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Comparative analysis on the antimicrobial action of honey, orange peel and chlorhexidine against E.faecalis, streptococcus mutans and Staphylococcus aureus.

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Abstract

Background:
Antimicrobial activity of honey is exhibited due to it's high sugar content which prevents the growth of the bacteria. Similarly, antimicrobial activity of orange peel is due to the presence of flavonoid-like substances. Chlorhexidine is a standard product which shows both bacteriostatic and bactericidal effects.

Aim:
To compare the antimicrobial activity of honey, orange peel and chlorhexidine against E.faecalis, Streptococcus mutans and Staphylococcus aureus.

Materials and Method:

Honey, orange peel extract and chlorhexidine were obtained commercially. Enterococcus faecalis, Streptococcus mutans and Staphylococcus aureus were taken from the microbiology lab. The antibacterial activity of the extracts will be screened using agar well diffusion assay and compared with 0.2% chlorhexidine.

Results:
The test compounds showed antimicrobial activity against E.faecalis and Streptococcus mutans , but not against Staphylococcus aureus. Chlorhexidine exhibited a higher zone of inhibition.

Conclusion:
Based on the study, it can be concluded that honey shows a bactericidal effect against streptococcus mutans and bacteriostatic activity against Staphylococcus aureus. However, orange peel showed a bacteriostatic effect against streptococcus mutans. Chlorhexidine being gold standard showed a bactericidal effect against streptococcus mutans and staphylococcus aureus. Further studies using herbal products should be used as an alternative tool by carrying out with higher concentrations and in order to get better antimicrobial effects so as to incorporate them in mouthwashes as well as periodontal dressing in the form of topical application to decrease the bacterial load in the oral cavity.

Keywords:
Flavonoid, agar well diffusion assay, chlorhexidine, bacteriostatic, bactericidal, innovative technique.

Introduction

Microorganisms play a major role in our body as they cause a multiple of diseases. One of the most common pathogens being Staphylococcus aureus is said to cause a variety of bacterial infections at the level of blood stream, lower respiratory tract, skin and soft tissue.[1] Penicillin was found to be effective at the earlier stages however increased usage of the same drug led to the development of resistance towards it by the bacteria . This bacteria has a long association with nosocomial infection. The pathogenicity is expressed by the exotoxins which are present . Streptococcus mutans is yet another pathogen which is the common commensal of oral cavity leading to dental caries. This pathogen has an ability to form biofilm which sticks onto the tooth surface leading to cavitation [2]. It possesses multiple high affinity surfaces that enable colonisation even in the absence of sucrose. This complex multi-functional adhesion mediates bacterial attachment to the tooth's salivary pedicle . E.faecalis is another dangerous pathogen which is commonly seen in endodontic infections. It has the ability to invade into the dentinal tubules, causing nutritional deprivement.[3], It also suppresses the activity of the lymphocyte leading to the endodontic failure.[4] It possesses serine protease, gelatin and collagen binding protein that helps to bind to the tubules of the dentin. Inorder to control the growth of these microbes causing diseases, certain antimicrobial products are devised for specific treatment . Generally, these products are made up of chemical materials like chlorhexidine which show side effects but at the same time depict their high antimicrobial properties .

Chlorhexidine bisbiguanide formulation with cationic properties was developed in the late 1940s for the use of broad antimicrobial spectrum [5]. The substantivity of the chlorhexidine is attributed to the controlled release system. The presence of beta cyclodextrin with hydrophilic outer surface and hydrophobic inner core enables them to form inclusion complexes by trapping small amphiphilic molecules in their core which inturn regulates and controls the amount of CHX released. The higher percentage of chlorhexidine shows a greater antimicrobial activity.[6] The major disadvantages caused by CHX is staining, and in few people, desquamative lesions in the oral mucosa.[7] In order to avoid these problems, people are now in

search of medicinal properties . One such medicinal property is orange peel. Very high population in our country, after consuming the orange, consider the peel to be a waste product. Many researches have conveyed that orange peel has its own antimicrobial properties against various microorganisms . The antioxidant property is present in the plant materials due to many active photochemical which includes the vitamins, flavonoids, terpenoids, carotenoids,coumarins,lignin, saponin, plant sterols etc. the citrus fruits and their juices are an important source of the bioactive methanol [8]. Similarly, another medicinal property is honey which also exhibits a good antimicrobial activity against a wide range of bacteria . A good zone of inhibition was obtained in the case of honey when reacted with the methicillin resistant staphylococcus aureus [9]. A synergistic effect was achieved upon the application of honey together with the antimicrobial agents in both gram negative and gram positive bacteria [10]. In ancient times, honey was used for increasing the wound healing process. Thus, the aim of the study is to compare the antimicrobial effect of honey, orange peel and chlorhexidine against Staphylococcus aureus, Streptococcus mutans and E.faecalis.Our team has extensive knowledge and research experience that has translated into high quality publications.[11–23],[24–28 29 30].

