



## THE ANTIBACTERIAL EFFECT OF FLAXSEED EXTRACT ON SELECTIVE ORAL PATHOGENS - COMPARATIVE IN VITRO STUDY

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Article Received on  
21 August 2018,

Revised on 10 Sept. 2018,  
Accepted on 01 October 2018

DOI: 10.20959/wjpps201811-12548

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### ABSTRACT

**Introduction:** Flaxseed from the flax plant (*Linum usitatissimum*), which has been cultivated for domestic use since prehistoric times. This study aims to investigate presence of antibacterial effect of flaxseed extract against selected oral pathogen in-vitro. **Materials and Methods:** This is a laboratory experimental in vitro study using selected oral pathogens cultured in nutrient agar. The pathogens then were inoculated in nutrient based broth and incubation for 24hours. Flaxseed extract efficacy was tested by measurement of the zone of inhibition. The antibacterial activities were compared with streptomycin as positive control and DMSO as negative control. The statistical analysis was done by using SPSS. **Results:** There is positive antibacterial effects of flaxseed extract against *Streptococcus mutans*,

*Streptococcus Pyogenes*, *Pseudomona Aerigenosa* and was comparable in efficacy to that of Streptomycin. **Conclusion:** This study concludes that flaxseed extract has antibacterial activity ageist selective oral pathogens. The implication of this result will be useful in propagating the use of natural based product as therapeutic drug against the chemical

synthetic products. This study recommends the use of natural herbal product to have the equal potential while negating the unwanted side effects towards humans' body.

**KEYWORDS:** Flaxseed, Oral Pathogens, in vitro, Antibacterial.

## INTRODUCTION

Flax seed (*Linum usitatissimum*), which has been cultivated for domestic use since prehistoric times. Its use as a dietary supplement becomes more popular nowadays. Extraction of flaxseed protein did showed an antibacterial activity against many gram-negative bacteria<sup>[1]</sup> but only a number of researches investigating the effect of this extract on oral pathogens which naturally present in the human oral microbiota and consider as the primary pathogenic species responsible for dental caries.<sup>[2]</sup> Flaxseed extract is potential source of natural antimicrobial compounds that can be used to treat many oral health problems with fewer side effects than that of commonly used synthetic based antimicrobial compounds.<sup>[3]</sup>

Lignin extract of flaxseed has a potential sources of antioxidant and antimicrobial agents.<sup>[4]</sup> Lignan extracts were found to be most effective antibacterial against the Gram positive bacteria such as *Streptococcus aureus* compared to *Klebsiella* sp and *Shigella* sp.<sup>[5]</sup>

Finding new natural compounds with promising antimicrobial activities has derived by isolating proteinaceous materials from plant sources.<sup>[6]</sup>

## Literature Review

Flaxseed is the seed from the plant *Linum usitatissimum*. this plant had fascinated man's interest for its benefits for human health.<sup>[7]</sup> It is rich source of dietary fiber, micronutrients, and the essential fatty acid, Consuming flaxseed may help in cancer protection. Recent studies suggest that lignans within flaxseed have anti angiogenic properties.<sup>[8]</sup> Flax seed contains lignin, secoisolariciresinoldiglucose (SDG) and it is the richest dietary sources of SDG.<sup>[9]</sup> The lignan constituents of flax seed also is claimed to possess in vitro antioxidant properties and has efficacy for the treatment of breast cancer.<sup>[10]</sup>

The higher the flow rate, the higher the rate of extraction of lignans but it needs larger water volumes.<sup>[11]</sup> The advantage of this method include increase of temperature accelerated extraction in which it helps in reducing solvent volume and time to achieve equilibrium.<sup>[12]</sup>

Alpha-linolenic acid (ALA) and gamma tocopherol present in Flax seed, are two forms of vitamin E in which it was shown to decrease the human estrogen receptor-positive breast tumors (MCF-7) growth. (American Institute for Cancer Research, 2016). Flaxseed oil have antibacterial potential against *Staphylococcus aureus*, and *Escherichia coli* K-12.<sup>[13]</sup>

