ANTIMICROBIAL AND SYNERGISTIC EFFECTS OF MISWAK, NANO-SILVER DRUG, AND CHLORHEXIDINE ALONE AND THEIR COMBINATIONS UPON CERTAIN ORAL MICROBIOTA

Sakeenabi Basha, Amal Adnan Ashour, Nayef H. Felemban, Enas T. Enan, Mohammed Fareed Felemban, Amal Ahmad Alyamani, and Sanaa M. F. Gad El-Rab

1Department of Preventive and Community Dentistry, Faculty of Dentistry, Taif University, Taif 26571, Saudi Arabia
2Department of Oral and Maxillofacial Surgery and Diagnostic Sciences, Oral Pathology Division, Faculty of Dentistry, Taif University, Taif 26571, Saudi Arabia
3Preventive Dentistry Department, Faculty of Dentistry, Taif University, Taif 26571, Saudi Arabia
4Department of Dental Biomaterials, Faculty of Dentistry, Mansoura University, Mansoura 35511, Egypt
5Department of Oral And Maxillofacial Surgery And Diagnostic Sciences, College of Dentistry, Taif University, Taif 26571, Saudi Arabia.
6Department of Biotechnology, Faculty of Science, Taif University, Taif 21974, Saudi Arabia.
7Department of Botany and Microbiology, Faculty of Science, Assiut University, 71516 Assiut, Egypt

The current study target to compare the antimicrobial efficacy of miswak, silver nanoparticles (AgNPs), and chlorhexidine with different concentrations alone and in combinations with each other against two bacteria Streptococcus mutans (S. mutans), Staphylococcus aureus (S. aureus), and one fungus Candida albicans (C. albicans). Manually prepared miswak extract, chlorhexidine gluconate (0.2% and 0.12% concentrations), and AgNPs (20 to 50 nm) were used. The following combinations were prepared and their synergistic effect were evaluated: AgNPs with chlorhexidine, miswak with chlorhexidine, and miswak with AgNPs. The antimicrobial efficiencies were defined using agar well diffusion method. The mean difference was tested using ANOVA. When tested materials were used alone, the mean diameter of inhibition zone (DIZ) of AgNPs, chlorhexidine and miswak against S. aureus and S. mutans were significantly higher than C. albicans. The AgNPs had greatest effect than the other two tested materials (AgNPs > chlorhexidine > miswak) upon tested bacteria. When tested combinations are used, the mean DIZ of AgNPs with miswak extract combination was significantly highest more than miswak with chlorhexidine 0.2% or 0.12% combinations at all the tested microbe levels. Mean DIZ of AgNPs with 0.2% chlorhexidine was significantly highest more miswak with 0.2% or 0.12% chlorhexidine combinations at all tested microbe levels. Finally, the combination of AgNPs with miswak extract has superior antimicrobial efficacy when compared to the other tested combinations. Therefore, AgNPs, and miswak extract can be used as a promising antimicrobial biomaterial in dental applications.

Keywords: AgNPs; miswak extract; chlorhexidine; combinations; antimicrobial efficacy.

INTRODUCTION

The dental biofilm is formed of microorganisms through an ordered sequence of events, resulting in a rich, well-ordered and functionally organized microbial community in the teeth. Nowadays, Oral biofilm is a dense environment for a large number of microbial species, which causes periodontal infections and dental caries\(^1\). Dental biofilms cause dental caries which is dominated by carbohydrate-fermenting bacteria. Dental caries considers as
multifactorial disease with high sugar diet and S. mutans as main etiological factors. Along with S. mutans, studies highlighted the role of C. albicans in increase of dental caries. Oral microorganism like S. aureus, S. mutans and C. albicans with other microorganisms forms dental plaque. The S. aureus, S. mutans and C. albicans strains are resistant to the currently used antimicrobials, and shifted the focus of research to other alternatives, offering therapeutic benefits and more inexpensive treatment. The AgNPs are metallic nanoparticles that serve as an antimicrobial agent with long term antibacterial activity, low bacterial resistance, high surface to volume ratio, and low volatility. Nano-sized silver particles penetrate into the cell membranes/cell wall by thiol groups or sulfur-containing proteins, ultimately resulting in the microbial DNA damage and cell death. Antimicrobial efficacy of miswak is related to its b-sitosterol, m-anisi acid, and chloride content and commonly used tooth cleaning tools. Chlorhexidine is commonly used mouth wash because of its efficacy against common oral pathogens. However, continuous use of high concentration of chlorhexidine is associated with dental complications like dryness of mouth, dental stains, changes in taste, and gingival irritation. Previously, limited research was conducted to check the antimicrobial efficiency of AgNPs, miswak, and chlorhexidine combinations against common oral microbes and researches highlighted the need for further studies in this regard. The current study was carried for the comparison of the antimicrobial efficiency of miswak, AgNPs, and chlorhexidine with different concentrations alone and their combinations against S. aureus, S. mutans, and C. albicans and one strain of C. albicans were used in this study to evaluate the antimicrobial activity. The microbes were isolated from patients visiting University Dental Hospital, Taif, Saudi Arabia. The isolated microbes were confirmed using standard microbiological methods. This study was a continuation of previous studies, so we relied on the same previous strains.

