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A Comparative Analysis of Various Sugar Substitutes - A Potential Application in Caries Prevention

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Abstract: Dental caries is one of the most established and worldwide disease of prehistoric and current times. Sugars and their substitutes play an important role in the prevention of dental caries, oral health preventive programmes have to go beyond focusing solely on relation between sugar intake and dental caries. In this study there is an attempt was made to compare various sugar substitutes and their antimicrobial properties. There was no report on the comparative analysis of various sugar substitutes and their antimicrobial properties. The present study is carried out by analysing the phytochemicals present in various sugar substitutes and also their antimicrobial activities by using *E. coli*, *Staphylococcus aureus*, *Vibrio cholerae*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*. In this study, palm sugar shows the better activity against oral bacteria than other sugar substitutes. Also, many people are using cane sugar in case of normal sugar but in our result, we proved that cane sugar and domestic sugar have similar activity against oral bacteria. Palm sugar contains lesser amount of reducing sugar which can be used as a substitute for domestic sugar. Additionally, palm sugar contains many good nutrients and minerals and thus, which can be included in our daily life.

Keywords: Dental caries, sugar substitutes, *E. coli*, *Staphylococcus aureus*, *Vibrio cholerae*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*. Cane sugar, palm sugar.

I. INTRODUCTION

Sugar substitutes are natural, as well as artificial and much popular these days because of their beneficial effect on dental, as well as general health. It is vital that the general population be educated to make informed decisions regarding the amount and type of sugar substitute choices. Recent scientific evidences indicate that routine and long-term consumption of beverages with non-nutritive sweeteners are associated with an increase in risk for 2 types of diabetes, cardiovascular disease, hypertension and stroke. Prolonged use of sugar substitutes contributes to an increased risk of dental caries. Many oral bacteria such as *arachnia*, *lactobacillus*, *leptotrichia*, *treponema*, *pseudomonas* have effect on different varieties of sugar substitutes. Cane sugar is obtained from sugar beet and the palm sugar is a sweetener derived from various varieties of palm tree, it is sometimes qualified by the type of palm as in coconut palm sugar and have slightly different composition while saccharine is non-nutritive or artificial sweetener which is made by oxidizing chemicals like phthalic anhydride and it looks like white crystalline powder. Saccharine is commonly used as a sugar substitute because it does not contain calories or carbs. Many oral bacteria utilize sucrose, glucose, fructose and other simple sugars to produce organic acids (lactic, acetic and propionic) in sufficient concentration to lower the pH of plaque to levels that may result in some demineralization of enamel. The dietary sugars diffuse into the plaque rapidly and are fermented to lactic and other essential acids or can be stored as intracellular polysaccharide by the bacteria. Here is an attempt was made to compare various sugar substitutes and their antimicrobial properties. There was no report on the comparative analysis of various sugar substitutes and their antimicrobial properties so the objectives of the present study are as follows;

- A. To analyse the preliminary phytochemicals, present in various sugar substitute such as cane sugar, palm sugar and saccharine.
- B. To know the antimicrobial activity of sugar substitutes against various oral bacterial species.
- C. To recommend the sugar substitute which can able to inhibit the oral bacteria.

II. MATERIALS AND METHODS

A. Collection of samples

For the present study, the sample is collected from a super market located in Coimbatore. The various sugar substitutes such as cane sugar, palm sugar and saccharine are used as sample for the phytochemical and antimicrobial analysis.

B. Preliminary Phytochemical Analysis

1) *Preparation of Plant Extract:* Each sugar samples were prepared by using standard procedures as described by Harborne *et al.* 1998 The sugar samples are prepared by using soaking method. 25g of each sugar samples were percolated in 200ml methanol for 24 hours with occasional shaking. Then the extracts were then filtered using Whatmann no. 41 filter paper. The organic solvent filtrates were concentrated in vacuum using a rotary evaporator, and the hexane extracts were dried using water bath to obtain crude extracts. They are collected and stored for further analysis.

C. Qualitative Analysis (Harborne *et al.*, 1998)

The extract was tested for the presence of phytochemicals such as alkaloids, carbohydrates, flavonoids, glycosides, phenol, protein, amino acid, saponins, steroids, tannins, terpenoids reducing sugar.

