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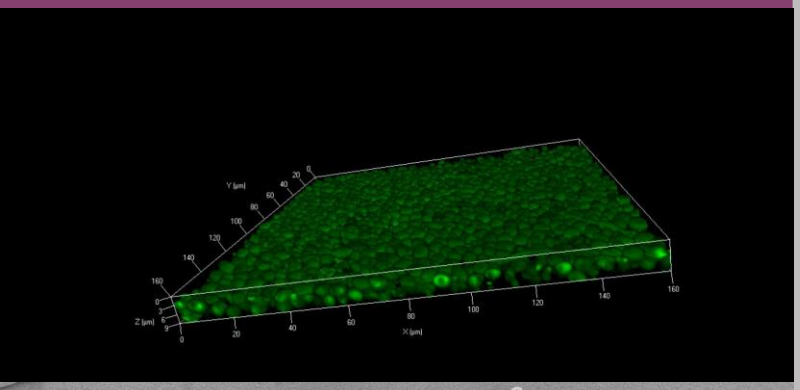

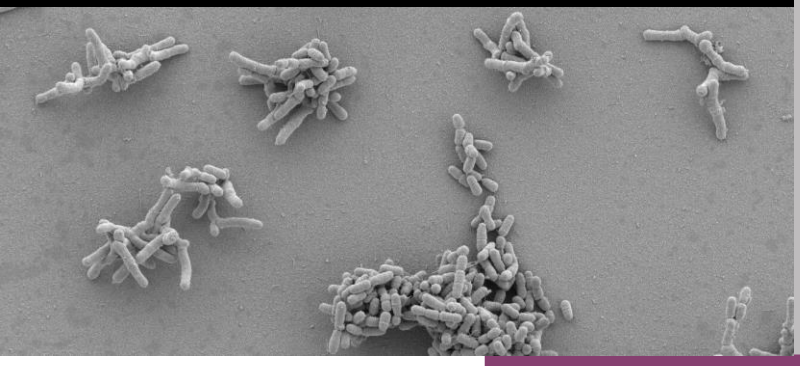

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Aims and Scope:

International Journal of Orofacial and Health Sciences (IJOHS) is a peer reviewed biannual international journal dedicated to publish high quality of scientific research in the field of orofacial sciences, health sciences and interdisciplinary fields, including basic, applied and clinical research. The journal welcomes review articles, original research, case reports and letters to the editor. Areas that are covered include but are not limited to dental sciences, oral microbiology and immunology, oral maxillofacial and craniofacial surgery and imaging, dental stem cells and regenerative medicine, dental biomaterial, oral maxillofacial genetic and craniofacial deformities.

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CONTENTS

ORIGINAL ARTICLES

Potential antibacterial effects of flaxseed and *Nigella sativa* extracts on *Streptococcus pyogenes*

Basma Ezzat Mustafa Alahmad[1*], Nurul Fatihah Mohamed Yusoff[2], Nazih shaban Mustafa[3], Pram Kumar A/L Subramaniam[3], Deny Susanti Darnis[2], Khairani Idah Mokhtar[1]

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Abstract

Antibiotic resistance is a major global problem, associated with inadvertent drug usage. Herbal interventions are a therapeutic strategy that warrants greater research attention. Flaxseed and *Nigella sativa* are well recognized original super foods that have demonstrated potent anti-microbial and anti-biofilm activities. In the oral cavity, the bacterial population is a result of the dynamic relationship between pathogens and commensals *Streptococcus pyogenes* is an important global human Gram-positive pathogen that causes a wide variety of acute infections, it is highly virulent since it has the ability overcome the host defence system. This in vitro study aims to evaluate antimicrobial activity of flaxseed and *Nigella sativa* extract against *S. pyogenes*. Ethanolic extract of flaxseed and *Nigella sativa* extracts were prepared and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against *S. pyogenes* was estimated. The results of this study show that both extracts exhibited antibacterial activity against *S. pyogenes*. Present study demonstrated the bactericidal activity of both extracts which can be an adjunct to the future natural anti-bacterial therapy.

