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The Effects of Fresh Fruit Extracts and Commercially Available Fruit Extracts on the Growth of *Streptococcus mutans* — An *In Vitro* Study

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ABSTRACT

Resistance of antibiotics towards pathogenic microorganisms has led to the continuous search of natural plant products as a potential alternative for synthetic antibiotic agents. Many plant produces have shown promising results towards this end. This has led to commercialization and packaging of plant produce especially fruits. However, much research needs to be conducted to assess the potential benefits of commercially available plant produce compared to the natural ones. Consumable food items have both positive and bad effects, and their antibacterial effects on various microorganisms found in the oral cavity are good. To comparatively evaluate the effects of fresh fruit extracts and commercially available juice of Apple, Guava and Pomegranate on the growth of *Streptococcus mutans*. The present study describes the antibacterial activity of three selected fresh fruit extract and their commercial counterparts (Apple, Pomegranate and Guava) on *Streptococcus mutans*. Antimicrobial activity of fruit juices (fresh and commercial) was tested by well diffusion assay by an inhibition zone surrounding the well. The aim of the study was to comparatively evaluate the antibacterial activity of three fruit juices on *Streptococcus mutans*. Analysis of the results revealed the highest antibacterial activity for Fresh fruit extract of Guava towards *Streptococcus mutans* with a mean diameter of inhibition zone (DIZ) value of 11.26 ± 0.53(P>0.01) and the least was exhibited for commercial juice of pomegranate with a mean diameter of inhibition zone (DIZ) 2.20 ± 0.25 (P>0.01). The results obtained in this study clearly demonstrated a significant antimicrobial effect of fresh fruit extract of guava against *Streptococcus mutans*. However, preclinical and clinical trials are needed to evaluate biocompatibility and other effects, but *in vitro* observation of fresh fruit extracts of selected fruits appears promising.

Keywords: Antimicrobial; Fruit Juice; Microorganism; Agar well diffusion method; Apple (Malus domestica); Psidium guajava; Pomegranate (Punica granatum)

1 INTRODUCTION

Oral diseases are universally prevalent and can be broadly divided into plaque and non-plaque diseases. Plaque diseases are by far, the most common form of oral diseases. Microorganisms reside in the oral cavity as commensals but under favourable conditions they thrive and become potentially dangerous to teeth and their surrounding structures [1]. Dental caries and periodontal diseases are harmful conditions associated with increase in the proportion of harmful oral bacterial flora. Traditionally chemoprophylaxis has been an important measure to prevent potential harmful consequences of these microorganisms. Over a period of time due to an extensive usage of conventional antibiotics, these microorganisms have developed resistance. This problem is further complicated due to the inability of the newer antibiotics to attack and destroy the resistant strains.

The concept of health promotion has prevailed since centuries and "Healthy Eating" is perceived to be an extremely important measure for health promotion [2]. Dietary habits are apparently changing with modernization and with evolution, drastic transformations in the dietary patterns have been observed [3,4].

There are many references to fruits having medicinal values in ancient literature. People in ancient times regarded fruits to be endowed with magic or divine properties. There has been a great demand of fruit juices in the treatment of various illnesses such as arthritis, heart diseases, muscle aches, and drug addiction [5]. Fruits contain variety of vitamins and minerals that contribute to the daily functions of the body. 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. The role of fruits and their juices in improving oral health is well documented. The industry has exploited this fact and has commercialised the concept with various brands claiming at least if not more health benefits than their naturally occurring counterparts. This present *in vitro* study is thus designed with an aim of evaluating the effects of fresh fruit extracts and commercially available fruit extracts on the growth of *Streptococcus mutans*.

*1.1 **Aim***

To comparatively evaluate the effects of fresh fruit extracts and commercially available juice of Apple, Guava and Pomegranate on the growth of *Streptococcus mutans*.

*1.2 **Objectives***

- Preparation of a standard fresh fruit extract and commercially available fruit extract.
- Obtaining and preparing microorganism strains.
- Inoculating and incubating the organism in specific media containing the fruit extracts and their commercially available counterparts.
- Assessing the effects of fruit extracts/juices on the growth of *S. mutans*.