Materials and Method

Stud setting:

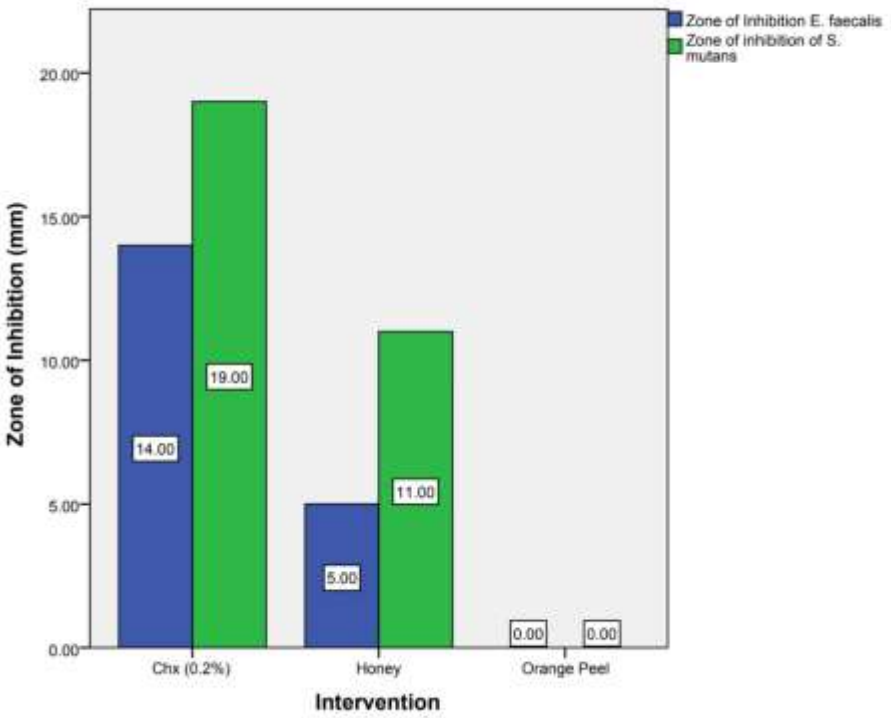
Honey, orange peel and chlorhexidine were obtained commercially. Staphylococcus aureus, Streptococcus mutans and E.faecalis were retrieved as fresh broth cultures from the Department of Microbiology. The antimicrobial activity of the extract was screened using agar well diffusion assay. 0.2% chlorhexidine was used as the control. Lawn cultures of the broth suspensions were made onto the sterile brain heart infusion agar plate and wells were cut for the test compounds and the control. 10µl of honey, orange peel and 0.2% of chlorhexidine were added to the respective wells. All the plates were incubated at 37 degree celsius for 24 hours. After the incubation time, the zone of inhibition was measured in millimeters and was recorded.

Results

Figure 1: The figure represents the zone of inhibition of the antimicrobial activity exhibited by orange peel, honey and chlorhexidine against Streptococcus mutans.

Figure 2: The figure represents the zone of inhibition of the antimicrobial activity exhibited by honey, orange peel extract and chlorhexidine against E.Faecalis.

Figure 3: The figure represents the zone of inhibition of the antimicrobial activity exhibited by honey, orange peel extract and chlorhexidine against Staphylococcus aureus.



Zone of inhibition(mm)

	Staphylococcus aureus	Streptococcus mutans	Enterococcus faecalis
Honey	-	11	5
Orange peel	-	-	-
Chlorhexidine	15	19	14

Graph 1: The graph represents the zone of inhibition exhibited by E.Faecalis and Streptococcus mutans. X axis represents the samples used and Y axis represents the zone of inhibition. Green colour denotes the zone of inhibition for Streptococcus mutans and blue colour denotes the zone of inhibition for E.Faecalis. From the graph, it has been inferred that chlorhexidine exhibits the highest zone of inhibition of 14mm and 19mm for E.faecalis Streptococcus mutans respectively.

