Hes·so
Haute Ecole Spécialisée de Suisse occidentale
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Master of Science HES-SO in Life Sciences

Novel Antibiotics from Unexplored Natural Sources

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CHEMICAL DEVELOPMENT & PRODUCTION

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In collaboration with **Inflamalps**

DESCRIPTION

Pathogen microorganisms (bacteria and fungi) have become more resistant to current antibiotics over the years. These dangers impose a significant social and economic cost on society, and their consequences will affect an increasing number of people and animals throughout the world.
Since Fleming's discovery of penicillin in 1928, a multitude of antibiotics have been discovered, and their widespread usage in humans and animals has resulted in a scenario in which pathogens are becoming able to overcome the effect of these molecules and succeed to proliferate in their presence.
The need for new antibiotics is now being recognized by health officials and governments. In an attempt to find new antibiotics, poorly studied sources, marine fungi and extremophilic bacteria, were explored.

OBJECTIFS

- Chemical Part:
  Perform initial analyses on extract of F24[1] and to further expand the extract library.

- Biological Part:
  Implement the Kirby Bauer Disc Diffusion Test in the HEIA laboratories.

- Cheminformatics Part:
  Development of Cheminformatics tools necessary for structural dereplication and identification of antibiotic molecules already described in the literature.

[1] Identification code of the extract of a microorganism whose name is confidential

Chemical Part Results

Extraction of cell cultures with ethyl acetate:

Of the 54 cell cultures, 30 were extracted and 24 still have to be extracted

F24 extract:

The GC-MS results conducted at the EPFL identified the molecule 2,2-dimethoxybutane (DMB) in fraction 3 of F24[1].
The DMB is active against gram-positive (*Lactobacillus Delbreuckii*) and negative (*E. Coli* clinical strain) bacteria while inactive against *Candida Albicanse*.
Since the extract of F24[1] is active against *Candida Albicanse* is was hypnotised that DMB is not solely responsible for the antibiotic activity of the extract.
The 2,2-dimethoxybutane (DMB) turned out to be instable in water and a GC-MS Single Mass Mode analysis allow to determine that DMB was an impurity present in methanol, highlighting the importance of analysing all the peaks in the blanks.

Biological Part

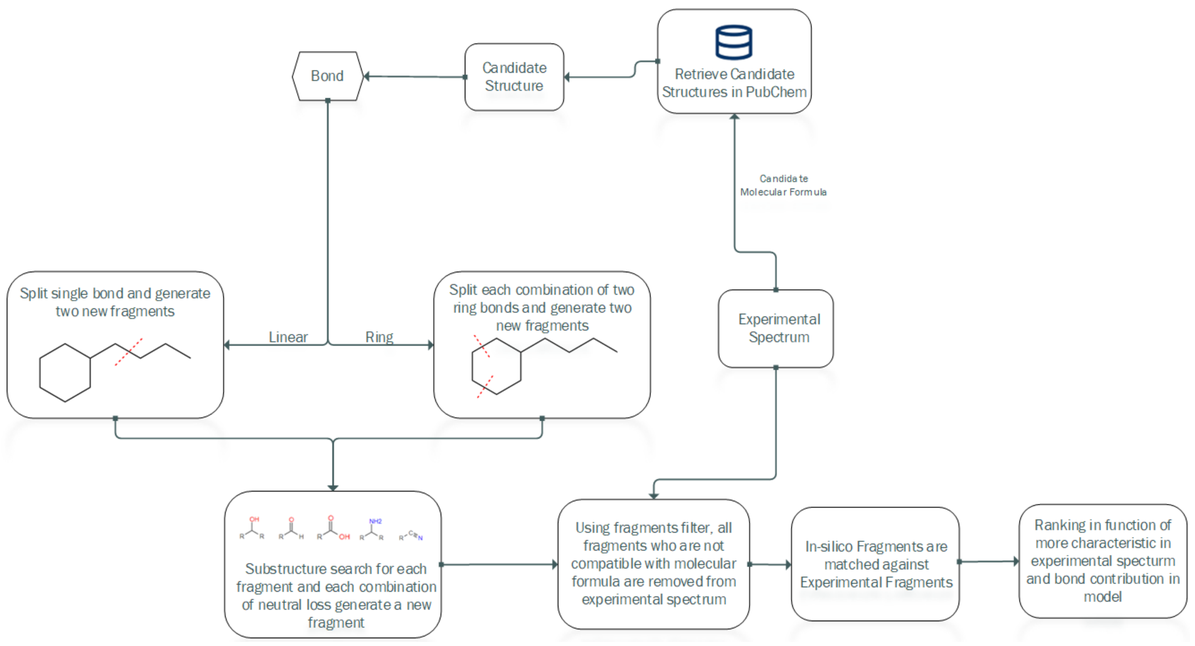
Results of Kirby Bauer Disc Diffusion Test are shown in the following table:

Antibiotic	Sample deposed on disc	Result
Ampicillin	2.5mg	16 mm
F24	0.1mg	0
DMB	16 mg	0
DMB	0.1 mg	0

Ampicillin has an inhibition diameter corresponding to that in the literature and no inhibition was observed for F24[1] extract and DMB.

Cheminformatics Part Results

An In-silico fragmentation algorithm was developed to overcome the lack of experimental mass spectra for most of the known structures, this algorithm uses a model that combines the Hierarchically Ordered Spherical Environment Codes of the fragmented bonds and the bond contribution, a formula derived from the description of fragmentation reaction kinetics.



Comparison of the algorithm performance (indicated as "Mass-Fragmentation") with that of the algorithms which took part in the CASMI contest in category 2 is show in the following table:

Ranking	Mass-Fragmentation	MS-Finder	MetfragCL	CFM-ID
Top 1	55%	11%	25%	14%
Top 5	82%	27%	44%	37%
Top 10	88%	38%	53%	50%

CONCLUSION

Initial analysis of the F24[1] extract identified 2,2-dimethoxybutane as the main compound. Subsequent analysis revealed that this molecule was derived from an impurity present in the analytical solvent (methanol), highlighting the need to have scrupulous control over which peak came from solvent.
The Kirby-Bauer test was implemented, but this method still needs further development to correct the problems still present and to determine the reproducibility of the results.
Finally, an In-silico fragmentation algorithm was developed to overcome the lack of experimental mass spectra for most of the known structures, for which the first tests performed showed that it could identify the correct structure in 55% of cases and rank it within the Top 5 in 82% of cases. The algorithm is available on GitHub at the address: https://github.com/cheminfo/mass-fragmentation.git