D. Antimicrobial Activity Study

Nutrient agar medium composition

Peptone- 0.5g

Yeast extract- 0.3g

NaCl- 0.5g

Agar- 3g

Distilled water- 100ml

pH- 7.2

Bacterial strains

E. coli

Staphylococcus aureus

Vibrio cholera

Klebsiella pneumonia

Pseudomonas aeruginosa

E. Pure Culture (Harborne *et al.*, 1998)

The pure culture of the clinically important microbial pathogens was obtained through performing streak Plate technique. A loop of full inoculum from culture was then transferred to 50 ml of the nutrient broth and incubated at room temperature (30°C) for further study. Nutrient agar medium prepared as per the above composition and sterilised by autoclaving. Then they poured into sterile petri dishes aseptically to uniform depth of 4mm and then allowed to solidify at room temperature (30°C). After solidification, the test organism was inoculated by sterile swab. This provides uniform surface growth of microorganism and is used for antibacterial sensitive studies. After that the well were made using cup borer (7mm). Add 50µl of plant extract samples, antibiotic (Chloramphenicol) and control (methanol) was introduced into each well. The petri dishes were incubated at 37°C for 24 hrs. The experiments were repeated under aseptic condition. After 24 hrs of incubation, the zone of inhibition produced by the different organism in different plates were measured.

III. RESULTS AND DISCUSSION

Sugar cane or cane sugar refers to several species and hybrids of tall perennial grass in the genus *Saccharum*, that are used for sugar production. The plants are two to six meters tall with stout, joined fibrous stalks that rich in sucrose, which accumulate in the stalk internodes. Palm sugar is a sweetener derived from any variety of palm tree, the plant is sometimes qualified by the palm, as in coconut palm sugar. While sugars from different palm may have slightly different compositions, all are processed similarly and can be used interchangeably. palm sugar contains 70% of sucrose Saccharine is an artificial sweetener with effectively no food energy. It is about 300-400 times as sweet as sucrose but has a bitter or metallic aftertaste, especially at high concentrations. Saccharine is used to sweeten products such as drinks, candies, cookies and medicines.

The present study has been carried out to assess the phytochemical screening and antimicrobial activities of sugars of *Saccharum officinarum* (cane sugar), *Arenga pinnata* (palm sugar), and Ortho- Sulphobenzoic acid imide (saccharine). The preliminary phytochemical analysis carried out for the presence of steroid, terpenoid, glycoside, carbohydrate, protein and reducing sugar in the methanolic extract of sugar substitutes such as cane sugar, palm sugar and saccharine. Among all the phytochemical tests, the presence of glycoside was confirmed in almost all the sugar substitutes. The higher amount of glycoside was observed in methanolic extract of palm sugar confirmed by the presence of dark yellow colour. Carbohydrate was also present in all the sugar substitute except saccharine, the presence was confirmed by the formation of dark brown ring at the junction of two solutions.

The presence of protein was observed in all the methanolic extract of sugar substitute but higher content of protein was confirmed in palm sugar by the presence of blue colour. The content of reducing sugar was seems to be higher in saccharine when compared to cane sugar and palm sugar. Methanolic extract of Palm sugar shows the presence of terpenoid in moderate amount was confirmed by the presence of light blue colour. The content of steroid was observed in methanol extract of cane sugar and sugar but it was totally absent in methanolic extract of palm sugar and saccharine. The present study reveals the absence of alkaloid, tannin, saponin, amino acid and phenol content in all the methanolic extract of sugar substitute tested.

Table-I: Phytochemical Analysis Of Various Sugar Substitutes

| SL. NO. | Phytochemicals | Sugar | Cane sugar | Palm sugar | saccharine |
|---------|----------------|-------|------------|------------|------------|
| 1 | Alkaloids | - | - | - | - |
| 2 | Tannins | - | - | - | - |
| 3 | Saponins | - | - | - | - |
| 4 | Steroids | + | + | - | - |
| 5 | Terpenoids | + | + | ++ | - |
| 6 | Glycosides | ++ | ++ | +++ | + |
| 7 | Flavonoids | - | - | - | - |
| 8 | Carbohydrates | ++ | ++ | +++ | - |
| 9 | Proteins | + | + | +++ | + |
| 10 | Reducing sugar | ++ | ++ | + | +++ |
| 11 | amino acid | - | - | - | - |
| 12 | Phenol | - | - | - | - |

+++ : most present ++ : medium present + : slightly present - : absent

A. Antimicrobial Activity

Antimicrobial activity of all the sugar substitutes such as cane sugar, palm sugar and saccharine were tested, the antimicrobial study was carried out by Agar well diffusion method in which the zone of inhibition was studied against various bacterial strains such as *E. coli*, *Staphylococcus aureus*, *Vibrio cholerae*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*. Among the various sugar substitute tested, methanolic extract of palm sugar shows maximum zone of inhibition against *Staphylococcus aureus* (4.5cm) followed by the methanolic extract of cane sugar, saccharine (Table- II, Plates- I& II). The palm sugar shows inhibition against the oral bacteria *Pseudomonas aeruginosa* whereas other sugar substitute lesser zone of inhibition. The antimicrobial activity against *E-coli* and *Vibrio cholerae* shows that palm sugar has maximum zone of inhibition (2.7 cm, 2.2 cm respectively). Saccharine shows higher zone of inhibition against *Klebsiella pneumoniae* (3.2). The standard used for antibacterial activity is Chloramphenicol used in the present study and the results were tabulated in Table-II.