Discussion

Honey depicts a good antimicrobial activity due to the high osmolarity. Many researches reveal that both in vivo and vitro studies on therapeutic properties of the honey have shown antibacterial activity against S.aureus . Medicinal properties of the orange peel reveal that it could be used for upset stomach, colic, diuretic, corunative, tonic to digestive system. The biologically active compound in the orange peel exhibits its antimicrobial property. A study by Akdemir revealed that orange peel extract was found to be effective against Klebsiella pneumonia. The antimicrobial activity of orange peel extract is due to the presence of tannins, saponins, phenolic compounds, essential oils and flavonoids . A secondary metabolite compound seen in the ethanolic extract was alkaloid. It acts against pathogenicity of the microbes. From the above results, it can be seen that chlorhexidine exhibits a higher percentage of zone of inhibition when compared to honey and orange peel. In the case of staphylococcus aureus, no significant result could be obtained. Honey is found to exhibit a considerable amount of zone of inhibition against the E.faecalis and Strep. mutans. Honey is said to inhibit the growth of a wide range of bacterial species in vitro . In the study of Jenkins et al, it showed that honey exhibited a potent microbial activity by inhibiting the growth of MRSA by not allowing the cell division to take place [31]. It is found to contain low harmful sweetener in the diet and is also confirmed to possess antimicrobial properties [32]. A contrast study reveals that when honey is used in dressings, they get diluted at one point of time and hence its action reduces. The property of the honey mainly depends on various factors like hydrogen peroxide, phenolic compounds, wound pH, pH of honey, osmotic pressure exerted by honey, level of exudate and the number of times of application. A recent study revealed that antibacterial properties of honey are due to the presence of MGO and an antimicrobial peptide, bee defensin-1. Orange peel has exhibited a bacteriostatic effect.This implies that half of the population alone is destroyed and these two products have the microbial ability to kill the rest half of the population when the concentration is increased [33]. Other researches have proved the antibacterial effect of ethanol components present in the orange peel against E.coli and Staphylococcus aureus [34]. A study of Cowan revealed that the presence of active compounds and the type of solvent in the citrus fruit peel exhibits its antimicrobial potency. Alkaloids, being the secondary metabolite compound, seemed to exhibit toxicity against the cell wall of the microorganisms.Chlorhexidine exhibits the highest percentage of zone of inhibition against strep.mutans, staphylococcus aureus and E.Faecalis. It could be a known fact of CHX exhibiting a greater antimicrobial activity as it is the standard product. The substantivity of chlorhexidine is due to the controlled release system. Beta cyclodextrin regulates and controls the amount of CHX released. Chlorhexidine molecule gets attached to the pellicle by one cation leaving the other free to interact with bacteria to colonise the tooth surface. In this study, chlorhexidine has shown a good antimicrobial property against s.aureus, strep.mutans and E.faecalis. Chlorhexidine serves a good purpose except for it not being a natural product. A study revealed that staining could be less if the concentration of chlorhexidine is less with more dilution. A contrast study of Parappa Sajjan, the most common side effect of chlorhexidine is brownish discoloration of the teeth, restoration and tongue. Regular usage of chlorhexidine causes temporary impairment of taste sensation. Due to the various disadvantages that are seen, these natural products like orange peel and honey are used. Another study revealed the honey showed significant antimicrobial property with different MICs against eight perio pathogens [35]. With the long time use of chlorhexidine, desquamative oral lesions in the oral mucosa

could be seen [36]. However, the limitations of the study include insufficient period of time to check with higher concentrations of the products, performing the activity a number of times would yield a better result. The wastes from these peels can be used as an effective and economical antimicrobial agents which does not produce any side effects.The future scope of the study is that both orange peel extract and honey can be formulated so as to be used in periodontal dressings, root canal irrigants, mouth rinses and toothpastes.

Conclusion

Under the limitations of study, it could be concluded that honey has demonstrated a good antibacterial effect with 5mm and 11mm zone of inhibition against E.faecalis and Streptococcus mutans. Whereas orange peel has exhibited good bacteriostatic effects against the bacteria. However, chlorhexidine being a standard agent as usual exhibited a good antibacterial effect against those bacteria. Further research needs to be carried out by increasing the concentration of these natural products so that good antimicrobial effects can be observed. Being a natural product, it produces no side effects and can be definitely employed into our clinical setup.

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Conflict of interest

The author declares no conflict of interest.

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