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ORIGINAL ARTICLE

Comparative Evaluation of Anti-Microbial Efficacy of Manuka Honey and Pomegranate Mouthwash with Chlorhexidine mouthwash on *P. gingivalis, T. forsythia, P. intermedia and Aggregatibacter actinomycetemcomitans* Periodontal Pathogens: An In-vitro Study

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ABSTRACT

Aim: To comparatively evaluate the antimicrobial efficacy of manuka honey and pomegranate mouth wash on gram negative periodontal pathogens of red, orange and green complex (*Porphyromonas gingivalis* {P.G.}, *Prevotella intermedia* {P.I.}, *Forshythus nucleatum* {F.N.}, *Aggregatibacter actinomycetemcomitans* {A.A.}) in in-vitro conditions.

Materials and methods: Nutrient agar plates were inoculated by rubbing sterile cotton swabs dipped into bacterial suspensions of *P. gingivalis, P. intermedia, F. nucleatum*, *A. actinomycetemcomitans* (overnight cultures grown at 37°C on nutrient agar) over the entire surface of the plate. After inoculation, 10 mm diameter five wells were cut into the surface of the agar using a sterile cork borer for each sample. Honey, pomegranate mouthwash, and chlorhexidine were added into wells in four different plates containing above mentioned four different bacterias. Plates were then incubated at 37°C for 24 hours. The diameters of zones of inhibition were measured using a Vernier calipers on all the plates. The mean score of zones of inhibition was calculated. Similar procedure for all different concentration was performed.

Results: 0.2% chlorhexidine had edge over the manuka honey and pomegranate mouthwash in inhibiting the growth of micro-organisms. *A. actinomycetemcomitans* showed the greatest zone of inhibition by manuka honey of 18 mm, pomegranate mouthwash 19 mm as compared to chlorhexidine getting 20 mm. Both were most effective against A.A. followed by *F. nucleatum*, *P. gingivalis*, *P. intermedia* as compared with 0.2 % chlorhexidine.

Conclusion: Manuka honey and pomegranate mouthwash can create new horizons in the field of chemical agents that can be used as an adjunct to mechanical periodontal therapy can be explored and compared with 0.2% chlorhexidine.

KEYWORDS manuka honey, pomegranate mouthwash, chlorhexidine, periodontal pathogens

INTRODUCTION

Periodontal diseases are among the most widespread oral bacterial diseases of mankind that affect 15–20% of the world's population, including Asia, eventually leading to tooth loss, if left untreated.[1,2] Although bacteria belonging to more than 630 different taxa exist in the oral cavity, only 10–15 bacterial species are recognised as potential periodontal pathogens.[3] Of them, *Porphyromonas gingivalis, Fusobacterium nucleatum*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* are recognised as the major pathogens for initiation and progression of destruction of tooth supporting structures.

Almost 25 centuries ago, Hippocrates, the Father of Medicine, stated "Let food be thy medicine and let medicine be thy food." Supporting this statement, at present, there has been an enormous interest worldwide in nutraceuticals, which are known to play a pivotal role in health management. Many different studies have shown the beneficial effects of a range of different fruits, vegetables, and spices. Dietary fibers are increasing in importance in chronic diseases, and reinforcing their place in the diet, to face these true 'epidemics'.[4]

Pomegranate or *Punica granatum*, is a plant native to India and other tropical and subtropical countries. It is one of the oldest and most consistently grown fruit trees in India. The healing

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property of pomegranates was discussed in one of the oldest medical texts, the Ebers Papyrus from ancient Egypt (1500 BC).[5] Pomegranate shrub belongs to the Punicaceae family which has been used as an astringent, hemostatic, antidiabetic, antihelmintic and also for diarrhea and dysentery.

Benefits of honey as a nutraceutical substance have an ancient history back to 2000–2200 BC in an Egyptian text. Avicenna (980–1037 AD), foremost Persian philosopher-scientist, also introduced honey as a natural remedy to cure wounds. Honey is supersaturated nectar processed by honeybee, Apis Mellifera, contains 40% fructose, 30% glucose, 5% sucrose and about 20% water. Manuka honey which originates from the manuka tree (*Leptospermum scoparium*) is sold as a therapeutic agent worldwide. The presence of Methylglyoxal in manuka honey contributes to its uniqueness and has been termed the unique manuka factor. Many researchers have studied the effect of honey on pathogens and on wound healing process in acute or chronic disease. Honey also has been an interesting topic for negotiation considering its safety, anti-oxidative, bactericidal, antinociceptive and wound healing properties.[6]

Till date chlorhexidine mouthwash is the gold standard in chemical plaque control as it has high substantivity. Due to its various adverse effect such as teeth staining, altered taste sensation, mucosal erosion, supra gingival calculus formation and rarely parotid swelling, chlorhexidine cannot be used for longer duration. Mechanism of action is by disruption of bacterial cell wall there by causing leakage which ultimately leads to death of bacteria.[9]

It would be of great interest of the current trend in periodontal treatment modality if pomegranate and manuka honey with its nutraceutical properties can act in inhibition or even better, destruction of the periodontal pathogens. Before including pomegranate and Manuka Honey in periodontal treatment modalities, it becomes mandatory to evaluate its anti-bacterial efficacy for periodontal pathogens and to compare the same with established local anti-microbial used that is 0.2% chlorhexidine.

