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RESEARCH ARTICLE

A COMPARATIVE EVALUATION OF THE EFFECT OF VIRGIN COCONUT OIL AND CHLORHEXIDINE MOUTHWASH ON PERIODONTAL PATHOGENS - AN INVITRO MICROBIAL STUDY

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ABSTRACT

Background: Periodontitis is a polymicrobial disease which effects bone and the supporting structures of teeth. The treatment for periodontal diseases has moved towards an antimicrobial model of disease management. With the threat of wide spread antibiotic resistance rendering many antibiotics useless against many diseases, there is an increased necessity to develop a novel antimicrobial based treatment for effective disease prevention. In this regard an invitro study was conducted comparing virgin coconut oil with standard chlorhexidine mouth wash (0.2%) on five periodontal pathogens.

Methods: An invitro study on the five putative pathogens of periodontal disease was conducted using minimum inhibitory concentration (MIC), maximum bacterial count (MBC) and time kill curve methods. The culture media used was Brain heart infusion broth.

Results: The results showed that all the organisms were resistant to virgin coconut oil, while there was varying degree of sensitivity to chlorhexidine.

Conclusion: The results of the current study showed that virgin coconut oil has no therapeutic effect in the treatment of active periodontal disease, while chlorhexidine was found to have bacteriocidal effect on against Prevotella intermedia, Porphyromonas gingivalis and Tenerella forsythia and bacteriostatic effect on against Fusobacterium nucleatum and Aggregatibacter actinomycetum commitans.

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INTRODUCTION

Periodontitis is a polymicrobial disease affecting the hard and soft tissue structures of the teeth (Kim *et al*., 2002). The main etiology being bacteria and bacterial byproducts which are capable of colonizing gingival and periodontal pockets and form a complex biofilm (Dimitri's N Tatakis and Purnima S Kumar, 2005). Mechanical debridement along with systemic antibiotics and local antimicrobial agents are frequently used methods of treatment to disrupt and eliminate biofilm (Rosema *et al*., 2008). Bacterial strains have exhibited resistance to several systemic antibiotics and it is challenging to maintain the therapeutic concentration of antimicrobial agents in oral cavity. Therefore various methods and treatment modalities have been researched and implemented in reducing the bacterial count in oral cavity (Addy, 1986; Kulkarni and Damle, 2003). Chlorhexidine gluconate is the best and most widely used antimicrobial agent (Shruti Balagopal *et al*., 2013) effective against gram positive and gram negative organisms, fungi (Silvia-Edith Calamari *et al*., 2011; Fernanda Campos Machadoa *et al*., 2010), yeasts and viruses. Its superior antiplaque activity is a result of its substantivity and pin cushion effect, but it also has limitation and side effects on a long term use. Research has been conducted on alternative antimicrobial agents for treatment of periodontal disease (Slots, 2002). Traditional essential oils have been used to cure many oral conditions. In Indian ayurvedic system oil pulling has been practiced for various oral and non oral diseases (Nilesh Arjun Torwane *et al*., 2014). With the recent knowledge and advances in the field of microbiology and periodontology we might be able to scientifically validate the ancient practices. If proven to kill or reduce periodontal pathogens we can justify its in cooperation in to modern dental practices. Coconut oil is considered as a unique physically functional food, with the added health and nutritional benefits (Bawalan, 2011). There are two types of coconut oil available in the commercial market as potential oil-pulling agents: Refined bleached deodorized coconut oil and Virgin coconut oil. Virgin coconut oil is a colourless odourless liquid. It's obtained by cold pressing the

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coconuts within 24-48 hours after harvest and avoidance of heat, light and air during processing and storage protecting the heat sensitive phytochemicals and preventing the hydrolysis of the oil into free fatty acids (FFAs) which leads to spoilage of oil (Mansor *et al*., 2012). Virgin Coconut Oil is also said to have many advantages, which include the health benefits from vitamins and antioxidants, it also has antimicrobial and antiviral activity (Ogbolu *et al*., 2007; Bartolotta *et al*., 2001).

