Antimicrobial activity of plant extracts against oral pathogens. Detection of cellular structural changes by FT-IR.

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Periodontal diseases and dental caries are common oral disorders in human population with a multifactorial etiology closely related with the development of dental plaque. The latter is composed of native oral microbiota and a is accumulated on teeth surface. Several antiplaque agents are used widely to inhibit bacterial growth [1,2]. However, these substances have adverse effects. In the current study, six plants extracts namely, chamomile, dittany, lemon balm, rosemary, saffron and sage, were tested as potential natural antimicrobial agents. The antimicrobial activity of plants extracts was studied towards Gram-positive strains belonging to Streptococcus species related to the oral health. Fourier transform infrared spectroscopy (FT-IR) was applied in order to evaluate the changes in the cellular composition of target bacterial cells after their exposure to extracts of both plants.

Sample preparation.
Plants were subjected to sequential extraction with petroleum ether, hexane, diethyl ether and methanol, as shown in figure 1. All extracts were evaporated under reduced pressure and dried using rotary evaporator. Dried extracts were stored in labeled screw capped bottles at -20°C.

Screening of plants extracts against oral pathogens.
Different concentrations of methanol extracts were tested against six Streptococcus strains by the well diffusion assay (WDA) as a preliminary screening test.
All plants extracts had a totally or partial antimicrobial activity. Str. mutans LMG 14558® and Str. salivarius LMG 11489® were more resistant. (Table 1).

Table 1: Antimicrobial activity of plant extracts towards six Streptococcus strains as determined by the well diffusion assay.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Strain Concentration (mg/mL)</th>
<th>Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Str. mutans LMG 14558®</td>
<td>Str. mutans LMG 14549®</td>
</tr>
<tr>
<td>Chamomile</td>
<td>184</td>
<td>14</td>
</tr>
<tr>
<td>Dittany</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Lemon balm</td>
<td>18</td>
<td>4</td>
</tr>
<tr>
<td>Sage</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
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Based on the results the methanolic extracts of lemon palm and saffron were selected for further investigation among these Streptococcus strains, namely Str. mutans LMG 14558®, Str. oralis LMG 14532® and Str. sobrinus LMG 14641®.

Figure 1. Tissue killing studies of methanolic extracts against S. mutans LMG 14558®, Str. oralis LMG 14532® and Str. sobrinus LMG 14641®. Antimicrobial activity was studied (in vitro killing assay) against target cells in the logarithmic phase of bacterial growth.

The viability of Streptococcus cells was studied for 24 hours of incubation with 28 mg/mL (final concentration) methanolic plants extracts. 99% cell death of Str. sobrinus LMG 14641® was achieved in 4 hours, while for Str. mutans LMG 14558® and Str. oralis LMG 14532® within 6 hours for both plants extracts (Figure 2).

FT-IR Analysis.
Fourier transform infrared spectroscopy (FT-IR) was applied in the respective time period, where 99% of cell death was achieved, in order to evaluate the changes in the cellular composition of cells.

The FTIR spectrum of a biological system like bacteria is complex and consists of broad bands (Figure 3) that arise from the superposition of absorption peaks of various contributing macromolecules (proteins, lipids, polysaccharides, and nucleic acids) [3]. The FT-IR spectra of control cells were compared with the spectra of incubated with methanolic extracts in four different regions.

Principal component analysis (PCA) of the second derivative transformed spectra was performed for each characteristic spectral region (Figure 4) [4].

Figure 6. PCA of second derivative transformed FTIR spectra of S. mutans LMG 14558® (A), Str. oralis LMG 14532® (B) and Str. sobrinus LMG 14641® (C) in the four characteristic spectral regions after their incubation with the control sample (Mean 70%) (M): 28 mg/mL methanolic extract of lemon balm (A), 28 mg/mL methanolic extract of sage (B).

Conclusions.
The results have shown that all plants methanol extracts consist of important secondary metabolites in the search for new effective antibacterial agents against the pathogens responsible for dental caries. lemon balm and saffron methanol extracts FT-IR analysis along with chemometric analysis (PCA) of incubated Streptococcus cells revealed significant differences in all regions of spectra that correspond to cellular structural components.

References: