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ANTIMICROBIAL ACTIVITY OF FRUIT JUICES ON ORAL BACTERIA

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ABSTRACT: To determine the Antimicrobial activity of fruit juices on the bacterial flora of mouth. The study is done to analyze whether the fruit juices helps in reducing the oral bacterial count which is constantly increasing. Fruits and vegetables are the main sources for essential nutrients. They also have a series of bio active components which have multiple effect on human general health. One such effect is, its Antimicrobial activity. More than 700 species of bacteria, fungi and parasites are present in the oral cavity as commensals. Among the fruit juices which are frequently consumed are apple, pomegranate and guava exhibit have an inhibitory effect on the oral bacterial flora and prevent them from causing dental caries and other oral lesions. The study is done using natural crude extracts of the fruit juices. After extraction they were sterilized by autoclaving at 121^0c for 30 minutes. This is to mimic the natural environment of the mouth when the fruit juices are consumed. The study helps in better understanding the antimicrobial effect of fruit juices on the oral flora.

INTRODUCTION: Oral diseases are the major concern of the world. Certain microorganisms play an important role in causing these oral diseases. Dental caries is an common complication associated with the increase in the proportion of the oral bacterial flora [1]. These microorganisms reside in the oral cavity as a commensal but when favorable conditions occurs they cause damage to the tooth and its surrounding structures. The microorganisms are constituents of the biofilms which are present on the tooth structure [2]. Dental prophylaxis is a cleaning procedure, which is one of the most frequent procedures performed in dental clinics to thoroughly clean the tooth. This process is done by removal of substances like tartar, calculus, plaque and debris.

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That helps in inhibiting or preventing the progression of periodontal diseases and gingivitis. Periodontal diseases are caused by bacteria which colonize the gingival tissues and supporting structures, either supra-gingival or Sub-gingival. The prophylactic measures also include administration of antibiotics to prevent harmful consequences of the microorganisms. Over the period of time due to the extensive usage of the conventional antibiotics, the microorganisms have developed resistant towards these. The problem is compounded due to the inability of the newer antibiotics to attack and destroy the resistant strains of the bacteria. It is documented from ancient times that active compounds of plant origin have been used as medicines to treat various diseases and microbial infections [3].

Some of the fruit juices have proven to exhibit significant antibacterial effect due to their mineral content such as iron and other biologically active substances. So these fruit juices which are abundantly available and have the property of withholding the minerals, bioavailability can be

recommended for as an anti- infective agent in natural medicine for the treatment of infectious dental diseases [4]. For a long period of time medicine importance of fruit juices have been increasing and has become valuable source to maintaining human health as they have great significance on therapeutic value [5].

The antimicrobial activity of apple is significantly influenced by the phenolic compounds and the flavonoids present in it [6]. In response to microbial infections in plants, they produce these which are hydroxylated phenolic substances which has antimicrobial effect on various microorganisms [4].

Medicinal use of guava has been demonstrated in various scenarios. The important constituents of guava are vitamins, tannins, phenolic compounds, flavonoids, etc. The two important flavonoids quercetin and guaijaverin are known for their antimicrobial and anti-oxidant effect. Guaijaverin inhibits the growth of streptococcus mutants as it increases hydrophobicity which an important factor for the oral pathogens to adhere to the tooth structure [7]. This inhibit plaque deposit and inhibit the growth of microorganisms without disrupting the homeostatis of the oral cavity. Quercetin on the other hand disrupts the cell membrane, inactivates the extracellular proteins and form irreversible products [7]. These prove to be bacteriostatic. It also contains vitamin c which improves healing property.

Pomegranate contains polyphenols, tannins, ellagic acid and anthocyanins like Delphinidins, Cynanidins [8]. These inhibit the formation of tartar by obstructing the activity of microorganism that cause them.[9] The tannins present in the pomegranate can cross the cell wall and form complexes of higher molecular weight to disrupt the polyglycans synthesis need for adherence to tooth structure and also increase the bacterial lysis [10].

Pomegranate, Apple and Guava are few tropical fruits which can be taken naturally or in processed form are of high medicinal value and are part of the normal diet. Apple (*Malus domestica*) belongs to the rose family and genus Malus. The flavonoid content in the apple has antimicrobial activity [4]. Guava (*Psidium guajava*) is a small tree in the Myrtle family. The phytochemicals of the guava fruits has an antifungal and anti- bacterial effect [7]. Pomegranate (*Punica granatum*) is a shrub of the family Lythraceae. The polyphenols present in them bind and form compounds which provide anti-infective effects [9, 10].