Chemical constituents in coconut fruit include:-

- Lauric acid (Pehowick *et al*., 2000)

It is a strong antiviral, antibacterial and antiprotozoal agent.

- Myristic acid

Used as a flavouring agent and is important to stabilize many proteins.

- Caprylic acid and Caproic acid

Are potent antifungal agents

- Oleic acid

Antioxidants that help remove free radicals in the body.

- Linoleic acid

Potent anti-oxidant, anti-carcinogen, a powerful immune system enhancer.

The proposed anti microbial properties of Virgin coconut oil are due to the monoglyceride monolaurin (Hierholzer and Kabara, 1982). These are small-medium chain (C-6 to C-12) produced on hydrolysis of Virgin coconut oil by lipases. The mechanism of action as proposed after a review of many lipid studies was that because of the small size of these lipids they are readily dissolved in the lipid phase and penetrated the cell membranes. They both physically disrupted the bacterial membrane (Durai *et al*., 2008; Fife, 2008) and also inhibited the enzymes involved in energy production and nutrient transfer. This lead to reversible and irreversible changes that caused microbial cell death. Considering the antimicrobial effect of other oils (Oil pulling a wonderful therapy, 2011; Thaweboon *et al*., 2011; Karach, 1992), including sesame oil (Ram *et al*., 1990; Hasan *et al*., 2000; Kato *et al*., 1998; Sirato-Yasumoto *et al*., 2001), sunflower oil (Cooney *et al*., 2001; Sechi *et al*., 2001), palm oil (Umuahia *et al*., 2015), rice bran oil (Dey Arpan, 2013) and soy bean oil (Karthikeyan Ramalingam *et al*., 2011), it was proposed that small amounts of saturated fatty acid, i.e. lauric acid, in these oils may play a role in their antimicrobial properties. Though there are many studies conducted on the efficacy of virgin coconut oil in various fields of medicine, there are no documented studies on specific periodontal pathogens and its efficacy in reducing the bacterial count.

In this study an attempt has been made to

- Use the current knowledge of causative pathogens of periodontal disease and its actual susceptibility to virgin coconut oil.
- Compare its efficacy with standard chlorhexidine. (Chlorhexidine being a broad spectrum antibacterial and antifungal agent is a very effectiveness in treating periodontal disease. Brecx *et al*., 1992; Leard A, Addy, 1997; Brecx *et al*., 1993; Hennessy, 1973; Emisilon, 1977; Budtz-Jorgensen and Löe, 1972; Grenier, 1996; McBain *et al*., 2003)

MATERIALS AND METHODS

- This is an invitro study on the five putative pathogens of periodontal disease that is Tenerella forsythia, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia and Aggregatibacter actinomycetum commitans.
- The minimum inhibitory concentration (MIC), minimum bacterial count (MBC) and time kill curve were assessed.
- The study was carried out in the department of microbiology, at Maratha Mandal dental college, Belgaum
- The culture media was Brain heart infusion broth (BHI).

Procedure for MIC, MBC (for anaerobes)

- Nine dilutions of each drug was done with Thioglycollate broth for MIC. (Fig:-1)
- In the initial tube 20 micro liter of drug was added into the 380microliter of Thioglycollate broth.
- For dilutions 200microliter of Thioglycollate broth was added into the next 9 tubes separately.
- Then from the initial tube 200microliter was transferred to the first tube containing 200 micro liter of Thioglycollate broth. This was considered as 10^{-1} dilution
- From 10^{-1} diluted tube 200microliter was transferred to second tube to make 10^{-2} dilution.
- The serial dilution was repeated up to 10^{-9} dilution for each drug.
- From the maintained stock cultures of required organisms, 5microliter was taken and added into 2ml of Thioglycollate broth.
- In each serially diluted tube 200microliter of above culture suspension was added.
- The tubes were incubated for 48-72 hours in anaerobic jar at 37°C and observed for turbidity
- From the MIC dilutions tubes, first 3 or 5 tubes were plated (which was sensitive in MIC) and incubated for 24 hrs then next day the colony count was taken.
- MBC was done to see whether there was bacteriostatic or bactericidal effect of the extract (Drug) against the organism.
- If there is no growth then - its bactericidal effect
- If there is minimal growth then - its bacteriostatic effect.

