources (28 800-369 000μg/100 g) of any food (Barbary et al., 2010). Flaxseed lignan is a constituent of up to 13 mg / g of flaxseed (Hall et al., 2006). Barbary et al. (2010) also found that there were different kinds of lignans saw as present in limited quantities are matairesinol, pinoresinol, and isolariciresinol.

Flaxseed lignan is a natural source of substantial bioactive phytoestrogens that exhibit a wide range of biological properties. Lignans are secondary metabolites, synthesised by the oxidative dimerisation of two units of phenylpropanoid (Saleem et al., 2005). The term lignans represents a group of phenylpropanoid dimers, in which all the central carbon (C8) of their propyl side chains binds the phenylpropane units (Sarajlija et al., 2012). Lignan allocating to the a dietary group of phytoestrogens with

major pharmacological properties including antimicrobials (Barbary et al., 2010; Saleem et al., 2005) anticancer (Herchi et al., 2011), antioxidant and antiviral interventions (Masashi, 2018; Herchi, et al., 2011). The previous study recorded optimum flaxseed extraction of lignans at 70 percent ethanol, 40°C and 28 hours extraction time (Zhang et al., 2007).

Both omega 3 and lignans appear to have anti-cancer properties. Animal studies have demonstrated that omega-3s can decrease the size and number of tumour cells. One recent human study showed that flaxseed shrinks breast cancer tumours. Lignans have a wide range of biological actions and potential for example antimicrobial also to beside mycobacteria and oral pathogen (Saleem et al., 2005; Silva et al., 2007; Silva et al., 2009).

2.1.3 Antimicrobial Action of Flaxseed

Flaxseed (*Linum usitatissimum*) has been related to many antibacterial activities. However, previously no antiviral studies on flaxseed have been published to the extent of our knowledge. Many studies correlated flaxseed antimicrobial activities with the existence of natural polyphenols in general, as well as glucosylated lignans (such as SDG or SMG), and aglycones (such as SECO or anhydro-SECO) in particular (Pag et al., 2014; Barbary et al., 2010). In addition, studies have shown that fatty acids served on microbial pathogens by slowing their growth and hence serving as the main components of antimicrobial food additives (Zheng et al., 2005; Freese et al., 1973). Furthermore, earlier studies have also revealed that residues from the flaxseed oil extraction process known as seedcake may contribute to antimicrobial activity as they

are associated with high phenolic acids that have antimicrobial properties (Zuk et al., 2014).

Also, flaxseed (*L. usitatissimum*) exhibited good antibacterial activity against several microbial strain including *Staphylococcus aureus* (ATCC 29737, ITCC8531), *Streptococcus agalactiae* (NCIM 2401), *Enterococcus faecalis* (ATCC 51299), *Micrococcus luteus* (ATCC 10240, ITCC9341), *Bacillus subtilis* (ATCC 6633), *Bacillus pumilus* (ATCC 14884), *Brevibacillus brevis* (ITCC7096), *Bacillus cereus* (ATCC 11778) and *Escherichia coli* (ATCC 8739) as compared with cefoperazone (Kaithwas et al., 2011). Flaxseed lignans were the most effective antibacterial against *S. aureus* and *Vibrio* sp. compared to Gram-negative bacteria such as *Klebsiella* sp. and *Shigella* sp. (Barbary et al., 2010).

2.2 *NIGELLA SATIVA*

2.2.1 *Nigella sativa* as Ancient Medicine

A large number of medicinal plants induce therapeutic potentials. *Nigella sativa L.* (Ranunculaceae) known commonly as “black cumin”, is a herbaceous plant that grows in Mediterranean countries and is also cultivated in Turkey. The black cumin herb goes by several different names, for example in old Latin it is called 'Panacea' which means 'cure-all' while in Arabic it is called 'Habbah Sawda' or 'Habbat el Baraka' which is translated as 'Seeds of Blessing'. Seeds of *Nigella sativa L.* have been employed for thousands year as a spice and food preservative.

N.sativa seeds and oils were being widely used to treat of various illnesses worldwide for decades. It is a beneficial effect on health in traditional Indian medicines

like Unani and Ayurveda (Sharma et al., 2005; Goreja, 2003). Among the Muslim community, it is regarded as one of the greatest forms of healing medicine available and included in the medicine of the Prophet Muhammad ﷺ (Aljawezjjah, 2001).

Figure 2.2 *Nigella sativa* Seeds

The oil and seed constituents have shown potential medicinal properties in traditional medicine (Ali and Bluden, 2003; Basha et al., 1995). In fact, *Nigella sativa* based oils are claimed to have potent anti-inflammatory, anticancer, antidiabetic, antimicrobial, antihistaminic and antihypertensive effects (Salem, 2005; Al-Rowais, 2002; Ali and Bluden, 2003).

2.2.2 Bioactive Components of *Nigella sativa*

The black *Nigella sativa* seed chemical composition is complex and includes amino acids, proteins, carbohydrates, fixed and volatile oils, alkaloids, saponins, and several other compounds. The screening of the oil samples by Thin Layer Chromatography (TLC) showed four main parts, given as follows thymoquinone, carvacrol, tanethole and 4-terpineol display respectable radical scavenge properties. These four constituents

and essential oil had variable antioxidant activity when checked for a non-specific hydrogen atom or electron donation in the 2,2'-diphenyl-p-picrylhdrazyl (DPPH) assay. The oil samples exhibited variable antioxidant property that was due mainly to these constituents' variable composition (Goreja, 2003; Ilhan et al., 2005).

There is a lot of biological activities of *Nigella sativa*. seeds contain a large number of fixed oils and the main constituent of the seed extract is thymoquinone (TQ). Several pharmacological effects have been attributed to the active principles of *Nigella sativa*. This includes thymoquinone, thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellicine, nigellimine-x-oxide, nigellidine and alpha-hedrin (Aljabre et al., 2005). The active component of *Nigella sativa*, TQ has many pharmacological properties such as antimicrobial, anti-inflammatory, antihypertensive, anticarcinogenic, antioxidant and hepatoprotective (Ahmad et al., 2014; Tariq. 2008; Shrivastava et al., 2011).

2.2.3 Antimicrobial Activity of *Nigella sativa*

Different researchers on *N.sativa* carried out comprehensive researchers using modern scientific techniques as it is believed to be a miracle herb that can heal numerous diseases and ailments. A number of *N.sativa* pharmacological activities were studied in the last few decades. (French et al., 2013)

N.sativa methanolic extract was found to exhibit anti-plaque action through powerful inhibition of *Streptococcus mutans*, thus also preventing dental caries. Alcoholic seed extracts have demonstrated antibacterial activity against the *Micrococcus pyogenes var.aureus*. It has also been shown to have antibacterial activity against *Shigella dysenteriae, S.sonnei, S.boydii, Vibrio cholera* and *Escherichia coli*.