Preparation of test solutions
Miswak extract
The roots of miswak were brought from the local markets, Taif City, KSA and ground into powder. The miswak was placed into a thimble, a thick filter paper tube with D x L = 35 x 150 mm and grade 603 (Carl Schleicher und Schull, Dassel, Germany), for extraction with methanol which was removed after extraction leaving a brownish oil. A methanol was added to dissolve the oil and to precipitate it again by adding diethyl ether and the precipitate with ether-methanol were separated by decantation. This step was repeated and finally, the miswak oily extract was kept in a refrigerator at 4ºC. Then, 50 mg/ml miswak extract was prepared.

Miswak extract characterization
Miswak extract was characterized using Fourier transform infrared analysis (FT-IR).

Silver nanoparticles
Chemically derived, commercially available AgNPs powder of size 20 to 50 nm (Alibaba Company, Shanghai Xinglu Chemical Technology Co., LTD, China) was purchased. The AgNPs (100 μg/ml) was prepared by adding 1 mg of AgNPs powder to 10 ml of normal saline.

Chlorhexidine solution
Commercially available chlorhexidine gluconate with two different concentrations (0.2% and 0.12%) were purchased (Perioshield mouthwash, Sunstar GUM-Butler, USA).

Preparation of solution combinations
Miswak and AgNPs combinations
Equal quantity of 50 mg/ml miswak extract and 100 μg/ml AgNPs were stirred together.

Study design, ethical approval, microbial strains used
An in-vitro study was conducted at University Dental Hospital and Department of Microbiology, Taif University, KSA. Ethical clearance was obtained from Taif University, Institutional review board (Ethical clearance number- 41-1107-00152). Two bacterial biofilm producing strains, S. aureus, S. mutans
**Chlorhexidine and AgNPs combinations**

Equal quantity of chlorhexidine with 0.2% and 0.12% concentration individually and 100 μg/ml AgNPs were stirred together.

**Miswak and chlorhexidine combinations**

Equal quantity of chlorhexidine with 0.2% and 0.12% concentration, individually and 50 mg/ml miswak extract were stirred together using sterile glass rod to get uniformly.

**Determination of antimicrobial activity**

The agar-well diffusion method was used to determine antimicrobial efficiency of the tested solutions [AgNPs, miswak, and chlorhexidine with 0.2% and 0.12%]. The inoculum (100 μl) of The bacterial strains or Candida (~10^6 CFU/ml) was put onto the surface of the Mueller-Hinton agar and Sabouraud dextrose agar, respectively. Wells (7 mm in diameter) were cut from the agar with a sterile borer and different volume (50, 100, and 200 μl) of each solution (AgNPs, miswak, and chlorhexidine) were delivered into them in addition to phosphate-buffered saline as negative controls. The inoculated plates of the tested microbes were incubated for 24 h at 37°C. The diameter of the inhibition zone (DIZ) of the tested microbes were obtained to determine antimicrobial efficiency of AgNPs, miswak, and chlorhexidine. Minimum Inhibitory Concentration (MIC) of AgNPs, miswak, and chlorhexidine was determined by broth micro-dilution method, each test was occurred in triplicates.

**Statistical analysis**

Mean difference was tested using One-way Analysis of variance (ANOVA) followed by Tukey Post Hoc using the Statistical Package for Social Science version 17 (SPSS Inc Chicago link). All statistical tests were two-sided, and the significance level was set at P< 0.05.

