Journal of Asian Scientific Research

Special Issue: 3rd International Malaysia-Ireland Joint Symposium on Engineering, Science and Business

journal homepage: http://aessweb.com/journal-detail.php?id=5003

EVALUATION OF VEGETABLE OILS AND THEIR RESPECTIVE FATTY ACIDS ON THE VIABILITY OF *STREPTOCOCCUS MUTANS*, A PERSISTENT ORAL PATHOGEN

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ABSTRACT

This study evaluated the in vitro antibacterial activity of vegetable oils and their respective fatty acid constituents on the growth of Streptococcus mutans. S. mutans is one of the primary etiological agents associated with the onset of dental caries, a disease which is considered to be an oral health epidemic in industrialized countries. Micro-broth dilution assays were carried out to assess the viability of the oral pathogen in the presence of olive oil, palm, oil, sunflower seed oil and coconut oil. Bacterial growth was not affected by any of the oils tested. A selection of MCFAs and LCFAs comprising capric, lauric, myristic, oleic and linoleic acid showed varying levels of bacteriostatic activity towards S. mutans, wherein lauric acid was the most effective MCFA and linoleic acid the most effective LCFA. The findings of this study indicate that vegetable oils tested do not directly inhibit the viability of S. mutans. The fatty acid content in these oils are present as triglycerides but when in free form do exact an inhibitory effect.

Keywords: Streptococcus mutans, Fatty acid, Dental caries, Antimicrobial, Oil pulling

INTRODUCTION

Streptococcus mutans is one of the most frequently isolated microbial pathogens from dental plaque and is recognized as one of the primary precursor agents in the onset of dental caries (Habibian *et al.*, 2002). Plaque related diseases such as dental caries are the most common oral diseases in humans and if left untreated can progress to inflammation, infection and death of vital pulp tissue (Allaker and Douglas, 2009). The tooth surface is a major habitat for *S. mutans* which is

rarely isolated before tooth eruption. *S. mutans* utilizes dietary sucrose as an energy source within the oral biofilm to produce organic acids and synthesize extracellular glucans, the latter of which allows the bacteria to firmly adhere to the tooth surface (Arunakul *et al.*, 2011). Continuous fermentation of dietary carbohydrates within the close proximity of the oral biofilm drastically reduces the pH and leads to the gradual demineralization of the dental hard tissue and the establishment of dental caries. The prevalence of this disease is high, affecting both children and adults it is regarded as the most common bacterial infection in humans to which they are susceptible throughout their lifetime (Halpin *et al.*, 2011).

The prevention of dental caries is traditionally targeted at the non-specific removal and control of dental plaque through the use of mechanical methods such as brushing and flossing as well as other oral hygiene products such mouth rinses enriched with antimicrobial properties (Chen *et al.*, 2011). Oil pulling is an age-old procedure widely recommended in alternative medicines, particularly Ayurvedic medicine, wherein a tablespoon of a selected edible oil is rinsed in the mouth for 3-20 minutes, a practice typically performed daily (Durai *et al.*, 2008). Oil pulling therapy has been used extensively as a traditional remedy for strengthening teeth and gums, preventing tooth decay, oral malodor, bleeding gums and cracked lips. Research (Asokan *et al.*, 2009) states oil pulling therapy to be equally as effective as Chlorohexidine in the reduction of plaque induced gingivitis, through the use of Sesame oil. The exact mechanisms of oil pulling therapy remain unclear although the antimicrobial activity of lipids has been extensively studied and are found to display broad spectrum activity against a number of bacterial and fungal pathogens *in vitro* (Bergsson *et al.*, 2002);(Huang *et al.*, 2011);(Sprong *et al.*, 2002);(Thormar and Hilmarsson, 2007). Vegetable oils are important sources of TAGs in the human diet and are important vehicles for the transport of fat-soluble vitamins, nutrients and antioxidants in the body (Saberi *et al.*, 2011);(Ruiz-Rodriguez *et al.*, 2010). The oral cavity is considered an excellent modeling system for investigating the anti-infective potential of foods and their respective constituents as the environment of the oral cavity is conditioned by the diet (Signoretto *et al.*, 2012). The objectives of this research are to evaluate the viability of the cariogenic bacterium *S. mutans* in the presence of Olive oil, Palm oil and Sunflower seed oil and Coconut oil using an *in vitro* microtitre plate assay. A selection of medium and long chain fatty acids representative of those found in the above vegetable oils will also be assessed for antimicrobial activity towards *S. mutans.*