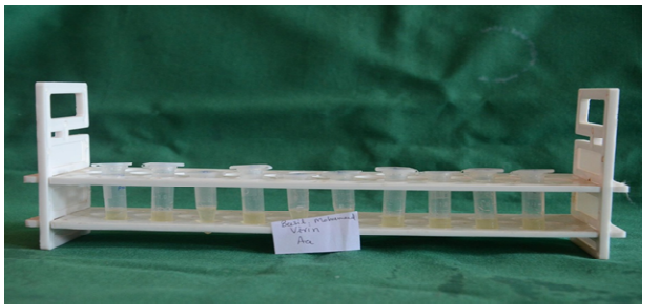

Fig. 1. 9 dilutions of V.C.O in Thioglycollate broth

Growth kill curve

- Equal quantity of broth and compound was mixed, and then immediately it was plated, this was noted as 0 hrs.
- Tubes were kept in CO_2 jar.
- After every 5mins, 10mins, 30mins and 2hrs, It was cultured or plated and incubated according to the growth requirement, i.e., in CO_2 Jar and anaerobic jar (Fig. 2).

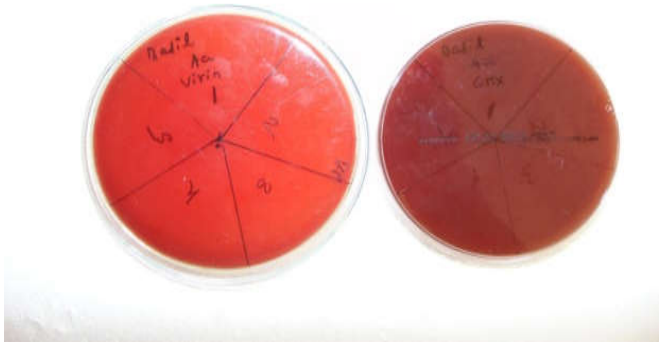

Fig.2. Aa cultured in CO_2 jar at 0.5,10,30 and 2 hours

RESULTS

The results were statistically analyzed using ANOVA. In the MIC results (Table 1) all the microorganisms were found to be resistant to virgin coconut oil, where as in relation to chlorhexidine (Fig. 3), Tenerella forsythia (T.f) was found to be sensitive up to 0.2%, Fusobacterium nucleatum (F.n) sensitive up to 0.4%, Porphyromonas gingivalis (P.g) sensitive up to 25% and Prevotella intermedia (P.i) and Aggregatibacter actinomycetum commitans (A.a) sensitive up to 6.25%. In the "MBC" results (table no:-2), chlorhexidine was found to have bactericidal effect on T.f, P.g and P.i, and bacteriostatic effect on F.n and A.a. Although in MIC, it was sensitive to most of the periodontal pathogens, there was growth of certain organisms. In the "time kill" results (Table no:-3), there was significant decrease in the count of organism with time from 0 mins to 2 hours in the chlorhexidine group when compared to virgin coconut oil for all organisms except P.g which was found resistant to both the groups (Fig 4). For all organisms except P.g there was significant reduction in the colony count at 2 hours. Also for P.g there was no significant difference in the colony count when compared with the chlorhexidine group as the organism was resistant to both virgin coconut oil and chlorhexidine. The most sensitive to chlorhexidine was T.f, F.n and P.i which showed no growth or very minimal colony count after 5 minutes. A.a though significant reduction in growth was seen with chlorhexidine, the colony count was 20 after 2 hours, which shows some resistance of A.a to chlorhexidine (Table 4)