Hence this study is done in order to evaluate the anti-bacterial property of pomegranate, apple and guava juice on *streptococcus mutants, lactobacilli* and *enterococci.*

MATERIALS AND METHOD:

Preapartion of fresh fruit juices: The fresh fruits such as apple, pomegranate and guava was purchased from the local market. These fruits were then thoroughly washed with water and dried at room temperature. The outer portion of the apple and guava were peeled off and then sliced before smashing. The outer skin was removed and the inner pulp of pomegranate was beat. These fruits were crushed in the grinder and the pulp was extracted. Fresh Fruit juices obtained from these fruits were then diluted with about 350ml of drinking water.

Primary filtration was done using a filter paper and funnel to remove the residual particles. This diluted fruit juice was then subjected to centrifugation using a centrifuge with 2000rpm and then secondary filtration was done using a membrane filter in a filter holder. It was then subjected to autoclaving at 121^0C for 30 minutes. This filtrate was then used for further study.

Preparation of culture: *Streptococcus mutants*, *Lactobacilli* and *Enterococci bacterial* species were isolated from the standard were used. Bacterial suspension was made with them to the turbidity matching 0.5 McFarland standards. This suspension was used for testing the antimicrobial activity of fruit juices. 2ml of each fruit juice extract were taken in 15 sterile cuvette and made into 3 sets of five each. Each 5 cuvette were used for 3 different bacterial species. First five for lactobacilli, second five for Standard strains of streptococcus mutants and the last five for enterococci. To all the cuvettes the respective bacterial suspension of 10ul were added and incubated for 1 hour.

A positive control was done for all bacterial suspension was done with sterile normal saline. The negative control was done with the fruit juice extracted without adding the bacterial suspension. After the incubation period from each cuvette 0.5ml is transferred to sterile cuvette containing 1.5ml of saline and made into whole volume of 2ml. From this 2ml, 10ul is transferred to specific culture media of each bacteria. From the cuvettes that contains streptococcus mutants 10ul was transferred to Mutant sangusa Agar(code), from the cuvettes containing lactobacilli 10ul was transferred to lactobacilli agar(code) and from the cuvettes containing enterococci 10ul was transferred to nutrient agar.

Then the samples were spread uniformly on the surface of the agar plates. The culture plates were then incubated for 24hrs at 37^0C aerobically before evaluation. After the incubation the plates were taken and the colony forming units were counted and the values were tabulated.

RESULT: The study revealed the extent of antimicrobial effect of fresh fruit juices of apple, pomegranate and guava against *streptococcus mutants, lactobacilli* and *enterococcus*.

It was found that there was a significant reduction in the number of colonies present on the culture of all the three pathogens along with the three fruit juices when compared with the positive control. The positive controls exhibited confluent growth whereas the negative control for each fruit juices shows no growth of any microorganism in them.

TABLE 1: THE TABLE SHOWS THE ANTIMICROBIAL ACTIVITY OF POMEGRANTE EXTRACT ON THE PATHOGENS

S.no	Streptococcus Mutants /ml	Lactobacilli /ml	Enterococcus /ml
1	0	186	1920
2	0	252	6048
3	0	210	2928
4	0	207	3274
5	0	180	3990
Mean	0	207	3632

The positive control showed confluent growth of Streptococcus, Lactobaccilli and Entrococcus bacteria.

TABLE 2: THE TABLE SHOWS THE ANTIMICROBIAL ACTIVITY OF APPLE EXTRACT ON THE PATHOGENS.

S.no	Streptococcus Mutants /ml	Lactobacilli /ml	Enterococcus /ml
1	27	537	9336
2	24	756	8328
3	45	1116	8545
4	75	1176	9267
5	60	1053	8684
Mean	46.2	927.6	8832

The positive control showed confluent growth of Streptococcus, Lactobaccilli and Entrococcus.

TABLE 3: THE TABLE SHOWS THE ANTIMICROBIAL ACTIVITY OF GUAVA EXTRACT ON THE PATHOGENS

S.no	Streptococcus Mutants /ml	Lactobacilli /ml	Enterococcus /ml
1	0	321	5184
2	0	372	7056
3	0	367	5764
4	0	297	6776
5	0	216	5820
Mean	0	314.6	6120

The positive control showed confluent growth of Streptococcus, Lactobaccilli and Entrococcus.