PREPARATION OF EXTRACTS AND MOUTHWASH

Extracts were prepared according to previous study done by Pai et al.[10]

Pomegranate fruits were obtained from the local market in Vadodara city. After washing, mesocarp was separated and then dried. Extract was prepared by boiling 10 grams of material in 100 ml sterile distilled water over a low flame for 15 min, then it was allowed to cool for 45 min and then filter paper was used to filter extract, after extract collection it was further diluted to obtain different concentration.

The concentration of pomegranate and manuka honey was obtained by volume by volume dilution. 100% pomegranate and manuka honey had 1 ml of pomegranate and manuka honey. And 75% pomegranate had 0.75 ml pomegranate and manuka honey, 0.25 ml distilled water. And 50% pomegranate had 0.50 ml of pomegranate and manuka honey, 0.50 ml of distilled water. Similarly, 25% pomegranate had 0.25 ml of pomegranate and manuka honey mouthwash and 0.75 ml of distilled water. And 12.5% pomegranate mouthwash had 0.125 ml of pomegranate mouthwash and manuka honey and 0.875 ml of distilled water.

Methodology

An in-vitro study was carried out in the Department of Molecular Biology and Immunology, Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences and Research Centre, Belgaum, Karnataka. Written permission was obtained from Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences and Research Centre, Belgaum, Karnataka for 'extract preparation' and 'microbiological analysis.' The study was started after Institutional Ethics Committee approval was obtained, the antimicrobial activity of these mouthwashes was done by disc diffusion method. Nutrient agar plates were inoculated by rubbing sterile cotton swabs dipped into bacterial suspensions of *P. gingivalis, P. intermedia, F. nucleatum, A. actinomyecetemcommitans* (overnight cultures grown at 37°C on nutrient agar) over the entire surface of the plate. After inoculation, 10 mm diameter five wells were cut into the surface of the agar using a sterile cork borer for each sample. Different concentrations of mouth washes, 0.2% chlorhexidine mouthwash and distilled water were added in the wells assigning each plate for each of the solution. After the incubation period, palates were read only if growth was confluent.

The diameter of zones of inhibition were measured for all the wells using a digital Vernier calipers. The mean score of zones of inhibition were calculated for each solution, respectively.

RESULTS

The study results showed that chlorhexidine had edge over the manuka honey and pomegranate mouthwash in inhibiting the growth of micro-organisms.

Against *P. gingivalis*, chlorhexidine showed a wider zone of inhibition of 18 mm as compared to 13 mm by manuka honey and 12 mm in pomegranate mouthwash, in the well which has 100% concentrations. The well with 75% and 50% concentrations showed 10 mm and 08 mm zone of inhibition with manuka honey and 9 mm and 8 mm with pomegranate mouthwash against *P. gingivalis*. Whereas, *P. gingivalis* were resistant against 25% and 12.5% manuka honey and pomegranate mouthwash.

Similarly against *P. intermedia*, chlorhexidine showed a wider zone of inhibition of 20 mm as compared to 12 mm by manuka honey and 14 mm by pomegranate

Table 1 Comparision of zone of inhibition by pomegranate mouthwash, manuka honey and chlorhexidine on various periodontal pathogens. (R = resistant)

Organisms		100%	75%	50%	25%	12.5%	Chlorhexidine (0.2%)
P. gingivalis	Pomegranate mouthwash	12	09	08	R*	R*	20
	Manuka honey	13	10	08	R*	R*	20
P. intermedia	Pomegranate mouthwash	14	08	08	R*	R*	20
	Manuka honey	12	08	08	R*	R*	20
A. actinomycetemcomitans	Pomegranate mouthwash	19	12	11	08	R*	20
	Manuka honey	18	11	10	08	R*	20
F. nucleatum	Pomegranate mouthwash	15	11	09	09	08	20
	Manuka honey	14	12	12	10	08	20

mouthwash, in the well which has 100% manuka honey and pomegranate mouthwash. The well with 75% and 50% manuka honey and pomegranate mouthwash showed 08 mm zone of inhibition. Whereas, *P. intermedia* were resistant against 25% and 12.5% manuka honey and pomegranate mouthwash.

Manuka honey and pomegranate mouthwash showed most equivalent zone of inhibition against *A. actinomycetemcomitans* as compared to chlorhexidine. Manuka honey showed 18 mm and pomegranate mouthwash 19 mm of zone of inhibition as compared to 20 mm of 0.2% chlorhexidine. 75%, 50% and 25% manuka honey wells showed 11 mm, 10 mm and 08 mm respectively. 75%, 50% and 25% pomegranate mouthwash wells showed 12 mm, 11 mm and 08 mm respectively *A. actinomycetemcomitans* were resistant against 12.5% manuka honey and pomegranate mouthwash.