METHODS

Bacterial Culture Conditions

A clinical isolate of *S. mutans* (3014 D59259), obtained from Prof. Martin Cormican of NUI Galway was used throughout these tests. Bacteria were maintained on Protect™ Bacterial Preserve beads at -80°C. A single bead from the frozen stock was used to inoculate a Brain Heart Infusion (BHI) agar plate supplemented with 5% defibrinated horse blood (Cruinn Diagnostics Ltd., Dublin, Ireland) and grown aerobically at 37°C for 48 h. A single colony from the agar plate was then used

to inoculate 20ml of BHI broth (Cruinn Diagnostics Ltd., Dublin, Ireland) and grown under aerobic conditions without shaking at 37°C for 18 h. A working culture of c. 10^8 colony forming units per milliliter (CFU^{-1}) was prepared by adding 1ml of overnight culture to 9ml of sterile BHI broth (Halpin *et al.*, 2011).

Antimicrobial Assessment of Vegetable Oils

Each of the vegetable oils (Table 1) were evaluated for antimicrobial activity using a 96 well microtitre plate assay. A 2% ethanol (EtOH) solution was prepared using sterile BHI broth and 100µl aliquoted into each well of a sterile 96 well plate. 200µl of each test sample were then added at varying concentrations ranging 3.3 mg/ml (w/v) to 416 mg/ml. 100µl of an overnight culture of *S. mutans* was added to each of the wells and for the control, 100µl of sterile broth. Optical density readings were recorded at 590nm at 2 h intervals for 12 h. Sample inoculums were removed prior to and following each assay for spread plates (n=3) to ensure the test was free from contamination.

Antimicrobial Assessment of Fatty Acids

Stock solutions of the fatty acids were diluted to the desired test concentrations in BHI broth and vortexed for up to a minute at the highest speed and tested immediately thereafter. The assay was carried out in the same format as the preliminary evaluation of the EtOH. 100µl of each concentration of the fatty acid solutions were seeded into the wells of a sterile 96 well plate (n=6). For the control, 100µl of sterile BHI broth was used. 100µl of an overnight culture of S. mutans was then added to each of the wells after which the plate was incubated at 37°C and read at 2 h intervals for 12 h.

Table-1. Fatty acid composition of vegetable oils, as determined by gas liquid chromatography (expressed as a percentage of total fatty acids). Adapted from Codex Stan 210-1999: Codex Standard for Named Vegetable oils (1999) and Codex Stan 33-1981: Codex Standard for Olive oils and Olive Pomace oils (1981)

ND – non detectable, defined as < 0.05%

Fatty acid composition	Palm oil	Sunflower seed oil	Olive oil	Coconut oil
Hexanoic acid ($C_{6:0}$)	ND	ND	ND	ND – 0.7
Caprylic acid ($C_{8:0}$)	ND	ND	ND	4.6 – 10.0
Capric acid ($C_{10:0}$)	ND	ND	ND	5.0 – 8.0
Lauric acid ($C_{12:0}$)	ND – 0.5	ND – 0.1	ND	45.1 – 53.2
Myristic acid ($C_{14:0}$)	0.5 – 2.0	ND – 0.2	ND – 0.5	16.8 – 21.0
Palmitic acid ($C_{16:0}$)	39.3 – 47.5	5.0 -7.6	7.5 – 20.0	7.5 – 10.2
Palmitoleic acid ($C_{16:1}$)	ND – 0.6	ND – 0.3	0.3 – 3.5	ND
Heptadecanoic acid ($C_{17:0}$)	ND – 0.2	ND – 0.2	ND – 0.3	ND
Stearic acid ($C_{18:0}$)	3.5 – 6.0	2.7 – 6.5	0.5 – 5.0	2.0 – 4.0
Oleic acid ($C_{18:1}$)	36.0 – 44.0	14.0 – 39.4	55.0 – 83.0	5.0 – 10.0
Linoleic acid ($C_{18:2}$)	9.0 – 12.0	48.3 – 74.0	3.5 – 21.0	1.0 – 2.5
α- Linolenic acid ($C_{18:3}$)	ND – 0.5	ND – 0.3	ND	ND – 0.2

Analysis of Growth Assays

In the presence of the FAs the following equation was used to calculate the percentile inhibition found throughout the assays using optical density values taken during early stationary phase of each assay:

(OD Control Growth) - (OD in the presence of Fatty acid) ×100
(OD Control Growth)

