Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 4, 2021, Pages. 10291 - 10296
Received 05 March 2021; Accepted 01 April 2021.

An In-Vitro Evaluation of the Efficacy of the Essential Oils from Leptospermum Scoparium and Melaleuca Alternifolia as an Antimicrobial Agent against P.Gingivalis

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Abstract.
Oral hygiene is essential to prevent the development of periodontal disease. In this contemporary period, there are concerns evolving regarding the use of antibacterial agents due to increasing resistance. The current study aimed to determine the antimicrobial efficacy of the essential oils - Manuka oil and Tea tree oil and Listerine against the periopathogen :*P.gingivalis* .
MATERIALS AND METHODS: Serial dilution method was carried out to analyze the MIC of the essential oils of Manuka and Tea tree oil at 10 different concentrations (100µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.12 µg/ml, 1.6 µg/ml, 0.8µg/ml, 0.4µg/ml, 0.2µg/ml) for P.gingivalis (ATCC 33277). The agar disk diffusion test for the same pathogen was carried out at various concentrations (100µg, 50µg, 25µg, 12.5 µg, 6.25 µg) of the above mentioned agents and the measurement of zone of inhibition was carried out in millimeters. The same procedure was repeated for the essential oil based mouthwash listerine.
RESULTS: The minimum inhibitory concentration values of Tea tree oil and Manuka oil against P.gingivalis was 0.8µg/ml and Listerine mouthwash was 1.6 µg/ml.Tea tree oil showed the strongest inhibitory activity producing inhibition zones of 42mm, 28mm, 21mm, 13mm, 5mm at the concentration of 100µg, 50µg, 25µg, 12.5 µg, 6.25 µg respectively. On the other hand, Manuka oil produced inhibition zones of 37mm, 25mm, 18mm, 10mm at the concentration of 100µg, 50µg, 25µg, 12.5 µg respectively and showed. The Listerine mouthwash, used as a control showed inhibition zones of 22mm, 13mm, 6mm at the concentration of 100µg, 50µg, 25µg respectively , no inhibitory effect was observed at the concentrations lower than the above mentioned, and the MIC value was 1.6µg/ml.
CONCLUSION: Both essential oils tested were effective against P.gingivalis. Following additional research on their clinical safety, they hold promising results in the future as topical agents to prevent and treat periodontal disease.
KEYWORDS: Antimicrobial activity, Manuka oil, Minimum inhibitory concentration, P.gingivalis, Tea tree oil.

Introduction

A group of pathologies that involve the tissues that sustain teeth are designated as periodontal diseases. Typical symptoms of periodontal diseases are clinical attachment loss, pathologic pockets, and bone

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resorption. The etiology, natural history and tissue reaction to therapy of these diseases vary, but their pathogenesis indicates common occurrences that can be modified by genetic factors and/or risk factors.[1]

The precipitating agents of periodontal disease are very well known to be dental biofilm and its byproducts. The correlation between dental biofilm and occurrence as well as severity of periodontal diseases are well established.[2]

Longitudinal reports have indicated that the elimination of bacterial deposits, tartar and proper instruction of oral hygiene techniques will effectively treat these conditions.[3]

The aim of periodontal therapy is to treat inflamed supporting tissues, decrease the number of pathogenic bacteria and remove diseased pockets. Conventional treatment requires complete removal of calculus and plaque, curetting the inflamed soft tissue, and removing the diseased cementum surface.

Periodontal diseases are associated with bacterial infections, therefore, antibacterial therapy appears to be an appropriate way of improving inflammatory tissue conditions. Previously, repeated use of multiple antimicrobials has led to the formation of multidrug resistant bacteria. The natural ingredients, thus, are coming to light. The use of a local drug delivery system has emerged successfully in reducing the distribution of therapeutic agents in the body. Mouth rinses, irrigating solutions and sustained release devices are some of the local delivery systems currently in use.[4]

Natural ingredients that have been used in folk medicine for thousands of years are claimed to be the latest source of antimicrobial agents and are thus finding growing utility in dentistry and medical arena. These drugs extracted from natural herbs seem to have less side effects relative to conventional medications, attributing to better application in reducing the microbial load.[5]

Plant extracts and essential oils have evoked curiosity not only as antimicrobial agents, but as anti-inflammatory and antipyretic as well. The versatility of these agents has paved way for their application in cosmetic sectors too. Therefore, their potential use as natural treatment sources are constantly a topic of research. Concentrated aromatic liquids derived from various parts of a plant like woods, spices, twigs, stems, bark, leaves, flowers, seeds, and buds produce these invaluable gifts from nature - known as essential oils.