**RESULTS AND DISCUSSION**

**Results**

**Determination of minimum inhibitory concentration**

The MIC was the lowest for AgNPs (25 μg/ml) and chlorhexidine 0.05% against S. mutans (Table 1). The mean diameter of inhibition zone (DIZ) of AgNPs against S. aureus (23.4 ± 0.5) was significantly (p= 0.04) higher than Candida albicans (19.8 ± 0.4) (Table 2 and Figure 1). The mean DIZ of miswak against S. aureus and S. mutans was significantly (p= 0.04) higher than C. albicans at 100 μl and 200 μl solutions (Table 2 and Figure 1).

**Table 1:** MIC of tested solutions against tested microbes

<table>
<thead>
<tr>
<th>Material name and concentration</th>
<th>Bacteria</th>
<th>Concentration and bacterial growth</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1/2</td>
<td>1/4</td>
</tr>
<tr>
<td>Miswak (50 mg/mL)</td>
<td>S. aureus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>S. mutans</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>C. albicans</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AgNPs (100 μg/mL)</td>
<td>S. aureus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>S. mutans</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>C. albicans</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>0.2% CHX</td>
<td>S. aureus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>S. mutans</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>C. albicans</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
**FT-IR analysis of miswak**

Alkaloids (salvadoline), benzyl isothiocyanate, benzyl cyanates, and sulfur (Table 3 and Figure 2) are more considerable in the extract of miswak, that are all responsible for the growth inhibition of bacterial and fungal strains (figure 3).

**Antimicrobial activity of test solutions**

The mean DIZ of chlorhexidine 0.12% against *S. mutans* was significantly (*p* = 0.04) higher than *C. albicans* at 100 µl and 200 µl solutions (Table 2).
Fig. 2: FTIR of miswak extract

Table 3: Peaks were obtained by miswak FTIR analysis and corresponding functional groups.

<table>
<thead>
<tr>
<th>Peak (cm⁻¹)</th>
<th>Functional group</th>
<th>Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>3381.73</td>
<td>• O–H stretch</td>
<td>• Phenols</td>
</tr>
<tr>
<td></td>
<td>• stretch vibration of N–H</td>
<td>• benzyl amides and three methylamine</td>
</tr>
<tr>
<td>2925.67</td>
<td>Aldehyde –CH</td>
<td>aliphatic compounds</td>
</tr>
<tr>
<td>2357.58</td>
<td>N=C</td>
<td>Isothiocyanate and isocyanate</td>
</tr>
<tr>
<td>1616.08</td>
<td>Alkene C = C</td>
<td>Aliphatic compounds</td>
</tr>
<tr>
<td>1403.98</td>
<td>C–H vibration in benzene ring skeleton</td>
<td>lignans, salvadorine, benzyl amides, benzyl cyanates</td>
</tr>
<tr>
<td>1093.42</td>
<td>C–H bending</td>
<td>Aromatic compounds</td>
</tr>
<tr>
<td>657.61</td>
<td>P = S</td>
<td>Compounds containing phosphorus and sulfur</td>
</tr>
</tbody>
</table>

Fig. 3: Inhibition zone of 1-AgNPs with miswak, 2- AgNPs with 0.12% CHX, 3- 0.12% CHX with miswak 4- Negative control against A- S. aureus, B- S. mutans and C- C. albicans.
Table 4: Mean diameter of inhibition zone (DIZ) of tested solutions against different oral microbes at 200 µl solutions.

<table>
<thead>
<tr>
<th>Microbes</th>
<th>AgNPs (100 µg/ml)</th>
<th>Miswak (50 mg/ml)</th>
<th>0.2% CHX</th>
<th>0.12% HX</th>
<th>ANOV A F value</th>
<th>ANOV A p value</th>
<th>Tukey post Hoc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 µl</td>
<td>200 µl</td>
<td>200 µl</td>
<td>200 µl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>23.4 (0.5)</td>
<td>20.4 (0.5)</td>
<td>21.4 (0.5)</td>
<td>19.2 (0.8)</td>
<td>7.12</td>
<td>0.03</td>
<td>AgNPs &gt; 0.2% CHX, Miswak &gt; 0.12% CHX</td>
</tr>
<tr>
<td>S. mutans</td>
<td>22.6 (0.5)</td>
<td>19.2 (0.5)</td>
<td>20.1 (0.1)</td>
<td>18.4 (0.5)</td>
<td>6.13</td>
<td>0.04</td>
<td>AgNPs &gt; 0.2% CHX, Miswak &gt; 0.12% CHX</td>
</tr>
<tr>
<td>C. albicans</td>
<td>19.8 (0.4)</td>
<td>15.6 (0.5)</td>
<td>16.6 (0.5)</td>
<td>14.4 (0.5)</td>
<td>6.19</td>
<td>0.04</td>
<td>AgNPs &gt; 0.2% CHX, Miswak &gt; 0.12% CHX</td>
</tr>
</tbody>
</table>

