Inhibition of dextran sucrase activity in *Streptococcus mutans* by plant phenolics

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*Streptococcus mutans* is responsible for causing dental caries in humans and utilizes sucrose for its growth. The dextran sucrase (EC 2.4.1.5) is responsible for sucrose metabolism, which exhibits both hydrolytic and glucosyltransferase activities. In this study, we examined the effects of the plant phenols, namely gallic, tannic and syringic acids and aqueous extracts of certain traditionally used chewing sticks (*Acacia arabica, Azadirachta indica, Pongamia pinnata* and *Salvadora persica*) for prevention of dental caries on hydrolytic activity of dextran sucrase in *S. mutans*. Gallic acid (4-5 mM) produced 80-90% inhibition of the enzyme, while tannic acid (0.2 mM) and syringic acid (5 mM) inhibited the enzyme activity 80% and 48%, respectively *in vitro*. The aqueous extracts of chewing sticks produced 35-40% inhibition of dextran sucrase activity at 5 mg phenol concentration. Kinetic analysis revealed mixed-type of enzyme inhibition by polyphenols, where both $K_m$ and $V_{max}$ were altered. The value of $K_i$ for tannic, gallic and syringic acids were 0.35, 1.6 and 1.94 mM, respectively. The enzyme inhibition by polyphenols was optimum at pH 7-7.5, while by plant extract was maximum at pH 5-6. These results suggest that plant polyphenols may find potential applications in the prevention and control of dental caries by inhibiting dextran sucrase activity in *S. mutans*.

**Keywords:** Dental caries, Polyphenols, Dextran sucrase, *Streptococcus mutans*, Plant chewing sticks.

Dental caries is the single most common biofilm dependent oral infectious disease worldwide. It results from the interaction of specific bacterial and salivary constituents with dietary carbohydrates in biofilms tightly adherent on the tooth surface. Oral biofilms harbor more than 700 bacterial species and most of the bacteria are non-pathogenic *Streptococci*. One of the oral inhabitants *Streptococcus mutans* has long been considered the primary bacterium involved in the initiation and progression of dental caries, one of the most prevalent infectious diseases. The ability of *S. mutans* to adhere firmly to tooth surfaces in presence of sucrose and to form acids by fermenting the dietary sugar has been associated with its caries-inducing potential. Adherence of *S. mutans* to the tooth surface involves two stages: an initial reversible interaction between the organism and the saliva-coated tooth surface and an irreversible stage which is mainly mediated by water insoluble glucan (IG) synthesized from the sucrose by the enzymatic action of glucosyltransferase (GTase).

Sucrose and starch, the main dietary carbohydrates in human diet are potentially more cariogenic in combination than either alone. Surface-adsorbed GTases increase the synthesis of structurally-distinct glucans, which provide enhanced *S. mutans* binding to apatetic surface. Thus, one way to prevent dental caries is by inhibiting the growth and adherence of *S. mutans* to the tooth surface. A variety of compounds have been employed for this purpose, but *S. mutans* is found to be resistant to many of the antibacterial agents. In addition, they may lead to side effects, including gastrointestinal problems. Thus, further research and development of natural antimicrobial agents is needed that are effective and safe for the host.

The green tea extract (GTE) has been reported to contain some cariostatic substances. The several polyphenolic compounds found in GTE can also suppress the growth of *S. mutans* and inhibit glucan synthesis from sucrose by GTases. Several plant extracts have been found to inhibit the growth of many oral bacteria, particularly *S. mutans* and thus may prevent the formation of dental caries. Plant chewing sticks commonly used by human populations in South Asian countries have been reported to inhibit the growth of *S. mutans*.

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Antibacterial effects of chewing sticks on cariogenic bacteria, such as *S. mutans* and inhibitory action on dental plaque formation have been reported. The use of plant-based alternatives for oral health has also been suggested. But, the underlying biochemical mechanism of actions is elusive.