Melaleuca alternifolia (Myrtaceae) essential oil originally found in Australia, known as Tea Tree Oil (TTO), has been used medicinally as an antimicrobial agent for many years, particularly in superficial skin conditions. On the other hand, Manuka oil, which is derived from the *Leptospermum scoparium* plant, has recently gained popularity because of its striking antibacterial properties.

Listerine is a commercially available mouth rinse - containing a mixture of essential oils that has anti - plaque properties and does not seem to induce tooth discoloration and alter taste sensation unlike chlorhexidine.[6]

This research was undertaken to determine and compare the anti- bacterial effectiveness of Manuka oil, Tea Tree oil and Listerine in different concentrations, on the periodontal pathogenic organism *Porphyromonas gingivalis*, in view of the increasing popularity of different herbs and their extracts.

MATERIALS AND METHODS

This in-vitro microbiological study was carried out in Department of Microbiology, SRM Institute of Science and Technology, SRM University, Potheri. The study protocol was discussed and accepted by the Institutional Ethical Committee and Review Board (1481 /IEC / 2018).

The *Porphyromanas.gingivalis* strain *ATCC 33277* colonies used in this study that were cultivated in planktonic form were procured from Department of Microbiology, Maratha Mandal's Research Laboratory, Belgaum.

The bacterial strains were maintained anaerobically in BHI media supplemented with 10% defibrinated horse blood, hemin and menadione.

Essential oils:

In this in-vitro study, antibacterial activities of phytochemical essential oils extracted from the plants, *Leptospermum scoparium* and *Me- laleuca alternifolia (Manuka* oil and Tea tree oil respectively), were evaluated against the periodontal pathogen *Porphyromanas.gingivalis* strain *ATCC 33277.*

Leptospermum scoparium (manuka) oil, *Melaleuca alternifolia* (tea tree) oil, were obtained from Great

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Earth, Southern Cross station (Melbourne, Australia), and used in this study .
The agent used as control for this study was Listerine Regular mouthwash manufactured by Pfizer Ltd., Kohalpur, India.
Two strategies were used to test the antimicrobial effectiveness of these essential oils, namely: Serial dilution and Agar Diffusion.

Procedure:

Serial dilution method (Anaerobes):

9 dilutions of each essential oil (control) were carried out with Thioglycollate broth for MIC. In the initial tube 20 microliter of essential oil was added into the 380 microliters of Thioglycollate broth. For dilutions 200 microliter of Thioglycollate broth was added into the next 9 tubes separately. Then from the initial tube 200 microliter was trans- ferred to the first tube containing 200 microliters of Thioglycollate broth. This was considered as 10-1 dilution. From 10-1 diluted tube 200
microliter was transferred to second tube to make 10-2 dilution. The se- rial dilution was repeated up to 10-9dilution for each drug (100µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.12 µg/ml, 1.6 µg/ml, 0.8µg/ml, 0.4µg/ml, 0.2µg/ml). From the maintained stock culture of the organism *P.gingivalis*, 5microliter was taken and added into 2ml of Thi- oglycollate broth. In each serially diluted tube 200 microliter of above culture suspension was added. The tubes were incubated for 48-72 hours in anaerobic jar at 37°C and observed for turbidity.

Agar diffusion method:

As a medium, modified nutrient agar was used. For the test cells, the plates were inoculated. The holes in the plates were drilled out. Using pipettes, three drops of varying essential oil concentrations (100µg/ml, 50µg/ml, 25µg/ml, 12.5 µg/ml, 6.25 µg/ml) were lowered into the punched holes. The plates were incubated in an anaerobic atmosphere for 18-26 hours. The efficacy of essential oil is seen by the size of the growth inhibition of microorganism region around the well, which was measured with the help of dial vernier caliper, and expressed as the diameter of this field.