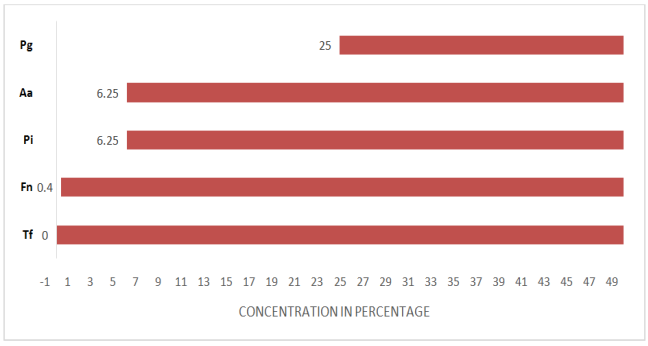

Fig. 3. Sensitivity of chlorhexidine against pathogenic bacteria

DISCUSSION

In the last century numerous evidences have emerged supporting the concept that, in susceptible hosts, bacteria causes periodontal diseases. Coconut oil has a unique role in the diet as an important physically functional food.

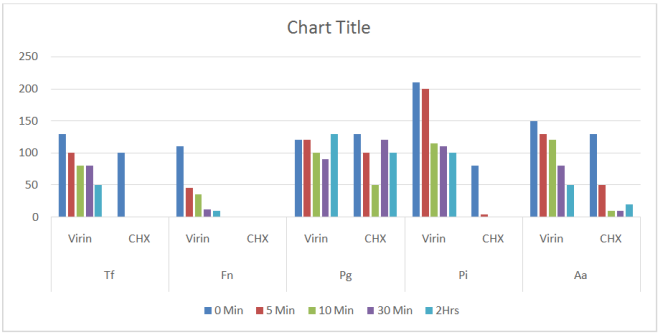

Fig. 4. Time kill results

MIC Results with dilutions from 100 – 0.2 (table no:-1)

Tf	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
Virin	R	R	R	R	R	R	R	R	R	R
CHX	S	S	S	S	S	S	S	S	S	S
Fn										
Virin	R	R	R	R	R	R	R	R	R	R
CHX	S	S	S	S	S	S	S	S	S	R
Pg										
Virin	R	R	R	R	R	R	R	R	R	R
CHX	S	S	S	R	R	R	R	R	R	R
Pi										
Virin	R	R	R	R	R	R	R	R	R	R
CHX	S	S	S	S	S	R	R	R	R	R
Aa										
Virin	R	R	R	R	R	R	R	R	R	R
CHX	S	S	S	S	S	R	R	R	R	R

MBC RESULTS (table no:-2)

Tf	3.12	1.6	0.8	0.4	0.2
Virin	110	113	120	136	142
CHX	NG	NG	NG	NG	NG
Fn	3.12	1.6	0.8	0.4	0.2
Virin	300	318	342	415	418
	6.25	3.12	1.6	0.8	0.4
CHX	8	12	18	26	30
Pg	3.12	1.6	0.8	0.4	0.2
Virin	210	236	242	300	312
	100	50	25		
CHX	NG	NG	NG		
Pi	3.12	1.6	0.8	0.4	0.2
Virin	312	326	412	446	468
	100	50	25	12.5	6.25
CHX	NG	NG	NG	NG	NG
Aa	100	50	25	12.6	6.25
Virin	82	98	102	114	>200
	100	50	25	12.5	6.25
CHX	68	76	92	104	136

Time Kill Results (Table no:-3)

Tf	0 Min	5 Min	10 Min	30 Min	2Hrs
Virin	130	100	80	80	50
CHX	100	NG	NG	NG	NG
Fn	0 Min	5 Min	10 Min	30 Min	2Hrs
Virin	110	46	36	12	10
CHX	100	NG	NG	NG	NG
Pg	0 Min	5 Min	10 Min	30 Min	2Hrs
Virin	120	120	100	90	130
CHX	130	100	50	120	100
Pi	0 Min	5 Min	10 Min	30 Min	2Hrs
Virin	210	200	115	110	100
CHX	80	4	NG	NG	NG
Aa	0 Min	5 Min	10 Min	30 Min	2Hrs
Virin	150	130	120	80	50
CHX	130	50	10	10	20

ANOVA ANALYSIS FOR TIME KILL (Table number:-4)