RESULTS & DISCUSSION

S. mutans was the primary test organism designated for each of the antimicrobial assays due to its cariogenic properties. As seen in Table 2, over a wide range of concentrations tested, the viability of *S. mutans* was not affected by any of the vegetable oils. At the highest concentration (416mg/ml) olive oil inhibited growth by 30% followed by palm oil which inhibited up to 27% of growth. Coconut oil and sunflower seed oil inhibited bacterial growth by 26% and 23% respectively. The functionality and the utilization of oils are dependent on their fatty acid composition. Triglycerides constitute the major fraction of vegetable oils although in this state have been found in-active in deterring microbial growth. Fatty acids are digestion products of triglycerides and are established antimicrobials capable of inactivating gram-positive bacteria *in vitro.*

Table-2. Minimum Inhibitory concentrations (50%, 90%) of vegetable oils as antimicrobial agents against *S. mutans* (n=6)
ND – Non detectable

Vegetable oil	Range (mg/ml)	MIC (mg/ml) 50%	MIC(mg/ml) 90%
Olive oil	3.3 - 416	ND	ND
Palm oil	3.3 - 416	ND	ND
Sunflower seed oil	3.3 - 416	ND	ND
Coconut oil	3.3 - 416	ND	ND

The sensitivity of *S. mutans* was further assessed in the presence of medium-chain and long-chain, saturated and unsaturated fatty acids (FAs). These FAs are representative of the dominant FAs present in each of the vegetable oils as tested in this study. Oleic and linoleic acid are long chain fatty acids (LCFAs) predominantly sourced in olive oil, palm oil and sunflower seed oil whereas lauric, capric and myristic acid are medium chain fatty acids (MCFAs) commonly sourced in coconut oil. The antimicrobial efficacy of the individual FAs is noteworthy as *S. mutans* growth was inhibited by both LCFAs and MCFAs, as shown in Table 3. The bacteria were unaffected by oleic and capric acid at the lowest concentration tested although at 10mM or above growth was reduced. Lauric and linoleic acid were the most active FAs followed by myristic and capric acid. Lauric acid was the most effective saturated FA tested displaying a dose-responsive effect on *S. mutans* and inhibiting < 100% of bacterial growth at 20mM, 10mM, 5mM and 2.5mM concentrations. It remains unclear the relationship between fatty acid (FA) structure and

antimicrobial activity although it has been suggested that the number of double bonds and their chain position may contribute to the inhibitory profile of the FA (Kabara *et al.*, 1972).

Table-3. Minimum Inhibitory concentrations (50%, 90%) of medium and ong chain fatty acids as antimicrobial agents against *S. mutans* (n=6)

ND – Non detectable

Fatty acid	Range (mM)	MIC (mM) 50%	MIC (mM) 90%
Capric acid	0.16 – 20	≤ 5	≤ 10
Lauric acid	0.16 – 20	≤ 1.25	≤ 2.5
Myristic acid	0.16 – 10	≤ 1.25	ND
Oleic acid	0.1 – 10	≤ 2	ND
Linoleic acid	0.1 - 25	≤ 1.25	≤ 2.5

Gram positive bacteria such as *Streptococci* are particularly susceptible to the antibacterial activities of fatty acids in comparison to Gram negative bacteria, which are less susceptible (Bergsson *et al.*, 2002). Although the mechanisms by which FAs exert their antimicrobial activity is not fully understood as of yet, disruption of the bacterial membrane wherein the permeability of the bacterial cell is compromised has been suggested (Huang *et al.*, 2011).

As previously stated the mechanisms behind oil pulling therapy remain unclear although a number of suggestions have been put forward such as saponification (Asokan *et al.*, 2009), wherein the vegetable fat is acted upon by salivary alkali and thus the 'soap-making' process is initiated. It is proposed that the cleansing action of the soaps aid in the reduction of plaque and subsequent infection processes. The viscosity of the oil may further contribute by inhibiting bacterial adhesion and plaque co-aggregation. *S. mutans* was exposed to a selection of vegetable oils in this study wherein the viability of the bacteria was unaffected; however upon exposure to the individual FAs of these oils, the viability of *S. mutans* was reduced. In light of these findings it may be suggested that the activation of lipid digestion may potentially initiate the release of antimicrobial FAs found in vegetable oils and aid in the reduction of cariogenic bacteria such as *S. mutans*. Though oil pulling therapy may not be used as a treatment for established dental caries, it may be used as a preventative home therapy to maintain oral hygiene.

ACKNOWLEDGEMENTS

This work has been funded by Athlone Institute of Technology under the President's Seed Fund award (2010 – 2014) and was carried out in the Biosciences Research Institute, Athlone Institute of Technology under the supervision of Dr. Damien Brady, Dr. Carmel Kealey and Prof. Neil Rowan.

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