RESULTS

In the present study the MIC and zone of inhibition of three different agents namely, Manuka oil, Tea tree oil and Listerine were tested at various concentrations for their antibacterial activity on gram-negative black pigmented *P.gingivalis* isolates.
Bacterial inhibition displayed by the plant extracts through utilization of serial dilution method is depicted in table 1. Both Manuka oil and Tea tree oil at the concentration of 0.8µg/ml showed antimicrobial effect on the bacterial cells of *P.gingivalis*. On the other hand, *P.gingivalis* displayed susceptibility to the mouthwash Listerine at the concentration of 1.6 µg/ml.
The zones of inhibition representing the antimicrobial activity of essential oils of Manuka oil, Tea Tree oil and the essential oil containing mouthwash are presented in table 2, 3 and 4 respectively. The zones of inhibition were measured in millimeters with the help of vernier caliper.
The zone of inhibitions of Manuka oil against *P.gingivalis* were found to be 37mm, 25mm, 18mm, 10 mm at the respective concentrations of 100 µg/ml (neat), 50 µg/ml, 25µg/ml , 12.5 µg/ml. No bacterial inhibition was observed in the concentration of 6.25 µg/ml (Table 2).
The zone of inhibition of Tea Tree oil against *P.gingivalis* were 42mm, 28mm, 21mm, 13mm, 5mm at the respective concentrations of 100 µg/ml (neat), 50 µg/ml, 25µg/ml , 12.5 µg/ml, 6.25 µg/ml (Table 3).
Lastly, the zone of inhibition of the control agent i.e Listerine mouthwash against the bacteria were 22mm, 13mm and 6 mm at the respective concentrations of 100 µg/ml (neat), 50 µg/ml, 25µg/ml. No bacterial inhibition was observed in the concentrations of 12.5 µg/ml, 6.25 µg/ml (Table 4).
The comparison between the zone of inhibition produced by Manuka oil and Listerine mouth wash at different concentrations has been illustrated in Graph 1. The various concentrations of the respective agents vary from 100 µg/ml to 6.25 µg/ml. The zones of inhibition are expressed in millimeters.
The comparison between the zone of inhibition produced by Tea Tree oil and Listerine mouth wash at different concentrations has been illustrated in Graph 2. The various concentrations of the respective

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agents vary from 100 μg/ml to 6.25 μg/ml. The zones of inhibition are expressed in millimeters.
The comparison between the zone of inhibition produced by Tea Tree oil and Listerine mouth wash at different concentrations has been illustrated in Graph 3. The various concentrations of the respective agents vary from 100 μg/ml to 6.25 μg/ml. The zones of inhibition are expressed in millimeters.

DISCUSSION:

In dentistry, both naturally and synthetically derived compounds are being used to prevent plaque biofilm-induced diseases.
The dental fraternity as a whole is in constant search of therapeutic materials that not only have a positive impact on periodontal health, but will also lack the usual adverse effects of antimicrobials currently in use. The phytochemical compounds derived from plants such as essential oils provide such an alternative.
In this study, two essential oils and an essential oil based mouth-wash which is commercially available were tested in varying concentrations against the periopathogen *P.gingivalis*.
In the initiation and progression of multiple periodontal disorders, in particular aggressive periodontitis, this microorganism was found to be typically involved.[7]
Our data revealed that both the essential oils, that is Manuka oil and Tea tree oil and the mouthwash Listerine had antimicrobial activity against *P.gingivalis* where Tea tree oil had maximum efficacy and Listerine the least.
P.gingivalis displayed sensitivity to Manuka oil and Tree tea oil at the concentration as low as 0.8 μg/ml as compared to Listerine which was 1.6 μg/ml.
Tea tree oil also exhibited the highest zone of inhibition at the concentration as low as 6.25 μg/ml against the periopathogen *P.gingivalis* followed by Manuka oil which showed its antibacterial activity upto the concentration of 12.5 μg/ml. As the concentration of each oil increased, the antibacterial efficacy also improved.
Listerine mouthwash lacked any antibacterial activity against *P.gingivalis* at 12.5 μg/ml concentration and lower.
Tea tree oil is harvested from the leaves and twigs of the *Melaleuca alternifolia* tree using steam distillation. Studies have demonstrated that it is able to prevent cellular respiration in micro-organism like *Eschercia coli* by altering the permeability barrier of it's cell membrane.[8]Similar to our analysis, it has been shown to provide a substantial permeability barrier of the bacterial cell membrane in various pathogens found in rootcanal environment.[9]Tea tree oil has also displayed significant adhesion inhibiting activity against *P. gingivalis.*[10]
There is a significant proportion of monoterpenes in *Melaleuca* oils, which attributes to its antibacterial action, while Manuka contained mostly sesquiterpenes.
Manuka (*Leptospermum scoparium*) tea trees are smaller vegetation plants scattered across New Zealand in greatly differing habitats, atmospheric conditions and population densities. Classic medical uses of manuka white leaf gums include active or passive sedatives, antipyretic and cough suppressants. A seed capsule decoction could also be used to cure inflammatory diseases. This agent could be used to relieve oral and throat sores by using it as a mouth- wash or gargle.
In a report by **Shapiro et al,**[11]investigating the antimicrobial effects of certain potent essential oils, Tea tree oil demonstrated MIC of 0.11 μg/ml towards *P.gingivalis,* this value in our report is analogous to the MIC value (0.8 μg/ml) of Tea tree oil against the same microorganism.
The antimicrobial activity of essential oils on cariogenic and periodontopathic bacteria, including *P.gingivalis* [12], was evaluated by **Takarada et al** [10]. They reported that periodontopathic bacteria had been completely destroyed by exposure to 0.2 percent manuka oil and tea tree oil for 30 seconds. These observations are in accordance with our research.
Nilima Thosar et al[13]in their research, measured the minimum inhibitory concentration (MIC) of five essential oils against oral pathogens, one of which was Tea Tree oil. *Staphylococcus aureus, Enterococcus fecalis, Escherichia coli* and *Candida albicans* were the pathogens used for the analysis. With a mean MIC of 17.12 ± 31.25, tea tree oil had quite a strong inhibitory effect. While our study focuses on the periopathogen *P.gingivalis*, this particular study indicates that the antibacterial efficacy of tea tree oil against other essential oral pathogens is significant.