2 SUBJECTS AND METHODS

An *in vitro* study was designed and conducted to comparatively evaluate the effects of fresh fruit extract and commercially available juices of Apple, Guava and Pomegranate on the growth of *Streptococcus mutans*. Ethical clearance was obtained from the Institutional Ethics Committee bearing a registration number IECKVGDCH/SS15/2019-20.

*2.1 **Selection Criteria***

- **Inclusion criteria**
  - Locally available fruits (apple, guava, pomegranate).
  - Commercially available juice counterpart of the selected fruit.
- **Exclusion criteria**
  - Fruits known to have a high sugar content.
  - Fruits with known erosive properties (acidic).

*2.2 **Study Procedure***

*2.2.1. **Preparation of juices***

Fresh fruits bought from the market were brought to laboratory washed in running tap water; surface sterilized with 70% alcohol, rinsed with sterile distilled water and fresh juice was extracted using Soxhlet method.

*2.2.2. **Sample collection, preservation, and handling***

Fruit extracts were collected in glass vials coded, sealed and stored at a temperature of 4°C. Commercially available juices of apple, pomegranate and guava were directly purchased from the market. The fresh and their respective commercially available juices were then transported to an Institute of Microbiology for quantitative bacterial analysis. The analysis was done within 10 hours of juice extraction.

*2.2.3. **Test organisms***

The microorganism "*Streptococcus mutans*" was obtained and Strains were confirmed by culture on blood agar, MacConkey agar, and nutrient agar plate, Gram-staining, catalase and oxidase test, and biochemical characteristics. Strains were maintained in slants at 4°C till inoculation.

*2.2.4. **Preparation of bacterial inoculums***

For testing antimicrobial activity, *S. mutans* bacterial strains were adjusted equal to 0.5 McFarland standards by adding sterile distilled water. McFarland standards was used as a reference to adjust the turbidity of microbial suspension so that number of microorganisms will be within a given range.

*2.2.5. **Assessment of antimicrobial activity***

Antimicrobial activity of commercially available juice and fresh fruit extract of three selected fruits were tested by well-diffusion assay. Inoculums of each of bacterial strains were suspended in 5 ml of Brain Heart infusion broth and incubated at 37°C for 24 h. After incubation, 100 μl of inoculums was spread on sterile Muller-Hinton agar plates. Wells of 8 mm size was made with sterile borer into Muller-Hinton agar plates containing the bacterial inoculums and the lower portion was sealed with a little molten agar medium. 100 μl volume of the fruit extract (commercially available and fresh fruit extract) was poured into each well of inoculated plates. The plates thus prepared were left at room temperature for 10 min allowing the diffusion of the extracts into the agar. After incubation for 24 h at 37°C, the plates were observed. Antibacterial activity present on the plates was indicated by an inhibition zone surrounding the well containing the fruit juice. The zone of inhibition was measured by measuring scale in millimetre.

*2.2.6. **Statistical analysis***

Performed using R commander software. Descriptive data in the form of mean, standard deviation is presented. ANOVA and mean difference were calculated to assess the significant inter and intragroup difference. Multiple comparison was performed using Tukey post hoc analysis. P-values of less than 0.05 were considered statistically significant.

3 RESULTS

Analysis of the results revealed the highest antibacterial activity for Fresh fruit extract of Guava towards *Streptococcus mutans* with a mean diameter of inhibition zone (DIZ) value of 11.26 ± 0.53 (P>0.01) [Table 1] and the least was exhibited for commercial juice of pomegranate with a mean diameter of inhibition zone (DIZ) 2.20 ± 0.25 (P>0.01). Additional analysis to compare the inhibitory potential of fresh fruit extract compared to their commercial counterparts revealed that natural fruit extracts consistently had a statistically significant better zone of inhibition. Antibacterial efficacy of fresh juices was compared with their respective commercial counterparts, the results indicated that fresh fruit extracts had significantly better properties compared to their counterparts as shown in Table 2. Additionally, fresh guava extract was found to have the highest antibacterial activity compared to all other commercial as well as fresh fruit extracts used in this study.

