Gum Arabic: Can it replace sodium fluoride in toothpaste? “Comparison of their antibacterial activity against Streptococcus mutans”

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Abstract

Background: The effectiveness of fluoride in caries prevention is enhanced by its ability to reduce demineralization, promote remineralization, and antibacterial properties against streptococcus mutans. Interestingly, Gum Arabic has a remineralization effect similar to that of sodium fluoride.

Objectives: This study aimed to investigate the antibacterial activity of Gum Arabic processed by two methods against Streptococcus mutans and compare it to the antibacterial activity of various concentrations of sodium fluoride in order to compare their caries prevention capability.

Design: Antibacterial susceptibility tests for 200mg ethanolic extracts of mechanically ground and spray-dried Gum Arabic, as well as sodium fluoride at concentrations of 0.05% and 1.23% against S. mutans (ATCC 25175) were performed using the agar disc diffusion method. Chlorhexidine and Antibiotic multi-disc for gram-positive bacteria were used as positive controls, while ethanol 20% as a negative control. The diameters of the inhibition zones were measured for all the materials under investigation.

Results: The diameters of the inhibition zones of the spray-dried and the mechanically ground Gum Arabic were (10.67 mm & 9.67mm), respectively, almost approximating each other. In comparison, sodium fluoride (0.05% wash and 1.23% gel) showed inhibition zones of diameters (0mm & 2.33mm), respectively.

Gum Arabic, with both processing methods, exhibited significantly higher antibacterial activity against S. mutans than sodium fluoride (0.05%, 1.23%)

Conclusion: Gum Arabic, either mechanically ground or spray-dried, is a partially active antibacterial against S. mutans. Gum Arabic is a more potent antibacterial against S. mutans than sodium fluoride.

Keywords: Gum Arabic, Acacia Senegal, Anti-caries, Sodium fluoride, Streptococcus mutans, Antibacterial activity

INTRODUCTION:

Natural products can be a cost-effective approach to dental caries prevention, particularly in low-income countries where dental caries is widespread and resources are limited. With concerns over the side effects of antimicrobials and increasing antibiotic resistance, scientists are exploring natural alternatives. These products are known for being safe, easily accessible, and cost-effective. Many natural products have been studied for their effectiveness in preventing cavities and are even incorporated into dental products.

Gum Arabic, also known as Acacia Senegal, is a natural gum from Acacia trees. It contains non-viscous soluble fiber and is chemically composed of calcium, magnesium, and potassium salts of a polysaccharidic acid (Arabic acid). Traditionally, Gum Arabic has been used as an oral hygiene substance by many communities in the Middle East and North Africa. Its roots have been used to alleviate bleeding gums and pain from loose teeth by sucking on or applying small amounts to the affected area.

Gum Arabic (Acacia Senegal) nodules undergo different processing methods depending on the quality of the powder needed. Mechanically-milled Gum Arabic is created by grinding dried Gum nodules using machines. Meanwhile, spray-dried Gum Arabic is produced by making a solution of Gum Arabic, which is then pasteurized and sprayed into hot air. The water in the solution evaporates in the hot air, resulting in a dry powder of Gum Arabic.

Many studies have proven the antibacterial efficacy of Gum Arabic against various bacterial species. There is insufficient data to determine the antibacterial effect of Gum Arabic against S. mutans. According to literature, Gum Arabic has anti-caries properties and is just as effective as sodium fluoride in promoting teeth remineralization. This is an important finding as it suggests that alternative approaches to high fluoride preventive measures, such as Gum Arabic, may be viable. These reports...
could inspire researchers to explore different aspects of caries prevention for Gum Arabic. *Streptococcus mutans* is considered the initial causative agent of dental caries and the beginner for tooth destruction. Accordingly, the antibacterial effect against *S. mutans* is an essential aspect of caries prevention.

This study was carried out to investigate the antibacterial activity of Gum Arabic processed by two different techniques against *Streptococcus mutans*, and to compare it to the antibacterial activity of different concentrations of sodium fluoride (the gold standard for caries prevention).

**MATERIAL AND METHOD:**

**Collection of the Plant:** Gum nodules were collected and authenticated at the Herbarium of Medicinal and Aromatic Plants & Traditional Medicine research institute with code number G-1983-1- MAPTRI-H. After that, the nodules were air-dried at room temperature away from the sun. They were then processed using two different methods: spray drying and mechanically grinding with a pestle and mortar.

