IN VITRO CLOVE OIL ACTIVITY AGAINST PERIODONTOPATHIC BACTERIA

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ABSTRACT
Clove oil antimicrobial activity against periodontopathic (ATCC) bacteria was investigated "in vitro". Bacterial strains tested were: Prevotella intermedia, Prevotella melaninogenica, Poryphromonas gingivalis, Actinobacillus actinomycetemcomitans, Capnocytophaga gingivalis, and Fusobacterium nucleatum. The minimal inhibitory concentration (MIC) for the strains tested was determined using the method of broth dilution with the clove oil in serial concentrations. Results showed MIC of (4 µ g/ml) for Actinobacillus actinomycetemcomitans, Capnocytophaga gingivalis; and Fusobacterium nucleatum were (1 µ g/ml). Prevotella intermedia and Poryphromonas gingivalis showed (2 µ g/ml). But (MIC) for Prevotella melaninogenica was 18 µ g/ml. Some superinfectant organisms were also tested: Cadida albicans susceptibility to clove oil was demonstrated at a concentration of (24 µ g/ml). The MIC for Pseudomonas aeruginosa, and Staphylococcus aureus was (24 µ g/ml) but for Escherichia coli was (18 µ g/ml). All periodontal pathogens and superinfectants tested were susceptible to the clove oil. The positive results suggest that the clove oil should be further tested as an adjuvant to periodontal therapy.

INTRODUCTION
Human periodontal disease has been associated with a complex microbiota. The development of destructive periodontitis seems to be the result of specific infection¹. Gram positive coccoid bacteria have been related to periodontal health, while periodontal disease was associated with Gram negative rods and spirochetes². Many authors³ suggest that the presence of Actinobacillus actinomycetemcomitans, Bacteroides gingivis (Porphyromonas gingivis) and Bacteroides intermedius (Prevotella intermedia) is related to active periodontal disease. Other species like Fusobacterium nucleatum and Capnocytophaga sp.⁴ were also associated with the periodontics. Carrasco⁵,
reported that human periodontitis is initiated and perpetuated by a small group of bacteria that colonize the subgingival region, mainly Gram-negative, anaerobic or microaerophilic bacteria. Furthermore, most cases of human periodontitis are caused by Porphyromonas gingivalis, Bacteroides forsythus and Actinobacillus actinomycetemcomitans.\textsuperscript{6}

Because periodontitis is an infectious disease, and taking into consideration that some patients do not respond to conventional mechanical therapy, sometimes antimicrobial agents have been prescribed as adjutants to periodontal treatment\textsuperscript{7}. However, the emergence of pathogenic bacteria those are resistant to antibiotics, due to inappropriate systemic usage, has become a serious clinical problem and has necessitated the search for alternatives.

Clove oil is known for its antibacterial activity, which is due to several constituents, and will be tested as an alternative to conventional antibiotic therapy. Slots\textsuperscript{8} pointed out that in order not to contribute to a "coming plague", dentists should add the knowledge of an infectious disease specialist to their surgical skills. T.E Rams\textsuperscript{9} suggested that antibiotics should be used only.

**MATERIALS AND METHODS**

Minimal inhibitory concentrations (MIC) for clove oil [manufactured locally in United Arab Emirates (UAE)] against the tested strains were determined using the clove oil in serial concentrations: 0 (negative control), 0.0625, 0.125, 0.5, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 µg/ml.

Control plates with serial concentrations of ethanolic solution were also tested. The strains were inoculated by steer apparatus. All tests were performed in quadruplicate.

All strains were grown in Brain heart Infusion Agar (BHI-Difco) except for Candida albicans, which was grown in Saboraud agar (Difco), and incubated at room temperature for 4 days.

*Pseudomonas aeruginosa, E. coli* and *S. aureus* inoculated in Brain Heart Infusion agar were incubated aerobically at 37°C for 2 days. Brain heart enriched with hemin (1%-Sigma) and mendadione (0.01%-Sigma) was used to grow strains of: *P. intermedia, P. melaninogenica, P. gingivalis, A. actinomycetemcomitans, C. gingivalis* and *F. nucleatum*. The plates were incubated anaerobically (Gas-Pak-BBL) at 37°C for 7 days.
RESULTS

The Minimum Inhibitory Concentration (MIC) was determined as the lowest concentration of the clove oil, which inhibited the growth of the tested microorganisms.

The clove oil showed antimicrobial activity against all tested strains. (Table 1) presents the Minimal Inhibitory concentration obtained for each strain tested. All control plates, including those with different clove oil concentrations and the negative controls presented regular bacterial growth.