Dextranucrase (EC.2.4.1.5) in *S. mutans* exhibits catalytic activities, namely hydrolytic and glucosyltransferase. We have previously reported that plant polyphenols, such as gallic and tannic acids, are potent inhibitors of disaccharidases in mammalian intestine. However, the effect of these compounds on dextranucrase activity in *S. mutans* is not known. Also, although aqueous extracts of various chewing sticks have been reported to inhibit the growth of *S. mutans* in vitro, but the mechanism of action is not known. Thus, in the present study, we have investigated the effects of plant polyphenols (gallic, tannic and syringic acids) as well as aqueous extracts of some Indian traditional plant chewing sticks (*Acacia arabica, Azadirachta indica, Pongamia pinnata* and *Salvadora persica*) on in vitro dextranucrase activity in *S. mutans*.

**Materials and Methods**

**Chemicals**

Glycerol, NaCl, exogenous dextrose and Folin-Ciocalteu reagent were obtained from Merck Pvt. Ltd. India. Peptone and maleic acid from Himedia, agar from SRL Pvt. Ltd. and Sephadex G-25 was from Sigma Chemical Co, St. Louis, USA, were used.

**Growth of bacterial strain**

A lyophilized culture of *S. mutans* (MTCC 890) was obtained from microbial type culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. For revival, the strain was inoculated into Snyder’s medium B247 (2% dextrose, agar, peptone and 0.5% NaCl, pH 6.8) and the inoculated broth was incubated for 18-24 h at 37°C. Culture purity was checked regularly by microscopic examination.

**Cell growth**

The cells grown in batch culture (200 ml) were harvested by centrifugation at 10,000 rpm for 15 min and washed three-times with saline [0.9%(w/v)].

**Cell disruption**

Cell-free extracts of *S. mutans* were prepared by disrupting saline-washed cells by sonic oscillation. For sonic breakage, washed cells were suspended in 4 vols of 50 mM of sodium maleate buffer, pH 6.8. The suspension was disrupted in a sonic sonifier of frequency 20 KHz, with eight 30 s periods of sonic treatments at 6 amps. The suspension was kept cool in an ice bath during this procedure. The broken cell preparations were centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant fluid served as the cell-free extracts. The pellet was suspended in 4 vols of 50 mM of sodium maleate buffer, pH 6.8 and used for enzyme assays together with the pool fractions for further studies.

**Gel filtration of dextranucrase**

Chromatography was carried out using Sephadex G-25 column (1.5 x 15 cm), equilibrated with 0.02 M sodium maleate buffer, pH 6.8 and washing with the same buffer. Suitable amounts of the enzyme from *S. mutans* were applied on to the column and developed at 4°C with the same buffer at a flow rate of 40 ml per h. The fractions (3 ml) were collected and assayed for dextranucrase activity and protein.

**Preparation of plant extracts**

Samples of the commonly used chewing sticks Neem (*Azadirachta indica*), Kikar (*Acacia arabica*), Sukhchain (*Pongamia pinnata*) and *Salvadora persica* were bought from the open market and were identified by their colour and scent and recognized by agriculturist and vendor. The chewing sticks were shade-dried and cut into small pieces. Each successive 100 g quantity was put into a sterile screw-capped bottle containing 500 ml of 0.05 M sodium maleate buffer (pH 6.8), allowed to soak for an overnight at 4°C and the mixtures were centrifuged at 10,000 rpm for 10 min. The filtrates were then passed through a 0.2-micron filter to remove any bacterial and fungal contaminants. The solutions were concentrated by lyophilization and stored at -20°C in amber flasks.

**Enzyme assays**

Dextranucrase activity was assayed using standard reaction mixture containing 0.05 M sodium maleate buffer (pH 6.8), 0.1 M sucrose, enzyme and extracts in total volume of 0.5 ml. After incubation for 30 min at 37°C, the samples were assayed for glucose using the Glucostat kit (Reckon Diagnostic Pvt. Ltd.). Product formation was linear in the 30 min incubation period under assay conditions. The enzyme activity was determined as µmol sucrose hydrolyzed per min/mg of protein. Protein was measured by the procedure of Lowry *et al.* using bovine serum albumin as the standard. One unit of dextranucrase
activity was defined as the amount of enzyme required to release 1 µmol of glucose per min under standard assay conditions.