Against *F. nucleatum*, chlorhexidine showed a wider zone of inhibition of 20 mm as compared to 14 mm by manuka honey and 15 by pomegranate mouthwash, in the well which has 100% manuka honey and pomegranate mouthwash. The well with 75% and 50% manuka honey showed 12 mm zone of inhibition against *F. nucleatum.* The well with 75% and 50% pomegranate mouthwash showed 11 mm and 9 mm zone of inhibition against *F. nucleatum;* whereas, 25% and 12.5% showed 10 mm and 08 mm zone of inhibition with manuka honey and 9 mm and 08 mm zone of inhibition with pomegranate mouthwash.

DISCUSSION

The consensus reports of American academy of periodontology proved and suggested that the micro-organisms responsible for the periodontitis are as follows: *A. actinomycetemcomitans, P. gingivalis, P. intermedia, F. nucleatum.*[11] According to specific plaque hypothesis, by eliminating above mentioned species, periodontal disease would regress.[12] Thus periodontal health of the individual can be established with the help of the agents like manuka honey and pomegranate mouthwash along with the use of mechanical plaque control therapy.

The basic reason behind antimicrobial property might be due to the osmotic effect that is, hygroscopic nature of manuka honey pulls out the water content of the microorganism which leads to death of the microorganism. The acidic ph of manuka honey also acts as a bacteriocidial property against microbes, but the most important property of manuka honey can be said to be production of hydrogen peroxide, to which microorganisms are very sensitive and which leads to the cell death of the microbes.[13] Phenolic compounds are one of the organic compounds of pomegranate which is said to be having the antimicrobial activity. The antimicrobial mechanisms of phenolic compounds involve the reaction of phenols with microbial cell membrane proteins and/or protein sulfhydryl groups that cause bacterial death due to membrane protein precipitation and inhibition of enzymes such as glycosyltransferases.[14] Thus this study was done to investigate in vitro antimicrobial activity of manuka honey and pomegranate mouthwash against certain microbial isolates like *A. actenomyecetamcommitans, P. gingivalis, P. intermedia, F. nucleatum.*

Both the neutraceuticals promotes wound healing, Adiga et al. in 2010 showed that 100 mg/kg aqueous extract pomegranate and topical application on incised wound on rats resulted in significant improvement in healing,[15] but the perfect mechanism is still not clear. Manuka honey has been shown to promote synthesis of

cytokines by monocytes that have the potential to mediate the immune response. In responding to challenges such as infected wounds, neutrophils and macrophages utilise the presence of free radicals such as superoxide and hydroxyl to modulate the activity of other cells such as monocytes and platelets.[17] These in turn produce specific cytokines to signal activation of other cells. However, in a prolonged insult, such as a chronically infected wound, such stimuli may give rise to an excessive response and the ability to dampen free radicals may therefore contribute to the complex interaction that helps to resolve the state of chronic inflammation typifying these wounds.[16]

Manuka honey and pomegranate mouthwash when compared with chlorhexidine could not show better inhibition of periodontopathic microbial species. However, they were effective in inhibiting the growth of the pathogens. There may be few improvements can be made in this study like inclusion of more periodontopathic bacterias, minimum inhibitory concentration can also be found for the different periodontopathic bacterias.

Maintenance or improvement of oral health by use of natural products that also form a part of habitual diet seems to be a suitable way to preserve oral health. Patients will tend to be compliant as they are not burdened with added cost or time. However, this cannot be confirmed without appropriate studies. Additionally, proper utilisation of available natural resources to reduce the ever increasing prevalence of oral diseases in rural parts of developing countries can help to diminish the dangers of a poor dentist-population ratio.

Active ingredients from such potent natural resources should also be evaluated for their anti-inflammatory and immune-modulatory properties. It is recommended that the readily accessible natural products may be integrated with presently available synthetic materials that are used to maintain the oral hygiene. However, any natural product should be assessed for its safety and clinical application should not be done without sufficient scientific evidence. The mechanism of action of a particular natural product needs to be studied at a molecular level in order to assure the accuracy of application and consequently, the outcomes. Although none of the neutraceuticals used in this study are known to have side effects, oral retention and duration of action of these phytoplants is an important factor that needs further exploration.

CONCLUSION

Based on our present findings we can conclude that mauka honey and pomegranate mouthwash indeed possess antimicrobial activity against the periodontal pathogens *P. gingivalis, P. intermedia* and *A. actinomycetemcomitans*, however their ideal concentration and clinical efficacy need to be addressed in future clinical trials of short and long duration, along with their action on the periodontal biofilm. Thus, from this study a wide plethora of new dimension in nonsurgical periodontal therapy can open up, if both can be used as adjunct to nonsurgical periodontal therapy.

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