Table 1: One-way ANOVA, comparing different fruit juices to evaluate their efficacy

Group	Fruit	Number of Samples	Mean ± SD	P
Natural	Apple	5	8.90 ± 0.75	
	Guava	5	11.26 ± 0.53	>0.01
	Pomegranate	5	4.56 ± 0.43	
Commercial	Apple	5	5.18 ± 0.37	
	Guava	5	4.12 ± 0.19	>0.01
	Pomegranate	5	2.20 ± 0.25	

*p < 0.05, statistically significant

Table 2: Comparison between different fruit juices to evaluate their efficacy

Group	Mean±SD	P
Fresh Apple	8.90 ± 0.75	
Commercial Apple	5.18 ± 0.37	>0.01
Fresh Guava	11.26 ± 0.53	
Commercial Guava	4.12 ± 0.19	>0.01
Fresh Pomegrannate	4.56 ± 0.43	
Commercial Pomegrannate	2.20 ± 0.25	>0.01

*p < 0.05, statistically significant

Table 3: Tukey multiple comparisons of means

Types	Difference	Lower Limit	Upper Limit	P Value
NG-NA	2.36	1.462097	3.2579	0.0000003
NP-NA	-4.34	-5.24	-3.44	0
NP-NG	-6.7	-7.59	-5.8	0
CG-NG	-7.14	-8.04	-6.24	0
CP-NP	-2.36	-3.26	-1.46	0.0000003
CA-NA	-3.72	-4.62	-2.82	0
CG-CA	-1.06	-1.96	-0.16	0.014
CP-CA	-2.98	-3.88	-2.08	0
CP-CG	-1.92	-2.82	-1.02	0.0000105

*NG-Natural guava, *NA-Natural apple, *NP-Natural pomegranate, *CA-Commercial apple, *CG-Commercial guava, *CP-Commercial pomegranate

4 DISCUSSION

Antimicrobial resistance, considerable side effects, higher cost of therapy combinations and the emergence of previously uncommon infections are the results of improper usage of synthetic antimicrobial agents. Instead, plant chemicals are one of the most powerful and safe alternative chemotherapeutic agents to control many infections if they are supported by scientific based evidence. There is a wide increase in the field of research in search of herbal substitutes for synthetic antimicrobial agents especially amongst fruits and vegetables due to their potential biological and health promoting effects. Protective effects of fruits and vegetables have been attributed to their bioactive antioxidant constituents, including vitamins, carotenoids, and polyphenols. Among various antioxidants present in fruits and vegetables, polyphenol oxidase (including anthocyanins) has received much attention since being reported to have a positive influence on human health [6].

Apple (*Malus domestica*) a worldwide diffused fruit with many health benefits, mostly due to the presence of phenolic compounds. Apples also ranked second for total content of phenolic compounds, including quercetin, catechin, phloridzin, and chlorogenic acid, all of which are strong antioxidants and thus capable of counterbalancing free radical activities that may cause cell injuries [7]. The phytochemical composition varies greatly with the different varieties of apples and thereby helps in developing new antimicrobials against various infectious diseases.

Psidium guajava is a phytotherapy plant commonly known as guava or poor man's apple is proven for its antidiarrheal, antimicrobial, antiparasitic, antitussive, hepatoprotective, antioxidant, antigenotoxic, antimutagenic, antiallergic, anticancer, and antihyperglycemic effects. The leaves of the guava have been reported to be used for the maintenance of oral hygiene [8].