SD – Standard deviation, CHX - Chlorhexidine.

Fig. 4: Mean diameter of inhibition zone (DIZ) of tested solutions against different oral microbes at 200 µl solutions.

Antimicrobial activity of combination solutions

Table 5 and Figure 3 and 5 show the mean DIZ of the tested combination of solutions against different oral microbes at 200 µl solutions. Mean DIZ of AgNPs with miswak extract combination was significantly higher than miswak extract with chlorhexidine 0.2% or 0.12% combinations at all the tested microbe levels. The mean DIZ of AgNPs with 0.2% chlorhexidine was significantly higher than miswak with 0.2% or 0.12% chlorhexidine combinations at all tested microbe levels.

Table 5: Mean diameter of inhibition zone (DIZ) of tested solution combinations against different oral microbes at 200 µl solutions.

<table>
<thead>
<tr>
<th>Microbes</th>
<th>AgNPs + miswak (a)</th>
<th>AgNPs + 0.2% CHX (b)</th>
<th>AgNPs + 0.12% HX (c)</th>
<th>Miswak + 0.2% CHX (d)</th>
<th>Miswak + 0.12% CHX (e)</th>
<th>ANOV A F value</th>
<th>ANOVA p value</th>
<th>Tukey post Hoc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 µl</td>
<td>200 µl</td>
<td>200 µl</td>
<td>200 µl</td>
<td>200 µl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>29.6 (0.3)</td>
<td>30.4 (0.4)</td>
<td>28.4 (0.5)</td>
<td>24.3 (0.5)</td>
<td>23.2 (0.3)</td>
<td>7.16</td>
<td>0.03</td>
<td>b &gt; d, e a &gt; d, e c &gt; d, e</td>
</tr>
<tr>
<td>S. mutans</td>
<td>25.3 (0.5)</td>
<td>25.5 (0.4)</td>
<td>23.2 (0.3)</td>
<td>23.4 (0.4)</td>
<td>22.1 (0.2)</td>
<td>6.19</td>
<td>0.03</td>
<td>a &gt; c, d b &gt; d, e</td>
</tr>
<tr>
<td>C. albicans</td>
<td>24.8 (0.4)</td>
<td>25.4 (0.5)</td>
<td>22.2 (0.1)</td>
<td>19.4 (0.5)</td>
<td>18.4 (0.3)</td>
<td>5.23</td>
<td>0.04</td>
<td>b &gt; d, e a &gt; d, e c &gt; d, e</td>
</tr>
</tbody>
</table>

SD – Standard deviation, CHX - Chlorhexidine.
Fig. 5: Mean diameter of inhibition zone (DIZ) of tested solution combinations against different oral microbes at 200 µl solutions.

Discussion

Development of antimicrobial resistance to synthetic alternatives shifted the research focus towards natural medicines and inorganic antimicrobials like AgNPs. The present in-vitro study was the first to assess the antimicrobial efficacy of AgNPs, miswak extract, chlorhexidine gluconate alone, and their combinations against oral microbes.