In the oral cavity, the bacterial population is a result of the dynamic relationship between pathogens and commensals.<sup>[14]</sup> Non-beneficial microorganisms have a direct link to dental caries, periodontal disease and halitosis.<sup>[15]</sup> Dental caries or tooth decay is a progressive, irreversible microbial disease that is characterized by bacterial fermentation of carbohydrates resulting in acid production and the subsequent destruction of the hard tissues of the tooth.<sup>[16]</sup> *Streptococcus Mutans* is a facultative anaerobic, gram-positive coccus found in the human oral cavity and is a significant contributor to tooth decay<sup>[17]</sup>, It has caries-inducing potential was demonstrated in animal models.<sup>[18]</sup> Study by Kleinberg (2002) postulated that the frequent association of *Lactobacillus acidophilus* and *Streptococcus mutans* with caries activity gives precedence to them being specific cariogens. Another cited study also stated that *Streptococcus mutans* is the leading cause of dental caries worldwide and is considered to be the most cariogenic of all of the oral streptococci strain.<sup>[19]</sup>

Metwalli (2013) reported that *Streptococcus mutans* are considered to be a principle cariogenic organisms due to its inherent ability to produce large quantities of glucans as well as acid that easily exceeds the salivary buffering capacities and gives the bacteria a tipping leverage when competing against non-cariogenic commensal species at low pH environments.<sup>[20]</sup>

Another common oral commensal of interest is *Streptococcus pyogenes*. It has been recognized that *Streptococcus pyogenes* can survive incubation in human blood, and this has in part been ascribed to an anti-phagocytic effect of M and M-like proteins expressed at their surface.<sup>[21]</sup> This Gram-positive bacterium is responsible for a number of diseases like pharyngitis, impetigo, scarlet fever and erysipelas.<sup>[22]</sup> Kilsgard and colleagues in their report have mentioned that *Streptococcus pyogenes* is an important human pathogen responsible for substantial morbidity and mortality worldwide.<sup>[23]</sup>

Additionally, group A *Streptococcus pyogenes* (GAS) is an important public health problem causing significant morbidity and mortality worldwide. A recently cited study stated that GAS is transmitted between humans through close or direct physical contact, namely via

respiratory droplets.<sup>[24]</sup> *Streptococcus pyogenes* is reported as one of the most common bacteria causing acute pharyngitis.<sup>[24]</sup> The study added that upper respiratory tract is colonized by *Streptococcus pyogenes* and it is highly virulent since it has the ability overcome the host defence system.<sup>[24]</sup>

Another oral commensal, *Pseudomonas aeruginosa* is a Gram negative, aerobic, rod shaped bacterium with unipolar motility.<sup>[25]</sup> *Pseudomonas aeruginosa* is Gram negative bacilli that is resistant to extremes of temperatures and also could survive and propagate in varied environment.<sup>[26]</sup>

Study in 2000 named *Pseudomonas aeruginosa* as a primary bacteremia pathogen in burn victims, urinary tract infection and hospital acquired pneumonia patients placed on respirators.<sup>[27]</sup> some researches stated that *Pseudomonas aeruginosa* has become resistant to many antimicrobial agents, coupled with numerous advancements in medical and surgical technology.<sup>[28]</sup>

The colonization prevalence of *Pseudomonas aeruginosa* is high in healthy subjects, Colonization is common in the respiratory tract of mechanically ventilated patients, in the gastrointestinal tract of the patients receiving anticancer chemotherapy and on the skin of burn patients.<sup>[29,30]</sup>

The most frequently associated with human infections within the genus of *Pseudomonas* is *Pseudomonas aeruginosa*.<sup>[31]</sup>

## MATERIALS AND METHODS

### Study Design

This study is a quantitative descriptive study conducted in a laboratory (in-vitro) condition.