**Preparation of extract**:\One hundred and fifty grams of each type of Gum Arabic was soaked in ethanol (70%) for three days with daily filtration. The solvent was evaporated and both extracts were inserted in a freeze dryer till complete dryness.

**Antibacterial susceptibility test:**

Antibacterial susceptibility test was performed by Agar disc diffusion method with the following steps:

1. **Preparation of the media:** Brain Heart Infusion (BHI) agar was prepared according to the instructions. Each 20 mL of the freshly prepared and autoclaved BHI agar was poured into a sterile petri dish and maintained at room temperature to cool down. Before use, the plates were incubated at 35 °C for 48 hours, and the sterility was checked.

2. **Preparation of discs:** Filter paper discs of 6 mm diameter were prepared from Whatman filter paper No. 1, placed in a petri dish, and sterilized in a hot air oven at 160 °C for two hours.

3. **Preparation of extracts solutions:** 200 mg of each extract was dissolved in 1 ml of 20% ethanol (Reagents Duksan, Ethyl Alcohol, absolute, product No.6923, 2.5L) and mixed well using a vortex to ensure complete dissolution.

To prevent any unexpected synergistic effect of the solvent (ethanol 20%) on the antibacterial activity of the extract:

1. A disc impregnated with ethanol 20% was placed on a plate inoculated with *S. mutans* and incubated at 37°C for 24 hours under anaerobic conditions. The solvent was used after no inhibition zone was observed around the disc.

2. Ethanol 20% itself served as a negative control.

4. **Reactivation and inoculation of Streptococcus mutans:** Standard *S. mutans* (ATCC 25175) was placed into 5ml of brain heart infusion (BHI) broth and incubated at 37 + C for 48 hours. Afterward, a sterile swab was used to transfer *S. mutans* from the BHI broth onto a blood agar plate. The plate was then incubated in anaerobic conditions at 37°C for 48 hours.

5. **Preparation and standardization of inoculum suspension:** Three to five well-isolated colonies of the same morphological type were selected from a blood agar plate culture and transferred with a sterile loop into a tube containing 5 ml of BHI broth. The broth was incubated at 37°C for 24 hours.

The turbidity of the *S. mutans* suspension was adjusted to match that of a 0.5 McFarland standard (1.5 × 10⁸ CFU/mL) using BHI broth for dilution.

6. **Antibacterial susceptibility test:**

A sterile cotton swab was used to streak BHI Agar plates with the adjusted bacterial suspension.

Three sterile discs were immersed in 10 µL of each of the following solutions:

- Spray dried Gum Arabic extract solution.
- Mechanically ground Gum Arabic extract solution.
- Fluoride 0.05% (Tebodont-F Mouthwash 250 mL, tea tree oil with 0.05% sodium fluoride)
- Fluoride1.23% (Iont, 1.23% APF Fluoride Gel – Mint parfait, 17 oz., Manufacturer code: 56-00006)

Ethanol (20%) (Reagents Duksan, Ethyl Alcohol, absolute, product No.6923, 2.5L) was a negative control. Chlorhexidine 0.2% (Clenora mouthwash, Chlorhexidine Gluconate BP 0.2%w/v) and readymade multiple antibiotic disc (Figure 1) (Axiom laboratories, multidisc for Gram-positive isolates, Code No. 001, Mantola Pahar Ganj- New Delhi-110055) were used as positive control.

After saturating the discs with solutions, they were transferred to the plates inoculated with *S. mutans*. These plates were then incubated at 37°C for 24 hours under anaerobic conditions. The plates were monitored for the appearance of a transparent area surrounding each disc, which is known as an inhibition zone. The measurement of the diameter of each inhibition zone was taken.

**RESULTS:**

The data were analyzed with SPSS 20 software, and the Least Significant Difference (LSD) test with a significance level of ≤ 0.05 was used to compare the two types of Gum Arabic and various fluoride concentrations.
One-sample t-test was conducted to determine if there was a significant difference between antibiotics and Gum Arabic, sodium fluoride, and chlorhexidine. The significance level was adjusted to 0.01 based on Bonferroni criteria.

The inhibition zones of the tested materials (Figures 2-8) provide insight into their antibacterial activity. The measurements of the inhibition zones are present in (Table 1).