Our study showed superior antibacterial efficiency of miswak against tested S. aureus and S. mutans strains than antifungal efficiency against C. albicans because of the presence of Alkaloids (salvadorine), benzyl isothiocyanate, benzyl cyanates and sulfur. These data are agreeing with Abhary and Al-Hazmi. These results were in agreement with previous studies which reported different zone of growth inhibition against different oral microbes because of discrepancy in the membrane permeability of the studied microorganism. Also, Constituents of miswak such as cyanides, chlorides, sulfur, and fluorides, possess an antimicrobial efficacy by inhibiting oxygen uptake and disrupting the transport system, and disrupting the bacterial cell wall. A recent systematic review showed that chlorhexidine was more effective against oral microbes than miswak extract with a mean difference of 0.19 (P=0.04, 95% CI: 0.01 to 0.37). In contrast to this, the present study showed no significant difference in antibacterial efficacy of miswak extract and chlorhexidine. However, antifungal activity against C. albicans was significantly higher for chlorhexidine gluconate at a concentration of 0.2% than miswak extract. In the current study, no significant difference was observed between antimicrobial efficacy of different chlorhexidine concentrations used, prompting the use of a lower concentration of chlorhexidine (0.12%) to avoid the adverse effect of high concentration (0.2%). A combination of miswak extract and chlorhexidine with 0.2% and 0.12% concentrations showed significantly higher antimicrobial efficiency than when the solutions are used alone. This indicates that the combination of two solutions synergized the antimicrobial efficiency of solutions. Ashour et al. reported that each solution of miswak extract and chlorhexidine (0.2%) with glass ionomer cement showed antibacterial activity.

Previous in-vitro research showed that nano-sized silver particles penetrate the microbial cell membranes/cell wall by thiol groups or sulfur-containing proteins, ultimately by damaging the microbial DNA and leading to cell death. In agreement with the recently conducted study by Panpaliya et al., present result showed that AgNPs has significantly higher antibacterial and antifungal efficacy in comparison to chlorhexidine tested concentrations. This may be attributed to the nano-size of silver particles which can penetrate a deeper layer of microbes leading to its destruction. Though, chlorhexidine has good antimicrobial properties, because of its...
size, it cannot penetrate deeper layers of microbes\textsuperscript{31}. When AgNPs were combined with chlorhexidine, the antimicrobial efficacy was enhanced (scheme 1) in comparison to using the solutions alone. Chlorhexidine interacts with the negatively charged cytoplasmic membrane. Nano-sized silver particles penetrate the microbial cell membranes and/or cell wall. The current result is agreeing with a recently conducted study by Ashour et al.\textsuperscript{30} showed the incorporation of chlorhexidine and AgNPs enhanced its antimicrobial efficiency against oral microorganisms compared to each solution of chlorhexidine and AgNPs individually.

Our study results exhibited that the combination of AgNPs and miswak extract components significantly enhanced their antimicrobial efficiency (scheme 2) due to synergized effect of solutions. AgNPs, and miswak extract can be used as a promising antimicrobial agent in dental applications instead of AgNPs and chlorohexidine because of using chlorohexidine cause dryness of mouth, dental stains, changes in taste, and gingival irritation\textsuperscript{32}\&\textsuperscript{33}.

The limitation of the current study is its \textit{in-vitro} nature; it was not possible to mimic all the oral conditions in the lab environment.

\begin{center}
\textbf{Scheme 1:} CHX (as a cationic agent) interacts with the negatively charged cytoplasmic membrane. Nano-sized silver particles penetrate the microbial cell membranes/cell wall.
\end{center}

\begin{center}
\textbf{Scheme 2:} Benzyl isothiocyanate, sulfur, and benzyl cyanates interact and penetrate cytoplasmic membrane. Nano-sized silver particles penetrate the microbial cell membranes/cell wall
\end{center}
Conclusion
To conclude, the present result exhibited the combination of miswak, and AgNPs with chlorhexidine increases the antimicrobial efficiency of combined solutions against common oral microbes. AgNPs, and miswak displayed insignificant antimicrobial activity in comparison to AgNPs, and chlorohexidine so we advise to use AgNPs, and miswak as a promising antimicrobial agent in dental applications.

Funding
This work was funded by Deanship of Scientific Research, Taif University (research group project No. 1/439/6084), Taif, Saudi Arabia. The authors declare that the funding bodies had no role in the design of the study, the collection, analysis, and interpretation of data, or in writing the manuscript.

Institutional Review Board Statement
The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of Taif University (protocol code-41-1107-00152 and 1/12/2019).

Acknowledgement
We would like to express our gratitude to Deanship of Scientific Research, Taif University, Taif, Saudi Arabia for financial support under the research group project number (1/439/6084).

REFERENCES


15- R. Mishra, K.T. Chandrashekar, V.D. Tripathi, A. Hazari, B.S. Sabu, A. Sahu, "Comparative evaluation of efficacy of 0.2% sodium hypochlorite (Hi Wash) mouthwash with 0.2% chlorhexidine mouthwash on plaque-induced gingivitis: A clinical trial", J Indian Soc Periodontol, 23(6), 534-538 (2019).