To study effect of pH, the enzyme activity was assayed using universal buffer (pH 5-8) containing 0.1 M sodium acetate, 0.1 M sodium maleate and sodium dihydrogen phosphate (pH 5-8) as reported previously\textsuperscript{25}. The effect of polyphenols and plant extracts on the enzyme activity was studied using 0.2 mM tannic acid and 2.0 mM gallic acid or syringic acid and 5 mg phenol equivalent of plant extracts or as specified otherwise.

**Kinetic assay**

To study effects of polyphenols on kinetics of dextransucrase, the enzyme activity was assayed at different substrate concentrations (0.2-1.0 mM) in the presence (2 mM gallic acid or 0.2 mM tannic acid) or absence of polyphenols. The data were analyzed by Lineweaver-Burk plot and from the straight lines obtained, the kinetic parameters $K_m$, $V_{max}$, and $K_i$ were computed using programmable calculator.

**Phenol estimation**

The total phenol content of plant extracts was determined as described previously\textsuperscript{26}. Phenol content of the tissue was expressed as tyrosine or gallic acid equivalents (µg/mg of dry weight).

**Statistical analysis**

The results were expressed as mean ± S.D. Differences between groups were assessed by one-way ANOVA using SPSS software package for windows. Post-hoc testing was performed for inter-group comparisons using the least significant difference (L.S.D.). P-value changes <0.05 were considered significant.

**Results**

The effect of gallic and syringic acids (0-6 mM) and tannic acid (0-0.6 mM) was studied on dextransucrase activity in *S. mutans* in vitro (Fig. 1). Addition of 1 mM gallic acid or 0.1 mM tannic acid to the assay system reduced the enzyme activity from $0.231 \pm 0.009$ µmol/min/mg protein in the control to $0.208 \pm 0.020$ and $0.194 \pm 0.007$ µmol/min/mg protein, respectively. The 5 mM gallic acid or 0.5 mM tannic acid further reduced the enzyme activity to $0.024 \pm 0.001$ and $0.122 \pm 0.003$ µmol/min/mg protein, respectively. However, 1 mM syringic acid produced 48% inhibition of the enzyme activity ($0.131 \pm 0.005$ µmol/min/mg protein), which was further reduced to $0.122 \pm 0.006$ µmol/min/mg protein at 6 mM concentration. Thus, the enzyme inhibition was enhanced by increasing the concentration of polyphenols in the reaction medium.

The effect of aqueous extracts of some plant chewing sticks on dextransucrase activity was also evaluated. The plant extracts contained considerable amounts of polyphenols expressed on dry tissue weight as shown in Table 1, taking gallic acid or tyrosine as the standards. *A. arabica* contained high

![Graph](image)

**Table 1—Polyphenol content of plant chewing stick tissues**

<table>
<thead>
<tr>
<th>Plant chewing stick tissue</th>
<th>Polyphenol content (µg/g dry tissue)</th>
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<tbody>
<tr>
<td><em>Acacia arabica</em></td>
<td>As tyrosine: 0.452 ± 0.03, As gallic acid: 0.395 ± 0.01</td>
</tr>
<tr>
<td><em>Azadirachta indica</em></td>
<td>0.027 ± 0.02, 0.202 ± 0.03</td>
</tr>
<tr>
<td><em>Pongamia pinnata</em></td>
<td>0.074 ± 0.04, 0.360 ± 0.08</td>
</tr>
<tr>
<td><em>Salvadora persica</em></td>
<td>0.055 ± 0.02, 0.096 ± 0.08</td>
</tr>
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amounts of polyphenols (0.395-0.452 µg/g dry tissue) as compared to *A. indica*, *P. pinnata*, *S. persica* on the basis of tyrosine, when compared to gallic acid.