Table - II: Antimicrobial Activity Study Of Various Sugar Substitutes

| Sl. No. | Bacterial strain | Zone of inhibition of chloramphenicol control (cm) | Zone of inhibition of methanol control (cm) | Zone of inhibition of sugar (cm) | Zone of inhibition of cane sugar (cm) | Zone of inhibition of palm sugar (cm) | Zone of inhibition of Saccharine (cm) |
|---------|-------------------------------|--|---|----------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| 1 | <i>E. coli</i> | 3 | Nil | 2.3 | 2.3 | 2.7 | 2.6 |
| 2 | <i>Staphylococcus aureus</i> | 3.7 | Nil | 3.3 | 3.3 | 4.5 | 4 |
| 3 | <i>Vibrio cholerae</i> | 3.5 | Nil | 2.8 | 2.8 | 3.2 | Nil |
| 4 | <i>Klebsiella pneumoniae</i> | 3 | Nil | Nil | Nil | Nil | 3.2 |
| 5 | <i>Psuedomonas aeruginosa</i> | 1.5 | Nil | Nil | Nil | 2 | Nil |

Plate-I

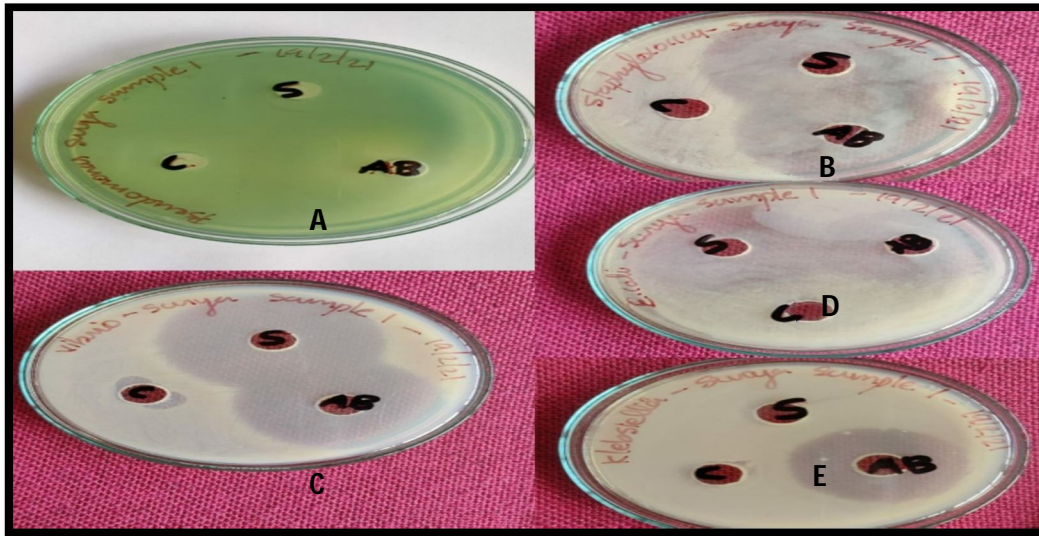
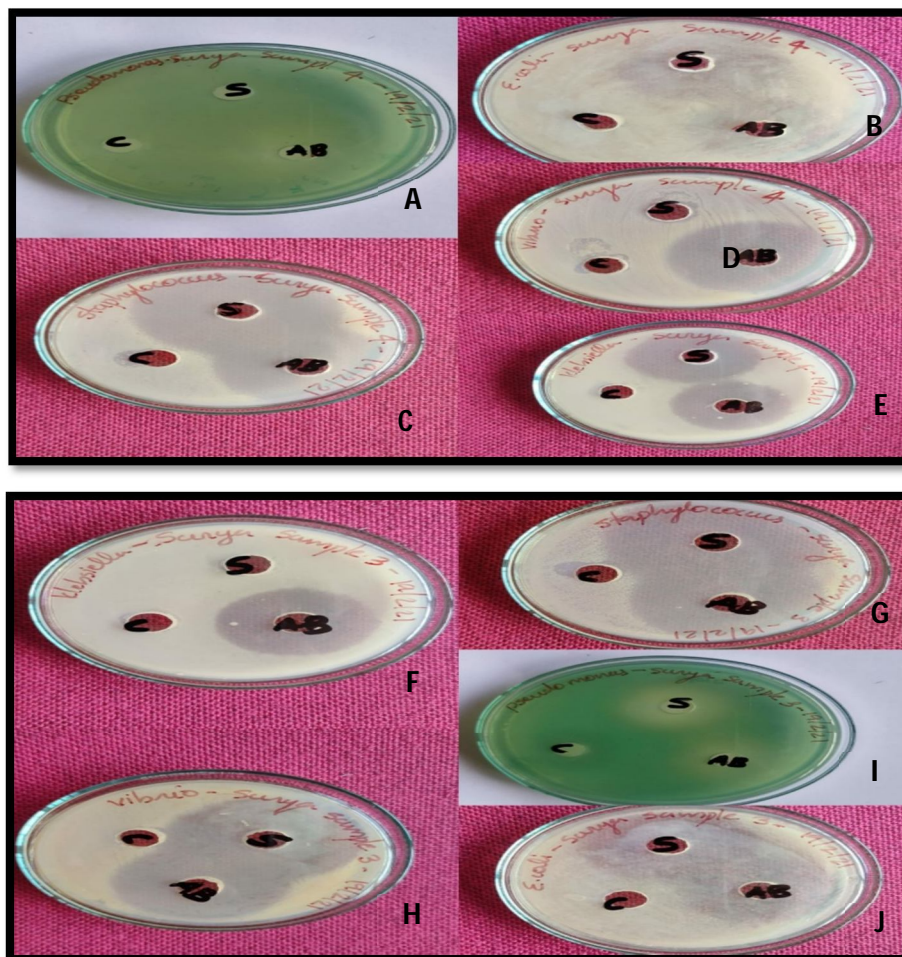


