Counter-Propagation-Artificial Neural Network and FT-MIR spectroscopy: the proper strategy for detailed antimicrobial activity analysis

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INTRODUCTION

Nowadays, microbial infections and resistance to antibiotic drugs have been the biggest challenges, which threaten the health of societies. Due to several pharmacological activities associated with artichoke (*Cynara cardunculus*), such as hepatoprotective, antioxidative, anticarcinogenic, hypocholesterolemic, antibacterial, anti-HIV, among others [1-3], aqueous and organic extracts from seeds, sheets and flowers were tested in two bacteria species: *Escherichia coli*, Gram (-) and *Bacillus subtilis*, Gram (+). High-throughput screening (HTS) associated with Fourier Transform Mid-Infrared spectroscopy (MIR-FTIR) is a very attractive technique that can be applied to screen new alternative drugs due to its sensitivity, accuracy, spectral quality, and reproducibility, making this technique attractive to measure biological processes as in microbiology. MIR-spectra theoretically enables the attainment of the cell metabolic fingerprint under different experimental conditions, as a result of drug influence. The clustering analysis of large experimental data was performed using unsupervised algorithms such as a Hierarchical Clustering Analysis (HCA), Principal Component Analysis (PCA), and Kohonen Self-Organizing Maps (SOM), considered one of the most popular Artificial Neural Networks. Kohonen maps and Counter-propagation Artificial Neural Network (CP-ANN) are increasing their uses related to different chemical issues and nowadays can be considered as two important tools in chemometrics, due to its ability of solving both supervised and unsupervised problems. MIR spectroscopy and ANN algorithm to recognize the promissory molecular profiles between different plant extracts, highlighting the biomolecules characterized by antimicrobial properties, allowing to develop a platform to discover new bioactive molecules.

MATERIAL AND METHODS

- Plant material: flowers, fresh leafs; dry leafs and seeds (~1.5 g) were extracted using water, ethanol, methanol, acetone, ethyl acetate [10% (w/v)]
- Antibiotics: Metronidazole; Kanamicin; Chloramphenicol; Clarithromycin; Ampicillin
- Antibacterial activity was evaluted using both strains: *Escherichia coli* (Gram -) and *Bacillus subtilis* (Gram +).
- Bacteria samples were incubated in different plant extracts.
- Mid-Infraed spectra data were obtained using the *High-throughput Screening*.
- Preprocessing algorithms were used for spectral data treatment:
- *Data mining algorithms Princiapl Component Analysis and Hierarchical Clustering Analysis*.
- Classification model using the Counter-Propagation-Artificial Neural Networks: Neurons (10x10); Epochs: 500; Validation – Monte-Carlo (20%); 10 iterations.

EXPERIMENTAL RESULTS

Escherichia coli

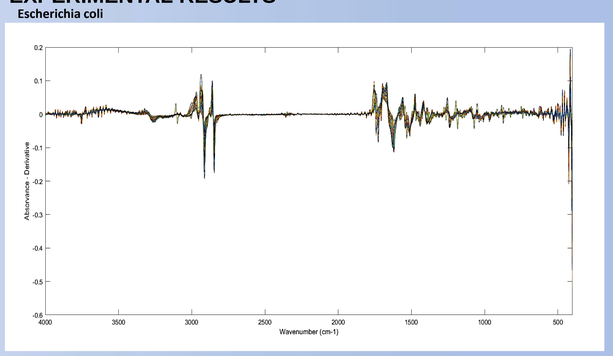

Figure 1A -Spectral data after 2nd derivative preprocessing procedure.

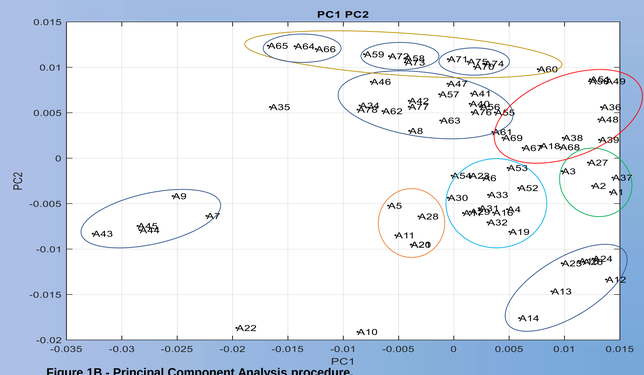

Figure 1B - Principal Component Analysis procedure.

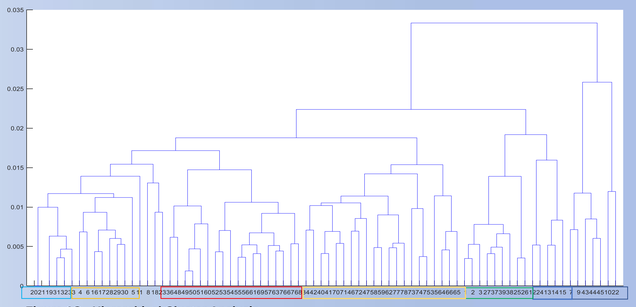

Figure 1C - Hierarchical Cluster Analysis

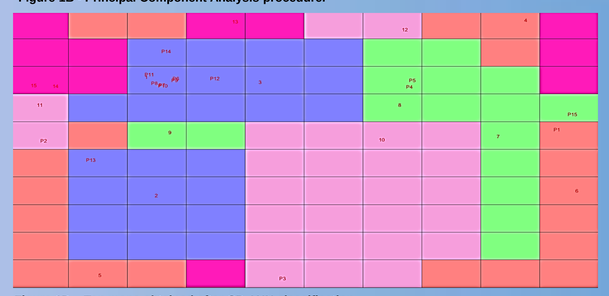

Figure 1D – Top maps obtained after CP-ANN classification.