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The antibacterial effects of Manuka essential oils have been illustrated by **Chien-Chia Chen et al**.[14]The oil was highly efficient at low concentrations (10 percent) to inhibit growth of the pathogens *S. aureus, S. sobrinus, S. Mutans* and *E. coli.*

In an in- vitro analysis by **Veenu Madaan Hans et al**[15], that tested the inhibition zone of different essential oils including Tea tree oil against *P.gingivalis* that was collected from plaque samples of chronic periodontitis patients, they observed that the TTO inhibition zone was 2.9 ± 0.356 mm in undiluted form while there was no inhibitory effect on the bacteria at 25 percent concentration. This particular finding was contradictory to our study, as TTO displayed inhibitory action against the bacteria at a concentration as low as 6.25 µg/ml with a zone of inhibition of 5mm. The difference in source of *P.gingivalis* might have contributed to the discrepancy in the result of our study.

Since periodontitis is a fairly widespread disease, there are questions about the adverse effects of many commonly used antimicrobial agents in the treatment of oral disease and the increased resistance of oral bacteria to antibiotics. For treatment purpose as well as disease prevention, alternative products that are safe but equally effective are essential. A strong substitute to regular commercial mouthwashes and antibacterial agents may indeed be essential oils, such as those investigated in this report.

LIMITATIONS:

Only one periopathogen *P.gingivalis* was tested for the antibacterial potency of these essential oils. The justification for it is the fastidious state of growth needed for these anaerobic periopathogens to grow.

The minimum bactericidal concentrations have not been evaluated for these essential oils.

CONCLUSION:

The antibacterial efficacy of essential oils holds promising results for its use in future in clinical settings as well as by patients for oral hygiene practices. Both Manuka and Tea tree oils exhibited significant antibacterial activity against *P.gingivalis.* Also, a proportional increase in its antibacterial efficacy was observed as the concentration was increased.Furthermore, additional in-vivo studies and clinical trials are vital to throw more light on their pharmacodynamics and pharmacokinetics and exclude any probable side effects, allergy, and adverse reactions with the tissues, to find potential use of them in oral products.