Plate- II



The results represent that palm sugar contains lesser amount of reducing sugar than other sugars so which is good for diabetic patients and which inhibit the growth of oral bacteria. Palm sugar contains a higher amount of glycoside, terpenoid, protein, carbohydrates.

Glycosides are medicines for treating heart failure and certain regular heartbeats, which is commonly used to treat heart related conditions. Terpenoids are used as medicine as an anti-inflammatory, expectorant, bronchodilator, and a local antiseptic. Proteins are better than carbohydrates for diabetes, because carbohydrates affect blood sugar levels so quickly, so there is a temptation to eat less of them and substitute more protein, if protein comes too much which risks heart's health. Palm sugar shows anti-cancerous property, it contains some palm phytonutrients which focused on Vitamin E. Other major phytonutrients are tocotrienol- rich fraction, carotenoids, squalene and co enzymes., which are responsible for the anticancerous property of palm sugar.

The quantity and quality of food residues that will either decrease or increase the numbers or the metabolic activity of oral bacteria; the amount and composition of saliva in the oral cavity; type of preventive and restorative care; immunologic response of the individual; and nutritional and dietary practices. There are many ways to implement adequate maintenance of oral health. Proper selection of a nutritionally balanced diet that does not provide an overwhelming challenge to oral health is one of them, but other preventive measures, especially, improvement of oral hygiene found to be more acceptable and more effective than dietary restrictions in decreasing caries prevalence. Sugar itself is non-corrosive to the enamel, according to the science division of the American dental association. Rather the bacteria in dental plaque metabolize sugar and release acid that can break the enamel down. Sucrose favors colonization by oral microorganisms and increases the stickiness of the plaque, allowing it to adhere in larger quantities to the teeth. This property along with the high specificity of the enzymes involved in the synthesis of the extracellular polymers has led some workers to regard sucrose as having a unique role in caries. The antimicrobial activity of various sugar substitutes reveals that cane sugar has lesser antimicrobial activity than palm sugar. Palm sugar shows inhibition against the oral bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* whereas other sugar substitutes does not show much inhibition. Also, saccharine which shows lesser activity than others and which conveys it is not good for oral health and also not for diabetic patients. These non-carcinogenic sweeteners are recommended by professionals in these clinical settings as an important adjunct in reducing dental caries risk in these individuals. From the present study, palm sugar is a good substitute for normal sugar, and which is the best among these substitutes and is good for oral health and also for sugar patients, which shows better antimicrobial activity.

IV. CONCLUSION

Sugars and their substitutes play an important role in the prevention of dental caries, oral health preventive programmes have to go beyond focusing solely on relation between sugar intake and dental caries. Recently, the role of sugar substitutes promoting remineralisation of enamel has attracted much attention. Thus, the dental profession needs to understand the general features and characteristics of sugar substitute to provide advice on oral health to patients as well as the general public. The use of a greater variety of confectionary containing sugar substitutes and development of new substitutes with high nutritional value are essential in the battle against caries. In this study, we concluded that, palm sugar shows the better activity against oral bacteria than other sugar substitutes. Also, many people are using cane sugar in case of normal sugar but in our result, we proved that cane sugar and domestic sugar have similar activity against oral bacteria. Palm sugar contains lesser amount of reducing sugar which can be used as a substitute for domestic sugar. Additionally, palm sugar contains many good nutrients and minerals and thus, which can be included in our daily life.

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