27- S. Naseem, K. Hashmi, F. Fasih, S. Sharafat, R. Khanani, “In vitro evaluation of antimicrobial effect of miswak against...


التأثيرات المضادة للميكروبات وتأثيرات التآزرية للمسواك والفضية النانوية، والكلورهيكسيدين بمفرده وтолيفاتهم على بعض الميكروبات الفموية

سكينة بي باشا - أم الميرف، عاشور إيناس - نافذ فلبين - محمد فريد فلبين - أحمد الفهمي - جاد الرب

قسم طب الأسنان الوقائي والمجتمع، كلية طب الأسنان، جامعة الطائف، الطائف 16571، المملكة العربية السعودية

قسم جراحة الفم والوجه والفكين وعلوم التشخيص، قسم أمراض الفم، كلية طب الأسنان، جامعة الطائف، الطائف 16571، المملكة العربية السعودية

قسم الأسنان الوقائي، كلية طب الأسنان، جامعة الطائف، الطائف 16571، المملكة العربية السعودية

قسم المواد الحيوية. كلية طب الأسنان، جامعة المنصورة، المنصورة، مصر 25111، المملكة العربية السعودية

قسم جراحة الفم ورئة الفكين وعلوم التشخيص، كلية طب الأسنان، جامعة الطائف، الطائف 16571، المملكة العربية السعودية

قسم التكنولوجيا الحيوية، كلية العلوم، جامعة الطائف، الطائف 16571، المملكة العربية السعودية

قسم النبات والموكروبولوجي، كلية العلوم، جامعة أسيوط، أسيوط، مصر 71516، المملكة العربية السعودية

تهدف الدراسة الحالية إلى مقارنة فعالية المضادات للميكروبات لكل من المسواك وحسيميات الفضية النانوية والكلورهيكسيدين بتركيزات مختلفة بمفردها وإلقاء الأقران مع بعضها البعض ضد تعداد من البكتيريا وهي: فيلودينية الطائرة والمكورات العنقودية الذهبية، فسفر واحد هو كابثيا البكتيريا. تم استخدام مستخلص المسواك المحضر بدوبا، وجداول الكلورهيكسيدين بتركيزات (0.2% و0.12%)، وجسيمات الفضية النانوية (0.005 نانومتر). تم تحضير التركيبات النانوية: جسيمات الفضية النانوية مع الكلورهيكسيدين، المسواك مع الكلورهيكسيدين، المسواك مع الميكروبات مع جسيمات الفضية النانوية. تم تأثيرها التأثيري وتم تحديد كفاءات المضادات للميكروبات باستخدام طريقة الاستشعار في حفر الأحبار. تم تحليل متوسط الفروق باستخدام منطقية التتالي لجسيمات الفضية النانوية والكلورهيكسيدين والمسواك ضد البكتيريا العنقودية الذهبية والمكورات العنقودية الذهبية، أعلى بشكل ملحوظ من كابثيا البكتيريا وكان لـ جسيمات الفضية النانوية تأثير أكبر من المادتين الأخرى المختبرتين (جسيمات الفضية النانوية) والكلورهيكسيدين المسواك) على البكتيريا المختبرة. كان متوسط قطر منطقة التثبيط لتركيز جسيمات الفضية النانوية مع مستخلص المسواك أعلى بكثير من تركيبات السواد مع الكلورهيكسيدين 0.2% أو 0.12% على جميع مستويات.
الميكروبات المختبرة. كان متوسط قطر منطقة التنثبيط لتركيبات جسيمات الفضة النانوية مع 0.2% كلورهيكسيدين أعلى بكثير من تركيبات المسواك مع الكلورهيكسيدين 0.2% أو 0.12% وذلك على جميع مستويات الميكروبات المختبرة. أخيرًا، فإن الجمع بين جسيمات الفضة النانوية مع مستخلص المسواك له فعالية فائقة كمضادات للميكروبات عند مقارنته بالتركيبات الأخرى المختبرة. لذلك، يمكن استخدام جسيمات الفضة النانوية ومستخلص المسواك كمواد حيوية واعدة مضادة للميكروبات في تطبيقات طب الأسنان.