The effect of aqueous extracts of chewing sticks on dextransucrase activity showed 18-37% inhibition of the enzyme activity (Fig. 2). Increasing the concentration of extracts from 0-6 mg phenol, the activity was decreased to 0.196 ± 0.007-0.152 ± 0.009 µmol/min/mg protein, compared to the control (0.231 ± 0.009 µmol/min/mg protein). However, optimum inhibition (35%) was noticed at 5 mg of phenol concentration of extract in case of *A. arabica* (0.152 ± 0.009 µmol/min/mg protein vs 0.231 ± 0.009 µmol/min/mg protein in control), while in case of *A. indica* and *S. persica*, it was 0.173 ± 0.005 µmol/min/mg protein.

Kinetics of enzyme inhibition by various polyphenols was also studied. Kinetic parameters obtained for dextransucrase activity in *S. mutans* in presence of gallic, tannic and syringic acids are given in Table 2. The presence of gallic or tannic acid essentially had no effect on the *K*<sub>m</sub> of the enzyme. However, the value of *V*<sub>max</sub> was reduced by 80-90%, as compared to the control. The values of *K*<sub>i</sub> for the enzyme inhibition by tannic, gallic and syringic acids were 0.35, 1.6 and 1.94 mM, respectively.

The data on the effect of pH on gallic, tannic or syringic acids interactions with dextransucrase activity in *S. mutans* are shown in Fig. 3a. An optimum enzyme inhibition was observed at pH 7.0. At pH 5.5, the enzyme activity was reduced to 0.104 ± 0.003, 0.153 ± 0.001 and 0.198 ± 0.001 µmol/min/mg protein in presence of gallic, syringic and tannic acids, respectively, as compared to control activity 0.250 ± 0.001 µmol/min/mg protein. However, at pH 8.0, the activity was 0.008 ± 0.001 to 0.017 ± 0.001 µmol/min/mg protein in presence of polyphenols.

As shown in Fig. 3b, the inhibition of dextransucrase by plant extracts was also pH-dependent. In contrast to polyphenols, which act as strong inhibitors of enzyme activity between pH 7-7.5 the optimum enzyme inhibition was observed
between pH 5-6. With an increase in pH from 5 to 8 of the assay system, there was a steep decline in the degree of enzyme inhibition by the aqueous extracts at pH 7.5-8.0, the enzyme inhibition was reduced by 47 -56%. However, around neutral pH, the enzyme activity was reduced to 0.116 ± 0.001-0.107 ± 0.001 µmol/min/mg protein in presence of A. Arabica and P. pinnata extracts, respectively and 0.161 ± 0.001-0.152 ± 0.001 µmol/min/mg protein in case of A. indica and S. persica, respectively as compared to the control 0.179 ± 0.001 µmol/min/mg protein.

Preliminary results showed that the inhibition by the plant extracts and polyphenols was reversible, since dilution of inhibitor concentration or dialysis to remove it essentially restored the enzyme activity to control levels (data not shown).

**Discussion**

A number of approaches have been used to control and prevent the formation of dental caries in the past. Among these include the inhibition of microorganism by plant lectins, use of antimicrobial agents such as chlorhexidine gluconate in the mouth rinses, which inhibits the growth of organism by binding to cell surface glycoproteins or by interfering in biofilm formation and in adhesion of microorganism to tooth enamel surface. However, long-term use of chlorhexidine gluconate as anticariogenic agent is limited, due to its disagreeable taste and propensity to stain the tooth brown.