References

1. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. Perio- dontol 2000. 1997; 14:216–48
2. Moore WE, Holdeman LV, Smibert RM, Cato EP, Burmeister JA, Palcanis KG, et al Bacteriology of experimental gingivitis in young adult humans. Infect Immun. 1982; 38:651-67.
3. Claffey, N., Loos, B., Gantes, B., Martin, M., Heins, P. & Egel- berg. The relative effects of therapy and periodontal disease on loss of probing attachment after root debridement. J Clin Perio- dontol. 1988; 15:163–69.
4. Lall, Winee Surabhi, Amit Alexander Charan, and Akhilesh Bind. "Antimicrobial activity of methanolic and acetonic extracts of Azadirachta indica, Saraca asoca and Curcuma longa." *International Journal of Medicine and Pharmaceutical Sciences* 3.2 (2013): 79-86.
5. Steinberg D, Friedman M. Sustained release drug delivery devices for treatment of dental diseases; In: Tyle P, Ed. Drug delivery de- vices: Fundamentals and applications. New York: Marcel Dek- ker.1998; 491-515.
6. Taheri JB, Azimi S, Rafaela N, Zanjani HA. Herbs in dentistry. Int Dent J 2011; 61:287-96.
7. Moran JM. Home-use oral hygiene products: Mouthrinses. Perio- dontol2000 2008; 48:42-53.
8. SHAMSUDDIN, BABA, and MOHAMMED MAZHARUDDIN KHAN. "ANTIMICROBIAL ACTIVITY AND ANTIBIOTIC SENSITIVITY OF LACTIC ACID BACTERIA ISOLATED FROM FERMENTED FOODS AND A COMMERCIAL PROBIOTIC: A COMPARATIVE IN VITRO STUDY." International Journal of Environment, Ecology, Family and Urban Studies (IJEEFUS) 9.4, Aug 2019, 59-66
9. Stingu CS, Jentsch H, Eick S, Schaumann R, Knöfler G, Rodloff A. Microbial profile of patients with

periodontitis compared with healthy subjects. Quintessence Int. 2012;43(2): e23-e31

10. Cox SD, Gustafson JE, Mann CM, Markham JL, Liew YC, Hart- land RP, et al. Tea tree oil causes K+ leakage and inhibits respi- ration in *Escherichia coli.* Lett Appl Microbiol. 1998;26:355-58.
11. Nilima Thosar, Silpi Basak, Rakesh N. Bahadure, and Monali Rajurkar. Antimicrobial efficacy of five essential oils against oral pathogens: An *in vitro* study. Eur J Dent. 2013 Sep; 7(1): S71–S77
12. Sudha, K., et al. "Determination Of Antimicrobial Activity Of Fruits." *Int J Med Pharm Sci* 7 (2017): 1-8.
13. Takarada K, Kimizuka R, Takahashi N, Honma K, Okuda K, Kato T. A comparison of the antibacterial efficacies of essential oils against oral pathogens. Oral Microbiology Immunology 2004; 19:61-64
14. Idris, Mohamed, et al. "Effect of temperature on partition behaviour and thermodynamic characteristics of bioactive phenolics transfer in aqueous two-phase extraction system and evaluation of antimicrobial activity." *International Journal of Chemical & Petrochemical Technology* 5: 1-8.
15. Shapiro S. Meier A, Guggenheim B. The antimicrobial activity of essential oils and essential oil components towards oral bacteria. Oral Microbiology and immunology 1994;9: 202-208.
16. Pan P, Barnett ML, Coelho J, Brogdon C, Finnegan MB. Determi- nation of in situ bactericidal activity of an essential oil mouth rinse using a vital stain method. Journal of Clinical Periodontology. 2000;27(4): 256-261
17. BORKATAKY, MUNMI, and K. A. U. S. H. A. L. Sood. "Antibacterial, antioxidant and cytotoxic activities of Cinnamomum tamala Nees. Leaves." *International Journal of Medicine and Pharmaceutical Sciences*: 55-62.
18. Nilima Thosar, Silpi Basak, Rakesh N. Bahadure, and Monali Rajurkar. Antimicrobial efficacy of five essential oils against oral pathogens: An *in vitro* study. Eur J Dent. 2013 Sep; 7(1): S71–S77
19. Chien-Chia Chen, Sui-Hing Yan, Muh-Yong Yen, Pei-Fang Wu, Wei-Ting Liao, Tsi-Shu Huang, Zhi-Hong Wen, Hui-Min David Wang. Investigations of kanuka and manuka essential oils for in vitro treatment of disease and cellular inflammation caused by in- fectious microorganisms. Journal of Microbiology, Immunology and Infection. 2014) xx;1e8
20. Veenu Madaan Hans, Harpreet Singh Grover, Himanshu Deswal, Preeti Agarwal. Antimicrobial Efficacy ofVarious Essential Oils at Varying Concentrations against Periopathogen *Porphyromonas gingivalis* Journal of Clinical and Diagnostic Research. 2016 10(9): ZC16-ZC19