Clove Oil against Candida albicans:

Clove Oil against Staphylococcus aureus:

Table (1): Minimum Inhibitory Concentration (MIC) of clove oil obtained for each strain tested Tests in quadruplicates.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>(MIC* - µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinobacillus actinomycetemcomitans</td>
<td>4</td>
</tr>
<tr>
<td>Capnocytophaga gingivalis</td>
<td>1</td>
</tr>
<tr>
<td>Fusobacterium nucleatum</td>
<td>1</td>
</tr>
<tr>
<td>Porphyromonas gingivalis</td>
<td>2</td>
</tr>
<tr>
<td>Prevotella intermedia</td>
<td>2</td>
</tr>
<tr>
<td>Prevotella melaninogenica</td>
<td>18</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>24</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>24</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>24</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>18</td>
</tr>
</tbody>
</table>
DISCUSSION

The antimicrobial activity of clove oil has been studied by several authors\cite{10}, and have investigated its activity towards oral pathogens.

Besides showing antimicrobial activity against periodotopathic bacteria, the clove oil did not demonstrate selection of superinfectant organisms.

The verification of the antimicrobial action of the clove oil is not surprising. The primary function of clove oil in the clove paste in Aphthous Ulcer treatment act as a biocide, being active against invasive bacteria, fungi and even invading larvae\cite{11}. The spectrum of activity is fairly broad, with action against Gram positive and Gram negative rods and cocci, yeast and fungi\cite{12}.

The present study has shown clove oil antimicrobial activity against the following periodontal pathogens: A. actinomycetemcomitans, P. intermedia, P. melaninogenica, P. gingivalis, C. gingivaïïs and F. nucleatum. Antimicrobial activity against Candida albicans, Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus was also demonstrated in this study, confirming previous results\cite{13}.

Different results were achieved by Nieva et al., \cite{14} that reported antimicrobial activity against Staphylococcus aureus, but no action against Pseudomonas aeruginosa and Escherichia coli. A possible explanation for these diverse results is the fact that clove oil composition is variable depending on the region and season that it is collected\cite{15}. Consequently, the active compounds may not be present in sufficient quantities or quality.

One of the limitations to clove oil use is the variability in composition and action as a consequence of variation in the flora of the region where it is produced. However, according to Bankova et al.\cite{16}, antimicrobial action is expected to be always present because of its vital importance as an antimicrobial agent in the bees wax paste, independently of the region where the clove oil is produced. In a recent study, Sforcin et al.\cite{17} did not find a seasonal effect on the antimicrobial activity of the Brazilian clove oil in its early collection period.

Clove oil mechanism of antimicrobial action, though not completely understood, seems to be complex and may vary according to its composition.
The compounds known to have antimicrobial action are mainly the flavonoids and cinamic acids\textsuperscript{18}.

Regarding susceptibility of the tested microorganisms, it seems that they were more susceptible to clove oil than to some antibiotics. Carrasco \textit{et al.} \textsuperscript{5} showed the MIC for Doxycycline, Tetracycline, Metronidazole, Ofloxaixin, and Amoxicillin were (8, 4, 8, 9, and 4\textmu g/ml respectively) against \textit{P. gingivalis, P. intermedia} and \textit{F. nucleatum in vitro}. When their MIC results are compared to ours, it is observed that frequently used antibiotics had greater MIC than clove oil against \textit{P. gingivalis, P. intermedia} and \textit{F. nucleatum}. These periodontal pathogens may be more susceptible to the antibiotics shown above.

Previous study on the susceptibility of \textit{A. actinomycomitans} to selected antimicrobial agents indicated that MIC 90\textmu g/ml to penicillin varied from 1.0 to 6.25\textmu g/ml, to amoxicillin from 1.0 to 2.0\textmu g/ml, to tetracycline from 0.5 to 8.0\textmu g/ml, to doxycycline from 1.0 to 3.1\textmu g/ml, and to metronidazole from 12.5 to 32\textmu g/ml\textsuperscript{19}. In the present study, the MIC of this pathogen to Clove Oil was 2\textmu g/ml.

Susceptibility tests of \textit{P. gingivalis} have shown that MIC90 to penicillin varied from 0.016 to 0.29\textmu g/ml, amoxicillin from 0.023 to < 1.0\textmu g/ml, metronidazole from 0.023 to 2.1\textmu g/ml\textsuperscript{20}. Interestingly, in present study MIC to clove oil was (0.5\textmu g/ml).

In 1990, Rams \textit{et al.} \textsuperscript{6} noted that some strains of \textit{S. aureus} isolated from the periodontal pocket were resistant to tetracycline, penicillin, metronidazole and erythromycin. Additionally, when the antimicrobial activity of 18antibiotics was tested against \textit{Enterobacteriaceae} and \textit{pseudomonadaceae}, only ciprofloxacin was able to eliminate these microorganisms from the periodontal pocket\textsuperscript{21}.

Our results showed that clove oil presented "in vitro" antimicrobial activity, not only against some periodontopathic bacteria (\textit{F. nucleatum, P. gingivalis, P. intermedia, P. melaninogenica, A. actinomycetemcomitans S. aureus, P. aeruginosa, E. coli} and \textit{Candida albicans}).

The antimicrobial action observed for the clove oil suggests its usage as an adjuvant to periodontal therapy. A step further should be given to verify if a dose sufficient to kill the target microorganisms can be reached within the
subgingival environment, without causing major local or systemic adverse effects.

REFERENCES