### Study Setting

This study was conducted at Microbiology Laboratory, Kuliyyah of Science IIUM, Kuantan, Malaysia.

### Methodology

This study used different types of bacteria included *Streptococcus pyogenes* (ATCC 19615<sup>TM</sup>), *Streptococcus mutans* (ATCC 25175<sup>TM</sup>), *pseudomonas aeruginosa* (ATCC 27853<sup>TM</sup>). All strains were procured from the American Type Culture Collection (ATCC,

Manassas, VA, USA). The study involves in vitro testing of flaxseed freeze dry extract against *Streptococcus mutans*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa* using agar-well diffusion method and comparing their antibacterial activities with the antibiotics (Streptomycin) as a positive control and dimethyl sulfoxide (DMSO) as a negative control. Flaxseed extract effectiveness is tested separately using zone of inhibition, done by bacterial counting of the inoculated suspension, bacterial viability will be microscopically confirmed by morphology and staining method.

The flaxseed was reduced to meal form which later was diluted in 99.8% ethanol within a Soxhlet chamber, following which the extract or filtrate containing flaxseed extract was collected and evaporated using a rotary evaporator under pressure at 60°C. The concentrated extract is freeze dried for 30minutes to remove remaining water residue. Then, it was stored 4°C until further use.

Consequently, the bacteria strains that were cultured on nutrient agar (NA) medium as well as nutrient based broth were used to inoculated the petri dishes by swabbing the agar with a sterile cotton swab soaked in selected pathogen (*Streptococcus mutans*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*). Additionally the petri dishes were divided into 4 portions, whereby each portion will house an impregnated disk in the centre. The plates were left to be dried before implanting the streptomycin or flaxseed extract or DMSO impregnated disks. The plates were then left to be incubated at 37°C for 18-24 hours. The plates incubated can be extended up to 48 hours if there is no growth on the agar. For control, streptomycin was used for analysis the bacteria susceptibility. After the plates were incubated, the diameter of the growth inhibition zones (in mm) around the impregnated disks were measured thrice and the mean value were calculated. The study was repeated three times to get an approximated value. The measurements are recorded and analysed statistically using GraphPad PRISM version 6.

## RESULTS

*Streptococcus mutans*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa* were successfully inoculated upon the agar petri dishes. The flora on the petri dishes was divided into 4 regions. In 2 regions, a couple of flaxseed extract (100µm) impregnated discs were placed. Consequently another disc impregnated with the positive control Streptomycin (50µm) was placed on another region. Finally in the last region, a disc containing DMSO

(100 $\mu$ m) was placed so that it will act as a negative control comparison. The divisions are clearly depicted in Figures 1,2 and 3.

Figures 1, 2 and 3: Zone of bacterial Growth inhibition Between Streptomycin against flaxseed extract. Blue circle shows positive control while red circle shows flaxseed extract.

Figure 1, 2 and 3 depicts the differential zone of bacterial inhibition diameter between flaxseed extract against Streptomycin. the zone of inhibition of each bacterial strain around the flaxseed extract disc is lower when compared to positive control. We surmise that the difference between inhibition zone of positive control disc and flaxseed test discs is not significant.

Both Streptomycin and Flaxseed had exhibited stable mean zone of inhibition across different strain of bacteria. This may indicate that both are able to inhibit growth of all 3 types of bacteria.

## DISCUSSION

Oral diseases continue to be a major health problem worldwide.<sup>[32]</sup> This is troubling notion considering the age of modern medicine and the many advances that we have seen in this field in the last century. Dental caries and periodontal diseases are among the most important global oral health problems, although conditions such as oral and pharyngeal cancers and oral tissue lesions also pose a significant health concerns.<sup>[33]</sup> In most of these oral diseases incidence, the implication of microbial aetiology is considered the greatest in majority of the globe. Traditional or herbal based healing is a path that is currently gaining a lot of attention in both commoners and scientists alike. Numerous traditional medicinal plants have been evaluated for their potential application in the prevention or treatment of oral diseases.<sup>[3]</sup>