The use of certain plant chewing sticks, including those derived from A. arabica, A. indica, P. pinnata, S. persica is common in various South Asian countries, since they are found to be effective against several types of cariogenic bacteria present in human oral cavity. But, chemical basis of their actions is unknown. In the present study, we investigated the effect of gallic, tannic and syringic acids as inhibitors of the enzyme activity than gallic acid. However, syringic acid, a structural analogue of gallic acid was relatively less effective in inhibiting the enzyme activity (40-48%), which was possible due to the fact that syringic acid lacks free -OH groups in the benzene nucleus of the molecule. The presence of such a polyhydroxy constellation perhaps makes the molecule more effective inhibitor of the enzyme. Both gallic and tannic acids produced V-type effect (reduced Vmax by 80-90%) without affecting Km of the enzyme. However, syringic acid increased the value of Km, presumably due to difference in the interaction of polyphenols with enzyme protein. Further, enzyme inhibition by polyphenols was optimum around alkaline pH, whereas plant extracts inhibited dextransucrase activity at acidic pH (5-6), suggesting that other molecules present in plant chewing sticks might be involved in this phenomenon. These findings also suggested high affinity of polyphenols for dextransucrase, thus may have a potential to inhibit bacterial growth. The observed inhibition of dextransucrase by gallic and tannic acids was similar to that reported for mammalian disaccharidases in rat intestine. However, kinetically the inhibition observed in the present study. Non-competitive-type of inhibition of dextransucrase is also reported by pyridine analogues.

The present results showed that 4 mM gallic or 0.4 mM tannic acids strongly inhibited (80-90%) the dextransucrase activity in S. mutans. The tannic acid was nearly 10-fold more effective in suppressing the enzyme activity than gallic acid. However, syringic acid, a structural analogue of gallic acid was relatively less effective in inhibiting the enzyme activity (40-48%), which was possible due to the fact that syringic acid lacks free -OH groups in the benzene nucleus of the molecule. The presence of such a polyhydroxy constellation perhaps makes the molecule more effective inhibitor of the enzyme. Both gallic and tannic acids produced V-type effect (reduced Vmax by 80-90%) without affecting Km of the enzyme. However, syringic acid increased the value of Km, presumably due to difference in the interaction of polyphenols with enzyme protein. Further, enzyme inhibition by polyphenols was optimum around alkaline pH, whereas plant extracts inhibited dextransucrase activity at acidic pH (5-6), suggesting that other molecules present in plant chewing sticks might be involved in this phenomenon. These findings also suggested high affinity of polyphenols for dextransucrase, thus may have a potential to inhibit bacterial growth. The observed inhibition of dextransucrase by gallic and tannic acids was similar to that reported for mammalian disaccharidases in rat intestine. However, kinetically the inhibition of intestinal sucrase by polyphenol was competitive-type, in contrast to non-competitive inhibition of dextransucrase observed in the present study. The mechanism of inhibition of dextransucrase by plant polyphenols is unknown. But, studies with brush border sucrase have revealed that the binding of these compounds occur at the active site which donates a proton. It is likely that inhibition of dextransucrase activity by polyphenols might involve a similar mechanism, as enzyme contains a proton donor -COOH groups at its active site. The insoluble polysaccharide produced by the action of glucosyltransferase by
Streptococci is thought to be a key determinant in plaque accumulation and if tannins can stop glucan synthesis, the plaque formation can be reduced.26

The observed inhibition of dextran sucrase by plant extracts was also pH-dependent as the optimum enzyme inhibition was observed between pH 5-6, which is also the optimum pH for the growth of S. mutans.37 These findings were in contrast to the inhibition of intestinal sucrase by polyphenols, which are more effective in inhibiting the enzyme activity at acidic pH. The experiments using soluble and particulate dextran sucrase essentially showed similar degree of enzyme inhibition by polyphenols, as well as plant chewing extracts (data not shown). This suggested that micro-environment of the enzyme did not interfere with the inhibitory process in S. mutans. Although the aqueous extracts of chewing sticks were analyzed in terms of their phenol content compared to gallic acid or tyrosine, identification and characterization of some other unidentified compounds which might be responsible for the observed enzyme inhibition, may help in their application for the prevention and control of dental caries by targeting dextran sucrase activity in S. mutans which utilizes sucrose, a primary substrate for its growth and colonisation.

In conclusion, the data presented herein indicate that plant phenolics which are also common ingredients of edible foods are effective agents in inhibiting the dextran sucrase activity in S. mutans. These findings support the century old traditional use of chewing sticks for the control and prevention of dental caries.

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References