The diameters of the inhibition zones for the spray-dried Gum Arabic and the mechanically ground Gum Arabic were almost the same, with 10.67 mm and 9.67 mm, respectively. There was no significant difference in the antibacterial activity of the two forms of Gum Arabic against S. mutans. (Table 2)

Gum Arabic, with both processing methods, exhibited significantly higher antibacterial activity against S. mutans than sodium fluoride (0.05% wash) and (1.23% gel), but significantly lower antibacterial activity than chlorhexidine (Table 2).

Only Gentamycin, Cephalexin, and Cefotaxime from the antibiotics listed in Figure 1 showed antibacterial activity against S. mutans with a 15mm inhibition zone diameter for each. Gum Arabic, with both processing methods, showed lower antibacterial activity against S. mutans than Gentamycin (10 mcg), Cephalexin (300 mcg) & Cefotaxime (30 mcg). However, only mechanically ground type displayed statistically significant result (P-value= 0.004) (Table 3).

Chlorhexidine showed significantly higher antibacterial activity against S. mutans than sodium fluoride 0.05% and 1.23% (P=0.000).

Table 1: Measurements of inhibition zones of Gum Arabic and Sodium fluoride on plates inoculated with S. mutans

<table>
<thead>
<tr>
<th>Plant extract (200mg/ml)</th>
<th>Inhibition zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1</td>
</tr>
<tr>
<td>Spray dried Gum Arabic</td>
<td>10</td>
</tr>
<tr>
<td>Mechanically ground Gum Arabic</td>
<td>9</td>
</tr>
<tr>
<td>Fluoride 0.05%</td>
<td>0</td>
</tr>
<tr>
<td>Fluoride 1.23%</td>
<td>7</td>
</tr>
<tr>
<td>Chlorhexidine (CHX) 0.2%</td>
<td>12</td>
</tr>
<tr>
<td>Ethanol 20%</td>
<td>0</td>
</tr>
<tr>
<td>GM (Gentamycin 10 mcg)</td>
<td>15</td>
</tr>
<tr>
<td>PR (Cephalexin 300 mcg)</td>
<td>15</td>
</tr>
<tr>
<td>CF (Cefotaxime 30mcg)</td>
<td>15</td>
</tr>
</tbody>
</table>

M1, M2, M3: Measurements of inhibition zones

N: Number of measurements

Table 2: A least significant difference (LSD) statistics to compare the antibacterial activity of two types of Gum Arabic against S. mutans versus different fluoride concentrations, using a significance level of 0.05.
Table 3: One sample t-test statistics to compare the antibacterial effect of Gum Arabic, Fluoride, and Chlorhexidine against *S. mutans* versus *Gentamycin*, *Cephalexin*, and *Cefotaxime* in multidisc antibiotic (Test Value = 15), using level of significance 0.01.

<table>
<thead>
<tr>
<th>Investigated material</th>
<th>Number of readings</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>t</th>
<th>P-Value</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spray dried Gum Arabic</td>
<td>3</td>
<td>10.67</td>
<td>2.08</td>
<td>-3.606</td>
<td>.069</td>
<td>Non-significant</td>
</tr>
<tr>
<td>Mechanically ground Gum Arabic</td>
<td>3</td>
<td>9.67</td>
<td>0.58</td>
<td>16.00</td>
<td>.004</td>
<td>Significant</td>
</tr>
<tr>
<td>Sodium fluoride (1.23%)</td>
<td>3</td>
<td>2.33</td>
<td>4.041</td>
<td>-5.429</td>
<td>.032</td>
<td>Non-significant</td>
</tr>
<tr>
<td>Chlorhexidine (0.2%)</td>
<td>3</td>
<td>14.00</td>
<td>2.646</td>
<td>-.655</td>
<td>.580</td>
<td>Non-Significant</td>
</tr>
</tbody>
</table>

Figure 2: Inhibition zones around discs saturated with spray dried Gum Arabic on a plate inoculated with *S. mutans*

Figure 3: Inhibition zones around discs saturated with mechanically ground Gum Arabic on a plate inoculated with *S. mutans*

Figure 4: Inhibition zones around discs saturated with Sodium Fluoride 0.05% wash on a plate inoculated with *S. mutans*

Figure 5: Inhibition zones around discs saturated with Sodium Fluoride gel 1.23% on a plate inoculated with *S. mutans*
DISCUSSION:
Alves et al.21, and Mulhtar & Ghori 22, proposed an activity scale based on the inhibition zone diameter. This activity scale has described an inhibition zone less than 9mm as an inactive product, 9-12mm as a partially active product, 13-18mm as an active product, and more than 18mm as a very active product. Based on this scale, the present study has concluded that Gum Arabic, either mechanically ground or spray-dried, is partially active antibacterial agent against S. mutans.