Flaxseed extract prove to have antibacterial effect against streptomycin. There are few studies comparing the antibacterial activity with other broad spectrum antibiotic such as Cefoperazon.<sup>[13]</sup> They mentioned that the anti-inflammatory and antimicrobial properties of the oil contribute to its therapeutic efficacy in mastitis. In addition, Al-Mathkhury et al., 2016 also conduct a study to investigate the antibacterial and antibiolfim activity of flaxseed oil extract on some locally isolated bacterial pathogens. The in vitro effect of flaxseed oil on bacterial isolates shows that there is no inhibitory effect noticed against *E. coli* or *E. faecalis*, perhaps it can be due to possession of resistance genes that need to be revealed.

Many research done previously to investigate the beneficial effect of flaxseed extract on general human health. The stem of flax plant can yields good quality fibers which having high strength and durability.<sup>[34]</sup> Flaxseeds also have nutritional characteristics and are rich source of  $\omega$ -3 fatty acid:  $\alpha$ -linolenic acid (ALA), short chain polyunsaturated fatty acids (PUFA), soluble and insoluble fibers, phytoestrogenic lignans (secoisolariciresinol diglycoside-SDG), proteins and an array of antioxidants.<sup>[35]</sup>

Flaxseed components such as ALA also play important role in inhibiting bacterial activities. Jung at al., 2015 study the mechanism of antibacterial activity of liposomal linolenic acid against *Helicobacter pylori*. Besides, it is also known that unsaturated fatty acids tend to have greater potency in inhibiting the growth of bacteria than saturated fatty acid with same length carbon chain.<sup>[36]</sup> Antibacterial effect of saturated and unsaturated fatty acids against *H. pylori* suggest that bactericidal activity increases with degree of unsaturation.<sup>[37]</sup>

Flaxseed also rich in secoisolariciresinoldiglucoside (SDG), the precursor of lignans, which has many favorable actions on human health. SDG has also anti-viral, antibacterial and anti-fungal properties, is an antioxidant and it has been shown to enhance immune system functioning.<sup>[38]</sup> Mazur et al. 1996 stated that flax plant contains up to 800 times more lignans than other plant foods. Plant lignans are phenolic compounds formed by the union of two cinnamic acid residues. Lignans are found within the plant kingdom and are present in almost all plants.<sup>[7]</sup> Thompson et al., 2005 examined, in a randomized double-blind placebo-controlled clinical trial, the effects of dietary flaxseed on tumour biological markers and urinary lignan excretion in postmenopausal patients with newly diagnosed breast cancer. The result shows that the dietary flaxseed did have the potential to reduce tumor growth in patients with breast cancer. Hashempur H et al., 2014 investigate the effect of flaxseed oil extract on mild and moderate carpal tunnel syndrome; they proved that flaxseed oil could be effective in the management of mild and moderate carpal tunnel syndrome, especially in improving the severity of symptoms and functional status. In addition, its effect on electerodiagnostic parameters, especially on the nerve conduction velocity, can be considered as a valuable point.<sup>[39]</sup>

Flax seed showed effective inhibitory effect against selective oral pathogens especially facultative anaerobe. Some bacteria like *Pseudomonas aeruginosa* are nosocomial pathogen that are very resistance and could survive and propage in varied environment.<sup>[40]</sup> Flaxseed is natural product that have positive action on those pathogens that commonly presence in oral

cavity. A good antimicrobial activity and marginal seal between tooth and temporary endodontic restorative material is required to minimize contamination of root canal systems during and after endodontic therapy.<sup>[41]</sup>

## CONCLUSION

This study has shown that flaxseed extract has antibacterial effect towards selective oral pathogens comparable to streptomycin bacterial. It is important to use natural based product compared to chemical synthetic based product in treating health problem so that it will be less side effects towards humans' body.

This study shows the effectiveness of flaxseed extract against anaerobic oral pathogens. The use of this product is more common and relevant in dentistry field.

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