TABLE 4: THE MEAN VALUE OF THE COLONY FORMING UNIT OF THE 3 ORGANISMS TREATED AGAINST THE FRESH FRUIT EXTRACTS.

	Streptococcus Mutants /ml	Lactobacilli /ml	Enterococcus /ml
Pomegranate	0	207	3632
Apple	46.2	927.6	8832
Guava	0	314.6	6129

DICUSSION: Nature has provided us with a wide range of botanical wealth and diversity in the types of plants that grow. Antimicrobial agents of plant origin have enormous therapeutic potential [1]. It is known that various bacteria are responsible for causing oral diseases.

There is a wide increase in the field of research in search of herbal substitutes the synthetic Anti-microbial agents. The gram negative bacteria are more resistant to the antimicrobial effect of the various fruit juices than the gram positive bacteria. This is due to the complexity in the cell wall structure of the gram negative bacteria. The gram negative bacteria contains an outer phopspholipid membrane along with the lipopolysaccharide components within it making it resistant to many environmental substances including synthetic and traditional antibiotics.

The gram positive contains layer of peptidoglycans which is does not form an effective barrier [11]. Many plant species are currently being used for their antimicrobial and antioxidant property to increase immunity to diseases.

Table 1 shows Streptococcus mutants was highly susceptible to the antimicrobial activity of pomegranate juice, whereas lactobacilli exhibited significant reduction and enterococcus showed moderate reduction in the colony count when compared with the positive control. Bacteria varies in their susceptibility to alteration in pH. Streptococcus mutants grows better in neutral and slightly alkaline pH. Since pomegranate extract has a high acidic pH which is not suitable for the survival and complete inhibition of streptococcus mutants takes place. Enterococcus shows tolerance to wide range of variation in pH and the reduction in the count is only moderate.

Table 2 shows antimicrobial activity of Apple extract, against the three groups of organism. It is not having antimicrobial activity as pomegranate. The mean value of Colony forming units of all the three organisms are higher than with the pomegranate extracts. As apple contains antioxidants and vitamins in them and hence they are directly not inhibitory to the bacteria but there is a reduction from the control values probably due to unfavorable environment and to an extent due to the presence of phenolic compounds.

Table 3 shows antimicrobial activity of guava extract, which is totally inhibitory to streptococcus mutants and a considerable reduction in lactobacilli count but not very effective against enterococcus. This has a definite beneficial effect in the oral cavity by reducing the mutants and lactobacilli count which are considered to be cariogenic.

Table 4 shows, the comparison of antimicrobial activity of the three fruit extract against the three organism tested. Among the three fruit extracts pomegranate have shown better antimicrobial activity against all the three organisms. It is found to be bactericidal to streptococcus mutants but it is bacteriostatic to lactobacilli and enterococcus in the in use consumable concentration of the fruit extract. The antimicrobial activity may increase when it is less diluted and it has to be further evaluated.

Among the three fruit extracts Apple extract has shown the least antimicrobial activity against the organisms tested, probably due to the presence of low concentration of phenolic compounds.

Guava juice has totally inhibited streptococcus mutants and moderately reduced lactobacilli and enterococcus count. The overall antimicrobial activity of fruit juices is to be considered beneficial in maintaining the oral hygiene. The controls used against all these fruit juices has shown confluent growth and the proportion of reduction with all the fruit extracts shows considerable antimicrobial activity thought they are not bactericidal they all, have bacteriostatic effect.

Regular consumption of fruits, apart from health benefits they help in maintaining good oral health and hygiene. In the in use concentration though they don't show bactericidal activity, it definitely checks the increase in bacterial load that will in turn reduce the risk of developing oral pathologies as well as improves the general health.

CONCLUSION: Summarizing the antimicrobial effect of the fresh fruit juices, the study implies that pomegranate fresh juice has the most significant effect on reducing the growth of the microorganisms followed by fresh guava and apples juices respectively. Hence the consumption of the fruit juices regularly can be a good prophylactic measure to maintain the oral hygiene and general health. Isolation and further evaluation of the phytochemical will yield to the better development of the therapeutic usage of many more plant extracts for various diseases. Incorporation of these agents in oral prophylaxis protocol can be practiced regularly without the harmful effect of the inorganic chemicals.

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CONFLICT OF INTEREST: No conflict of interest

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