Keywords: Antibacterial effect, flaxseed, Nigella sativa, Streptococcus pyogenes

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Introduction

Nowadays, there is a consumer preference for natural products over synthetic drugs. One of the main reasons for the same is to avoid the adverse effects of synthetic medications and the risks of bacterial resistance (David & Gordon, 2012). In the oral cavity, the bacterial population is a result of the dynamic relationship between pathogens and commensals *Streptococus pyogenes* may contribute to many human diseases, ranging from mild superficial skin infections to life-threatening systemic diseases. Infections typically begin in the throat or skin. Infections due to certain strains of *S. pyogenes* can be associated with the release of bacterial toxins that can lead to scarlet fever (Hammer, 2007). Other toxigenic *S. pyogenes* infections may lead to streptococcal toxic shock syndrome, which can be life-threatening (Hammer, 2007). The increase in the incidence of invasive *S. pyogenes* infection has frequently been associated with specific clones, which raises the

possibility that the rise of particularly virulent clones was responsible for this re-emergence - in particular, the MT1 clone which is dominant among invasive *S. pyogenes* isolates in most developed countries (Luca-Harari *et al.*, 2009). Variation in the distribution may lead to fluctuations in the severity of infections and in overall mortality rates. *S. pyogenes* infection may be observed in persons of any age, although the prevalence of infection is higher in children because of the combination of multiple exposures (in schools or nurseries, for example) and host immunity (Martin *et al.,* 2004). The prevalence of pharyngeal infection is highest in children older than three years and has been described as a 'hazard' in school-aged children (Martin *et al.,* 2004). Contemporary data suggested that invasive *S. pyogenes* infections incidence is around 2 to 4 per 100,000 population in developed countries (Steer *et al.,* 2012).

Numerous observational studies have described the frequencies of potential risk or predisposing factors in patients with invasive *S. pyogenes* disease, rigorous assessment through analytical means have been limited. The relative importance of these factors may change over time as the prevalence of the acute or chronic predisposing factors changes in frequency, such as influenza activity (Zakikhany *et al*., 2011). Infection of *S. pyogenes* in people lacking of teeth causes oral and maxillofacial cellulitis prior to sepsis. In this case, *S. pyogenes* originated from sinusitis leaked to oral cavity thus, leading to systemic infection through wounding of oral cavity mucosal lining. The study found that, the risk of odontogenic infection still there even among edentulous patients (Inagaki *et al.*, 2017). Penicillin remains the drug of choice for the empirical treatment of *S. pyogenes* infection, despite over sixty years of use. *S. pyogenes* has also remained uniformly susceptible to penicillin and resistance towards penicillin or other β-lactams which has been approved for the treatment of *S. pyogenes* (Spellerberg & Brandt, 2016).

Flaxseed and flaxseed oil (also called linseed oil) originated from the flax plant (*Linum usitatissimum*). Flaxseed protein extracts have demonstrated antibacterial activities against most tested microorganisms, especially Gram-negative bacteria. Meanwhile, flaxseed oil has been shown to have antibacterial potential against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* K-12 (Kaithwas *et al*., 2011). Evidently, flaxseed contains the highest content of lignin and secoisolariciresinol diglucose (SDG) among all grains, and is the richest dietary source of plant-based SDG (Liggins *et al*., 2000; Zhang & Xu, 2007). Flaxseed derivatives, such as defatted flaxseed meal or flax hulls, have higher concentrations (2.3 % and 4 % respectively) of SDG (Gaafar *et al*., 2013). Their usage as a dietary supplement is becoming more popular nowadays as a series of researches have highlighted its multitudinous effect on human health. However, there are still a lot of ongoing studies on the means of optimizing the beneficial effects of this called magic plant (Pan *et al*.,2009).

Nigella sativa L. (*Ranunculaceae*) – commonly as "black cumin" – is a herbaceous plant that grows in the Mediterranean countries and Turkey. It is known to have therapeutic potential; in fact, *sativa*-based oils are claimed to have potent anti-inflammatory, anticancer, antidiabetic, antimicrobial, antihistaminic, and antihypotensive effects (Al-Rowais, 2002; Salem, 2005). *N. sativa* contains many components that have pharmaceutical effects such as: thymohydroquinone, dithymoquinone, thymol, carvacrol, nigellicine, nigellimine-x-oxide, nigellidine, and alpha-hedrinhave (Aljabre *et al*., 2005). Thymoquinone is one of the main components of *N. sativa*

that has anti-microbial, anti-inflammatory, anti-hypertensive, anti-carcinogenic, antioxidant, and hepatoprotective effects (Tariq, 2008 & Ahmad *et al.,* 2013).

The present study has been conducted to evaluate the antibacterial effect of flaxseed and *N. sativa* extracts against *S. pyogenes* which is believed to be resistant to different types of antibiotics, the implication of this study will be useful in propagating the use of these natural based products as therapeutic medications.

