# 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 it is accumulated on teeth surfaces. Several antiseptic 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.

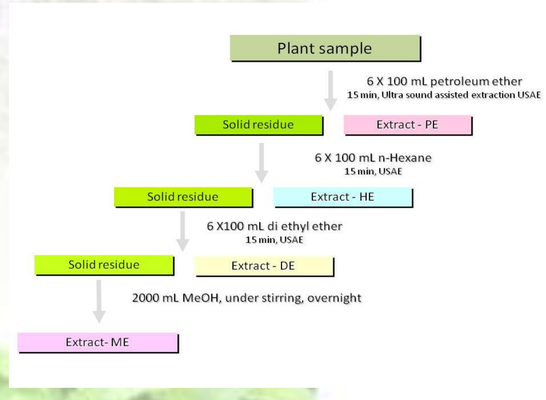


***Figure 1.*** *Schematic plan of extraction procedure*

**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$^T$ and *Str. salivarius* LMG 11489$^T$ were more resistant. (Table 1).
Based on the results the methanolic extracts of lemon palm and saffron were selected for further investigation against three *Streptococcus* strains, namely *Str. mutans* LMG 14558$^T$, *Str. oralis* LMG 14532$^T$ and *Str. sobrinus* LMG 14641$^T$.

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

| | | Inhibition (diameter, mm) | | | | | |
|---|---|---|---|---|---|---|---|
| **Plant** | **Strain/ Concentration (mg/mL)** | ***Streptococcus gordonii* LMG 14518$^T$** | ***Streptococcus mutans* LMG 14558$^T$** | ***Streptococcus oralis* LMG 14532$^T$** | ***Streptococcus salivarius* LMG 11489$^T$** | ***Streptococcus sanguinis* DSM 20068** | ***Streptococcus sobrinus* LMG 14641$^T$** |
| **Chamomile** | 280 | 16 | 17 | 14 | 8 | 15 | 14 |
| | 168 | 14 | 14 | 14 | 7 | 14 | 13 |
| | 84 | 14 | 7 | 13 | 7 | 11 | 11 |
| | 28 | 10 | 0 | 10 | 0 | 8 | 8 |
| **Dittany** | 280 | 26 | 19 | 26 | 17 | 18 | 17 |
| | 168 | 25 | 18 | 24 | 15 | 16 | 16 |
| | 84 | 16 | 7 | 16 | 9 | 14 | 15 |
| | 28 | 13 | 0 | 13 | 7 | 10 | 12 |
| **Lemon balm** | 280 | 17 | 9 | 14 | 13 | 15 | 10 |
| | 168 | 15 | 7 | 13 | 12 | 14 | 10 |
| | 84 | 12 | 0 | 12 | 10 | 12 | 7 |
| | 28 | 7 | 0 | 9 | 9 | 9 | 0 |
| **Rosemary** | 280 | 25 | 20 | 25 | 22 | 20 | 25 |
| | 168 | 24 | 18 | 24 | 20 | 19 | 24 |
| | 84 | 22 | 17 | 20 | 18 | 18 | 17 |
| | 28 | 18 | 11 | 16 | 11 | 13 | 13 |
| **Saffron** | 280 | 20 | 16 | 17 | 15 | 20 | 20 |
| | 168 | 18 | 15 | 16 | 15 | 19 | 18 |
| | 84 | 16 | 13 | 15 | 11 | 18 | 16 |
| | 28 | 12 | 7 | 13 | 8 | 13 | 14 |
| **Sage** | 280 | 20 | 19 | 15 | 8 | 14 | 17 |
| | 168 | 18 | 16 | 14 | 7 | 13 | 15 |
| | 84 | 16 | 7 | 14 | 0 | 12 | 14 |
| | 28 | 14 | 0 | 11 | 0 | 7 | 13 |

**Time killing studies of methanolic extracts against *Str. mutans* LMG 14558$^T$, *Str. oralis* LMG 14532$^T$ and *Str. sobrinus* LMG 14641$^T$.**

Antimicrobial activity was studied (*in vitro* killing assays) 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$^T$ was achieved in 4 hours, while for *Str. mutans* LMG 14558$^T$ and *Str. oralis* LMG 14532$^T$ within 6 hours for both plants extracts (Figure 2).