ORGANISM	F RATIO FOR TIME (a)	F RATIO FOR TREATMENT (b)	SIGNIFICANCE
Tenerella forsythia	8.06	32.53	a)P < 0.05 (there is significant difference with time) b)P<0.05 (there is very high significant difference with the different treatment methods)
Fusobacterium nucleatum	1.25	6.94	a)P <0.05 (there is significant difference with time) b)P<0.05 (there is significant difference with the different treatment methods)
Porphyromonas gingivalis	1.74	0.88	a)P > 0.05 (there is no significant difference with time) b)P<0.05 (there is no significant difference with the different treatment methods)
Prevotella intermedia	5.71	72.06	a)P < 0.05 (there is significant difference with time) b)P<0.05 (there is very high significant difference with the different treatment methods)
Aggregatibacter actinomycetum commitans	6.43	17.53	a)P < 0.05 (there is significant difference with time) b)P<0.05 (there is significant difference with the different treatment methods)

Besides the health and nutritional benefits, coconut oil has been shown to have anti-carcinogenic effects (Oyanagi *et al*., 2012) against colon tumors. What make coconut oil different from most other dietary oils are the basic building blocks, or fatty acids, present in the oil. The study by Kabara *et al*. (1972) and colleagues showed medium–chain (C-8 to C-14) fatty acids and their monoglycerides to have antimicrobial properties against a variety of organisms. The hydrolysis of VCO mainly produces 82% medium chain FAs mostly C-12(C-6 to C-14) FA which might explain its high antimicrobial activity (Sooryavanshi and Mardikar, 1994; Bruce Fife, 2000; DebMandal and Mandal, 2011; Alsberg and Taylor, 1928). A study done by Bergsson *et al*. (1999) using transmission electron microscopy a novel two colour fluorescent kit showed that monocaprin treatment of group B streptococcus damaged the inner plasma membrane, indicating its disintegration by the lipid. However the bacterial cell wall was intact. On scanning electron microscopy indicating that monocaprin can penetrate the extensive meshwork of peptidoglycans in the cell wall without visible changes and reach the bacterial membrane leading to its disintegration. In another research, it was found that enzyme modified coconut oil strongly inhibited the growth of most strains of Streptococcus bacteria, including streptococcus mutans - an acid producing bacterium that is a major cause of tooth decay (Coconut oil could combat tooth decay, 2012). Carpo *et al*. (2007) studied the antibacterial activity of monolaurin across different bacterial isolates and found it had high antibacterial activity. There was also significant and marked difference in resistance rates. Streptococcus aureus, coagulase negative Streptococcus aureus and Streptococcus species did not exhibit any resistance to monolaurin as opposed to varying resistance to antibiotics like penicillin, oxacillin, erythromycin, fusisdic acid, mupirocin and vancomycin (Asokan *et al*., 2009; Anand *et al*., 2008; Asokan *et al*., 2008). Though literature says coconut oil can be beneficial for oral health and can be used to treat periodontal diseases, there are no studies conducted on periodontal pathogens in literature. In this study an attempt has been made to use the current knowledge of causative pathogens of periodontal disease mainly the secondary colonizers and its actual susceptibility to coconut oil.

Limitations

It is an in vitro study. Bacterial susceptibility was found only for 2 hours. We have to analyze the therapeutic effect of V.C.O for longer duration of time. Initial colonizers were not included in the study which could play a major role in climax community. Single dilution method was used for all organisms and there was no repetition of dilution method which could have given us different interpretation.

Conclusion

The results showed that while chlorhexidine was found to be effective against all the five micro organisms (bacteriostatic against Fusobacterium nucleatum and Aggregatibacter actinomycetum commitans, and bactericidal against Prevotella intermedia, Porphyromonas gingivalis and Tenerella forsythia), all the strains of the microorganisms were resistant to virgin coconut oil. Hence based on the results of current study virgin coconut oil has no therapeutic effect in treatment of active periodontal disease. However further studies are being conducted in the department with repeated serial dilutions on the initial colonizers to understand the efficacy of virgin coconut oil in the treatment of periodontal disease, by inhibiting the first step in the formation of climax community.

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