Materials and Methods

Bacterial strains

Streptococcus pyogenes (ATCC®19615™) was used in this study. The cultures as obtained from the American Type Culture Collection (Manassas, VA, USA). Bacterial strain was stored in tryptic soy broth (TSB) with 20% glycerol at -80°C and used as required. Nutrient agar and nutrient broth (Merck) were used to culture the bacterial strains.

Flaxseed and *Nigella sativa* extracts

In collaboration with Philadelphia University, 500 grams of flaxseeds were ground using a dried blender and extracted using 99.8% ethanol in a Soxhlet chamber. The extract was collected and evaporated in a rotary evaporator under pressure at 60°C. Freeze-drying of the concentrated extracts was done for about 30 minutes to remove the water residues. The crude extracts were stored at 4°C pending further use.

The extracts of flaxseed were dissolved in 20% of dimethyl sulfoxide (DMSO) and filter-sterilized using a 0.22 µm PES syringe filter. The concentrations of the flaxseed extracts were 1, 5, 10, 20, 50, and 100 mg/ml. All extracts were diluted with DMSO to achieve the desired concentrations. Similar protocol was reflected for *N. sativa*.

Antimicrobial sensitivity tests

Bacterial growth

The bacteria were cultured on nutrient agar and inoculated in nutrient broth. The plates were incubated at 37°C for 18 to 48 hours. For broth media that were incubated for 24 hours, 10 µl from the bacterial stock was revived at 37°C to be used as the inoculum. The turbidity of the suspensions were adjusted to 1.5 to 3 x 10^8 cells/ml, which corresponded to an absorbance of 0.08 – 0.10 at a wavelength of 625 nm (Vanessa Maria Fagundes *et al.*, 2014).

Disk diffusion method

The sensitivity of *S. pyogenes* to the plant extracts was determined via the Kirby-Bauer disk diffusion method (Aqueveque *et al.,* 2006; Bauer *et al.,* 1966; Devi *et al.*, 2011) as well as the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. The nutrient agar was inoculated by swabbing with a sterile cotton swab that has been soaked in a bacterial broth. With a slight modification from previous studies, aqueous extract with 100 mg/mL concentration were pipetted with different volume (1, 5, 10, 20, 50 and 100 µl) onto sterile blank discs with 6 mm diameter (Oxoid, Badhoevedorp, Netherlands) and the discs were allowed to dry in the biosafety cabinet before being impregnated onto agar plate spread with inoculum (Revathi & Malathy, 2013). A standard antibiotic, penicillin was used as positive control for all tested bacteria while DMSO was used as negative controls. All agar plates were incubated in an incubator at 37°C for 18 to 24 hours. The positive control was penicillin while the negative control was DMSO. Susceptibility testing was performed in three biological replicates. The plates were observed for the presence of an inhibition zone. The diameters of the inhibition zones were measured (in mm) for each strain, and the mean values calculated. The absence of

inhibition zone was interpreted as absence of antimicrobial activity.

Statistical Analysis

The means and standard errors (SE) were calculated using Microsoft Excel 2010 (Microsoft Corporation, Redmond, CA, USA).

Result and Discussion

In this study, flaxseed and *N. sativa* extracts at concentrations of 5 to 100 mg/ml inhibited *S. pyogenes* which was similar to the positive control and this in line with the finding with Warnke *et al*., (2008). *N. sativa* showed inhibition zones to S. *pyogenes* at > 20 mg/ml concentration. This was similar to Hasan *et al*., (2013) in which the highest antimicrobial activity was recorded at 100 mg/ml. These plant extracts have considerable activity against Gram-positive bacteria but not Gram-negative (Alhaj *et al*.,2008).

The biological activities of the compounds from the plant extracts depend on the type of solvent that was used during extraction. The most commonly-used solvents were methanol, ethanol, and water (Parekh *et al*., 2009). In this study, the inhibition zones produced by the flaxseed and *N. sativa* extracts were not very high probably because of agro-climate factors, handling of the extracts, as well as the phytochemical ingredients of the extracts (Erdman *et al.*, 2007). Most active antimicrobial compounds were soluble in polar rather than nonpolar solvents (Parekh *et al*., 2009).