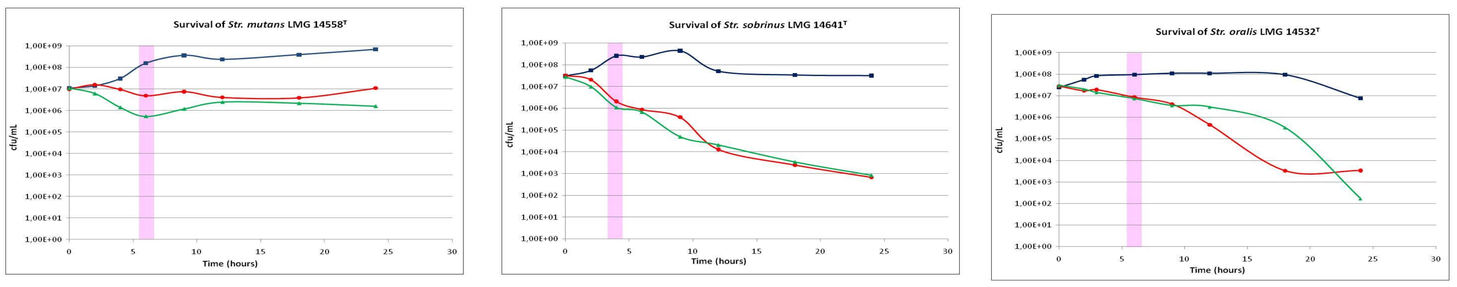


***Figure 2.*** *Time killing studies of logarithmic cells of Str. mutans LMG 14558, Str. oralis LMG 14532 and Str. sobrinus LMG 14641 after their incubation with the control sample (MeOH 70%) (●); 28 mg/mL methanolic extract of lemon balm (▲); 28 mg/mL methanolic extract ofsaffron (●).*

**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 cells in four different regions:

Region I- 3000– 2800 $cm^{-1}$ related to CH from fatty acids of the bacterial cell membrane

Region II- 1800– 1500 $cm^{-1}$ related to C=O and N–H from proteins

Region III- 1500– 1200 $cm^{-1}$ related to $PO_2$ from nucleic acids, as well as proteins and fatty acids

Region IV- 1200– 900 $cm^{-1}$: related to various absorptions of polysaccharides of the cell wall

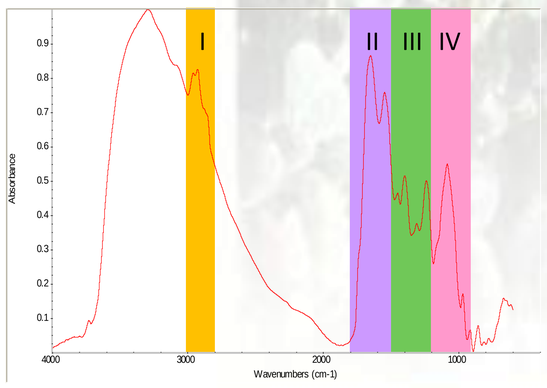


***Figure 3.*** *FT-IR spectra of Str. oralis LMG 14532$^T$ cells and the characteristic regions used for comparison*

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

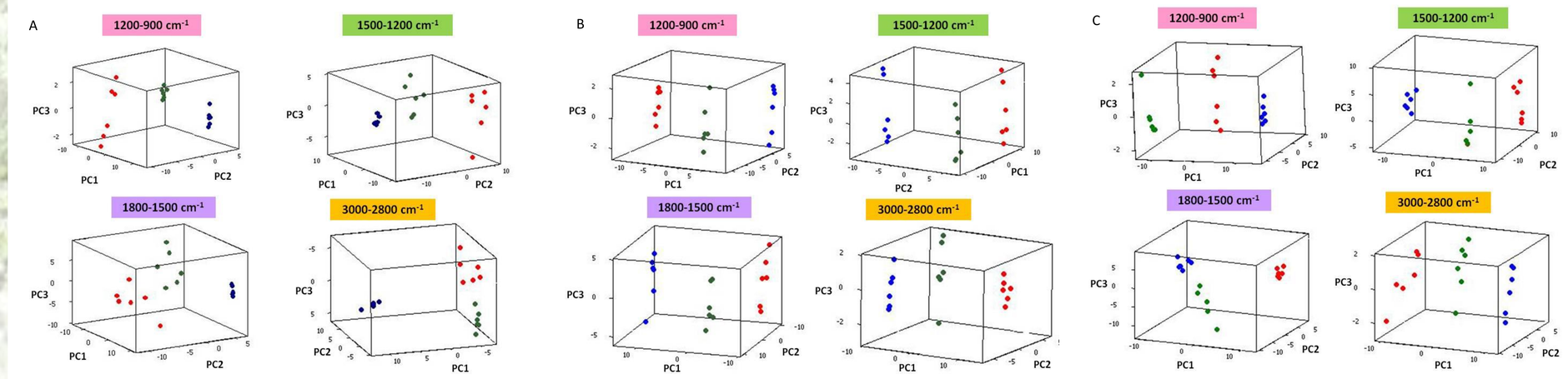


***Figure 4.*** *PCA of second derivative transformed FT-IR spectra of Str. mutans LMG 14558 (A), Str. oralis LMG 14532 (B) and Str. sobrinus LMG 14641 (C) cells in the four characteristic spectral regions after their incubation with the control sample (MeOH 70%) (●); 28 mg/mL methanolic extract of lemon balm (▲); 28 mg/mL methanolic extract of saffron (●).*

PCA revealed structural changes among cells treated with the extracts or the control sample. The significant differences were observed in characteristic spectral regions correlated to the above cellular structural components (Figure 4).

**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.

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