CPANN – model for *Escherichia coli* assay

- Error rate: 13%
- Non-error rate: 87%
- Accuracy: 77%

Drug type	Sensitivity	Specificity	Precision
Metronidazole	1.00	0.55	0.44
Kanamicin	0.00	0.92	0.00
Clarithromycin	1.00	0.86	0.33
Chloramphenicol	0.25	0.91	0.50
Ampicillin	0.00	1.00	0.00

Bacillus subtilis

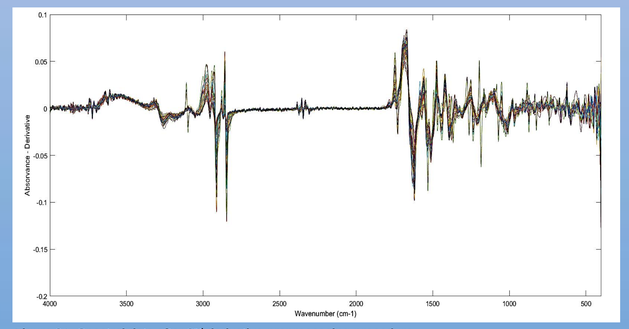

Figure 2A -Spectral data after 2nd derivative preprocessing procedure

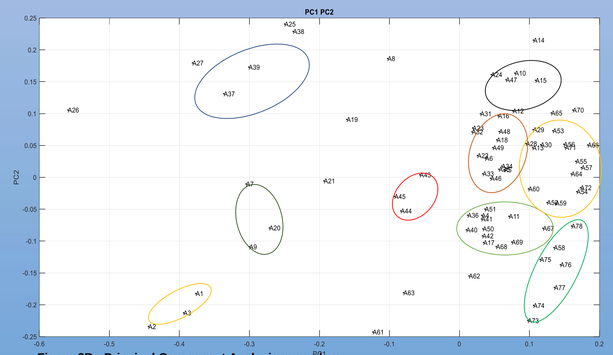

Figure 2B - Principal Component Analysis procedure.

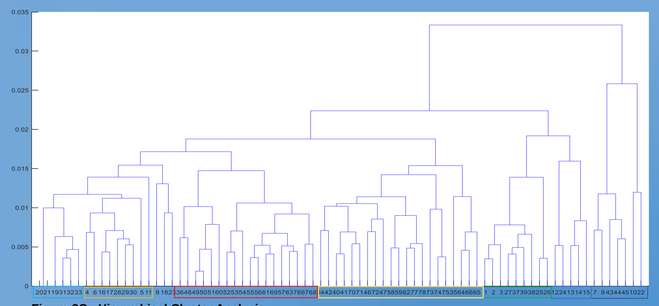

Figure 2C - Hierarchical Cluster Analysis

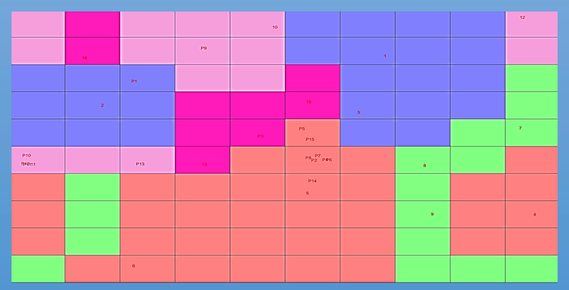

Figure 2D – Top maps obtained after CP-ANN classification

CP-ANN model

- Error rate: 18%
- Non Error rate: 82%
- Accuracy: 73%

Drug type	Sensitivity	Specificity	Precision
Metronidazole	0.33	1.00	1.00
Kanamicin	1.00	0.58	0.38
Clarithromycin	0.00	1.00	0.00
Chloramphenicol	1.00	0.83	0.60
Ampicillin	0.33	1.00	1.00

- Plant extracts were selected based on the previous PCA and HCA analysis, being characterized by higher proximity to the antibiotics.
- CPANN model were developed using the commercial antibiotics.
- The selected samples were used to test procedure using the model development by CPANN algorithm.

Validation procedure for the CP-ANN model was performed using the plant extracts:

- Aqueous flowers extract were classified with a significant specificity to clarithromicin antibiotic.
- Fresh and dry leafs and seeds were classified close to metronidazole antibiotics;
- The aqueous extract (*B. subtilis*) of the fresh and dry leafs were classified as kanamicin and chloranphenicol - both antibiotics inhibits the protein synthesis.

CONCLUSIONS

- Sensitivity, specificity, and accuracy for training, validation, and testing procedure the results obtained are very promising for this strategy.
- Both CP-ANN models are characterized by significant NER value: 87% - E. coli; 87% - B. subtilis.
- Based on the results obtained after the PCA, HCA and CP-ANN algorithms, many plant extracts can present antimicrobial activity which can be similar to some commercial antibiotics.
- MIR spectroscopy and Artificial Neural Network algorithm allowed to recognize the promissory compounds among, highlighting the biomolecules characterized by antimicrobial properties, allowing to develop a platform to discover new bioactive molecules, reducing time and costs related to the drug exploratory step.

References

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Acknowledgment: This work was supported by the DREAMS and FCT (Fundação para a Ciência e Tecnologia) funds.