The result of the current study on the antibacterial activity of Gum Arabic against S. mutans was different from that of Banjar et al. 13. The concentration of 200mg/ml Gum Arabic in the present study showed less antibacterial activity than the 40 mg/ml concentration used by Banjar et al. 13. The antibacterial activity of Gum Arabic in this study displayed an equivalent result to the concentration of 5mg/ml-10mg/ml of Gum Arabic used in the study performed by Banjar and colleagues 13.

It is observed that there is a distinct variation in the antibacterial effectiveness of Gum Arabic between the two studies. This variation can be attributed to the difference in the source of Gum Arabic used in the studies. The literature has mentioned that the chemical makeup and medicinal properties of Gum Arabic may vary depending on the source of gum nodules, the climate, the season, and the age of the trees from which gum nodules were collected 23. Moreover, the difference in bacterial virulence between the standard S. mutans (ATCC 25175) used in the current research and the isolated S. mutans used by Banjar et al. 13 may also play a role in this difference. To ensure consistency in different studies, Ali and colleagues recommended using a Gum Arabic preparation that is chemically well-characterized for research purposes 23. The present study supports this recommendation.

Although the objective of this study is not to compare the antibacterial activity of Gum Arabic to antibiotics and chlorhexidine mouthwash, the results of these comparisons are still worth discussing.

The study has agreed with the findings of Banjar et al. 13 that Gum Arabic has lower antibacterial activity against S. mutans than Gentamycin despite using different concentrations of Gum Arabic and Gentamycin in the two studies.

Gum Arabic, with both processing methods, has displayed lower antibacterial activity against S. mutans than chlorhexidine. This finding is consistent with a meta-analysis24 that considered chlorhexidine the most effective mouthwash compared to herbal alternatives. However, the study has contradicted the results of a clinical trial conducted by Dina Kamal and colleagues 15. Kamal’s team found no significant difference in S. mutans reduction after using Gum Arabic or chlorhexidine for 3 and 6 months. However, after 9 and 12 months of use, Gum Arabic mouthwash was found to reduce S. mutans count more than chlorhexidine 15.

Gum Arabic exhibited more potent antibacterial properties against S. mutans compared to 0.05% and 1.23% concentrations of sodium fluoride. This result is consistent with previous literature that considered natural products to have a more potent antibacterial effect than fluoride 25,36. It also supports studies considering fluoride a non-potent antimicrobial agent 37.

Fluoride is the gold standard in caries prevention. It inhibits tooth decay by enhancing remineralization and reducing demineralization 38. However, Gum Arabic has shown a similar remineralization effect to sodium fluoride 16, 17. Based on its antibacterial activity against S. mutans and its remineralization effect, Gum Arabic may considered superior to sodium fluoride in preventing caries.
There is scientific evidence that Gum Arabic can be an effective ingredient in toothpaste for improving periodontal health. Studies have shown that toothpaste containing Gum Arabic can reduce the plaque index, gingival index, and bleeding on the probing index. Trials with Gum Arabic gel and powder have also demonstrated significant improvements in plaque and gingival indexes for people with gingivitis, as well as a reduction in microbial counts comparable to those achieved with 1% chlorhexidine gel. Unsurprisingly, Gum Arabic has shown promising results in preventing caries. The era of fluoridated toothpaste may come to an end, and a new era of Gum Arabic toothpaste may begin. However, clinical trials are recommended to test its effectiveness as caries preventive measure.

**CONCLUSION:**

Gum Arabic, with both processing methods, is partially active as an antibacterial against *S. mutans*. Based on its antibacterial effect against *S. mutans*, it is considered superior to sodium fluoride in preventing caries.

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**Conflict of interest:** The authors have no conflicts of interest to declare.

**Availability of Data and Materials:** The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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