We have studied the antimicrobial activities of flaxseed and *N. sativa* extracts of various concentrations against *S. pyogenes*. The results are shown in Table 1. According to Table 1, the diameters of the inhibition zones of *S*. *pyogenes* in *N. sativa* and flaxseed extracts of 100 mg/ml were 6.33 ± 0.33 mm and 6.00 ± 0.0 mm, respectively. At the lowest concentration of the extracts (1 mg/ml), the diameters were 5.67 ± 0.33 mm and 6.00 ± 0.58 mm, respectively. The experiments were done in triplicates and the results expressed in terms of mean ± SE.

Antibacterial effects were demonstrated by the flaxseed extract at concentrations ranging from 5 to 10 mg/ml. From 20 to 100 mg/ml, the antibacterial effects of the flaxseed extract were the same. Evidently, the lignans of flaxseed (secoisolariciresinol) were effective against *S. aureus* and *Vibrio sp.* (Barbary *et al*., 2010). The *N. sativa* extract showed antibacterial effects at concentrations ranging from 1 to 100 mg/ml. In this study, it was effective against *S*. *pyogenes* bacteria. Evidently, a number of plant-derived compounds are more effective against Gram-positive bacteria then Gram-negative bacteria (Morsi, 2000; Ali *et al.*, 2001; Jones *et al.*, 2002).

Table 1. Inhibition zones of *S. pyogenes* in *Nigella sativa* and flaxseed extracts (n=3).

Test extract	**Positive control (mm)**	**Concentration of extract (mg/ml)**					
		1	5	10	20	50	100
Nigella sativa	31.7±1.67	5.67±0.33	5.33±0.33	6.67±1.20	5.67±0.33	6.67±0.33	6.33±0.33
Flaxseed	25.0±2.89	6.00±0.58	6.00±0.00	5.33±0.33	5.33±0.33	5.67±0.33	6.00±0.00

The Minimum Inhibitory Concentration (MIC) of flaxseed, *N. sativa* extracts were determined using resazurin based 96-well plate microdilution method. After the incubation period, columns with no colour changes (blue resazurin colour remain unchanged) were scored as (MIC) value. The result showed that *N. sativa*, flaxseed extract shared the same MIC which was 12.5 mg/ml on *S. pyogenes* (Table 2). Previous studies reported that MIC value for *N. sativa* extract was between <0.25 µg/ml and 1.0 µg/ml of *Staphylococci* species (Ayse *et al*., 2016 & Magdalena *et al.,* 2014). The difference may be due to the presence of various chemical compounds in this type of extract which affect the results of MIC towards *S. pyogenes*, and this may be due to the method of isolation and fractionation that provides a specific target of bioactive compound with antimicrobial properties (Shrivastava *et al.,* 2011). The antibacterial activity of flaxseed extract is associated with its ability to merge with bacterial cell wall thus, combating bacterial growth. Other than that, the existence of long-chain unsaturated fatty acids such as alpha linolenic acid and linoleic acid might contribute to the antimicrobial therapeutic efficacies of flaxseed (Barbary *et al*., 2010).

Table 2. Minimum Inhibitory Concentration (MIC) value (mg/ml) on *S. pyogenes*

Types of Extract		
Nigella sativa	Flaxseed	Penicillin
12.5	12.5	25

Conclusion

In conclusion, flaxseed and *Nigella sativa* extracts have the potential to be developed as antibacterial agents against *S. pyogenes*. However, in this study, the author suggest that these extracts should be explored in vivo to elicit a greater effect to the whole organism systems based on its toxicity, safe dosage as well as its effect on the normal microbiota in the future. Further investigations can be carried out on the synergestic effect since both extracts have good potential to be effective antimicrobial agents in the medical practice.

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All figures and illustrations could be original photographs, artwork or high-quality digital images (submitted as CMYK - 8 bits per channel in TIFF format). Images must be at least 600 by 450 pixels (proportional height) in size when in landscape orientation with a resolution of at least 300 pixels per inch. Graphs should be approximately 500 pixels wide so that all labeling can be read with data points clearly visible. Figures should be numbered

consecutively in the order in which they appear in the manuscript, using Arabic numerals. A list of figure legends must be included on a separate page following the illustrations. The legend should explain each figure in detail. All figures will be printed as black and white. Colour figures will only appear in the PDF file.

Page proofs

On acceptance of the manuscripts for publication, page proofs should be reviewed meticulously by the contributors. Changes made in proof should be limited to the correction of typographical errors. Proofs must be returned for publication with corrections and responses to queries on the date specified by the Editor.


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