Pomegranate (*Punica granatum*) fruit is widely consumed, fresh and in commercial products, such as juices, jams, and wine are known to possess enormous antioxidant activity. Pomegranate belongs to punicaceae family. The edible part of fruit contains considerable saccharides, polyphenol, and important minerals. Pomegranate contains polyphenols, tannins, ellagic acid and anthocyanins like delphinidins, cynanidins [9]. These inhibit the formation of tartar by obstructing the activity of microorganisms. The tannins present in the pomegranate can cross the cell wall and form complexes of higher molecular weight to disrupt the polyglycans synthesis needed for adherence to the tooth structure and increase the bacterial lysis [7].

In this study, antibacterial activity of fresh fruit extract and commercial juices of three fruits - Pomegranate (*P. granatum*), Apple (*M. domestica*) and Guava (*Psidium guajava*) was studied. Antibacterial potency was determined using Agar well-diffusion method indicated by an inhibition zone surrounding the well containing the fruit juice. The zone of inhibition was measured by measuring scale in millimetres. The present study showed that fresh fruit extract of Guava has maximum antibacterial efficacy among all three fruit juices (fresh fruit extract and commercial juices) against *S. mutans* with significant value of $P \leq 0.05$ but this was contrary to the finding of the studies conducted by Sahana Kritivasan and N. P. Muralidharan who implied that pomegranate fresh juice had a most significant effect on reducing the growth of microorganisms followed by fresh guava and apples juices respectively [10].

Statistically significant difference was observed for fresh apple extracts included in the study in accordance with the study conducted by Subasish Behera et al where a statistically significant antimicrobial effect was observed with apple fruit juice against *Enterococcus faecalis* and *Streptococcus mutans* reduction ($P<0.001$) [5] and Hassan Raza el al who concluded that apple is more helpful in antimicrobial and antioxidant activities than grapes and pomegranate [11].

Commercially available juice of pomegranate has shown the least antimicrobial activity against the organisms tested with a mean zone of inhibition of 8.9 mm. overall antimicrobial activity of fruit juices is to be considered beneficial in maintaining the oral hygiene. Regular consumption of fruits, apart from health benefits they help in maintaining good oral health and hygiene.

The difference in the antibacterial activity of juices and extracts may be due to their different components; soluble in aqueous and alcoholic media. Fröhling et al (2012) studied on TPC (total phenolic content) and antioxidant capacities of commercial nectars, suggesting that TPC in fruit juice are influenced by several factors such as selection of fruit variety to yield juice, processing methods and storage conditions [12]. Commercial fruit juices processing such as clarification, filtration and pasteurization would strongly affect the phenolic contents of the juices. Clarification and filtration, which aim to yield clear fruit juice, might remove part of the phenolic compounds that bound to the fibre and pectin (Candrawinata et al. 2012) [13]. Heat treatment could degenerate anthocyanins found abundantly in grape (Kechinski et al. 2010) [14]. Storage temperature at 4°C or lower over short period is optimum to preserve the antioxidants (Mgaya-Kilima et al. 2014) [15].

High antioxidant activity in guava juice is mainly attributed to its high content of vitamin C (Thaipong et al. 2006) [16]. Anthocyanin, resveratrol and hydroxycinnamate are reported as main constituents in grape juice (Mullen et al. 2007) [17]. A great variation in antioxidant activities for each type of fruit juices as compared to current literatures is mainly ascribed to the maturity index of fruits selected, technique used in juice extraction and parts of fruits used (peel, fresh, seed) (Burin et al. 2010; Gull et al. 2012; Sreekumar et al. 2014) [18–20]. Additionally, exposure to oxygen or light during sample handling and laboratory analysis might also influence the results (Wang & Xu 2007) [21].

5 CONCLUSION

Summarizing the antimicrobial effect of the fresh fruit juices, the study implies that fresh juice of guava has the most significant effect on reducing the growth of *Streptococcus mutans* followed by fresh extract of apple and commercial juice of apple respectively. Hence, the consumption of the fruit juices regularly can be a good prophylactic measure to maintain the oral hygiene and general health. However, preclinical and clinical trials are needed to evaluate biocompatibility and other effects, but *in vitro* observation of fresh fruit extracts of selected fruits appears promising. Isolation and further evaluation of the phytochemicals 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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