CATÓLICA PORTO
BIOTECNOLOGIA

Composition and antibacterial properties of extracts from agricultural by-products

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Outline

- Ginjinha: production and by-products.
- Extraction of high-added value compounds.
- Characterisation of the extracts.
  - Volatile compounds (terpenes).
- Antibacterial properties.
- Conclusions.

Ginja cherry and Ginjinha

- Ginja cherry: Portuguese cherry (*Prunus cerasus*, L. Rosaceae).
- Used to make Ginjinha, a traditional Portuguese liquor.
- By-products: stems and leaves.
  - Normally burned.
- Extraction of High Added Value compounds.

Extraction of High Added Value compounds

- Solvent extraction.
  - Water.
  - Ethanol:water 70:30.
  - Acetone:water 70:30.
  - Ethyl acetate:water 70:30.
- Extraction at room temperature (thermolabile compounds) for 24 h.
- Solvent removal by distillation (< 45 ^{o}C).

Previous work: polyphenols

Stems				
	Ethanol	Ethyl acetate	Acetone	Water
TPC	854.47	1113.21	559.15	517.32
Leaves				
	Ethanol	Ethyl acetate	Acetone	Water
TPC	140.72	1.82	135.88	166.72

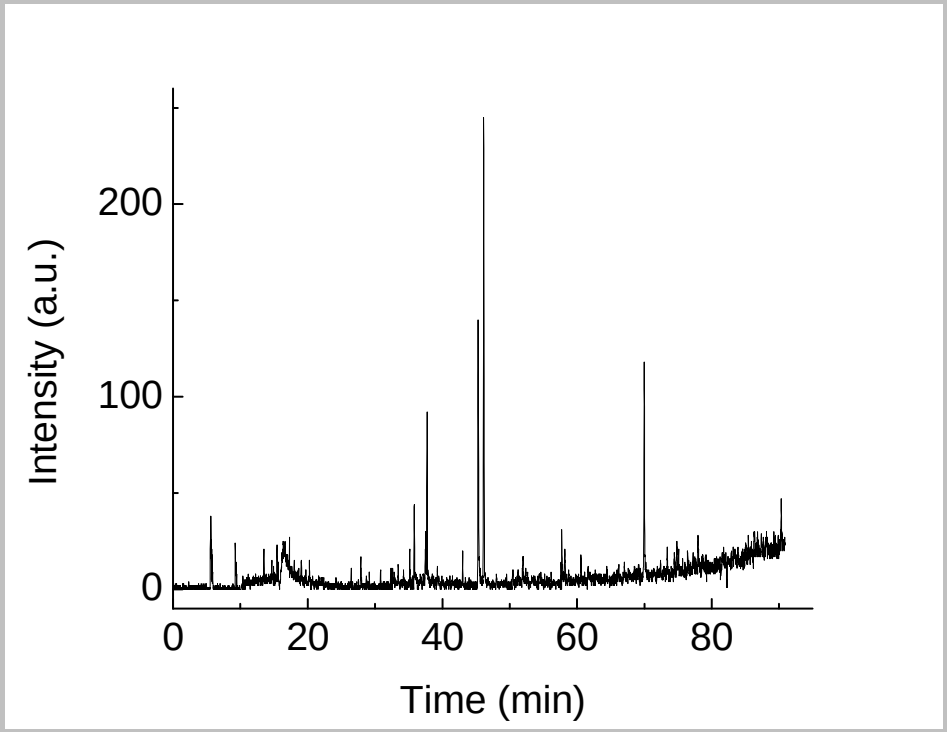
- TPC: Total Phenolic Content (μg/g dry plant).
- Stem > Leaves.
- Effect of the solvent.

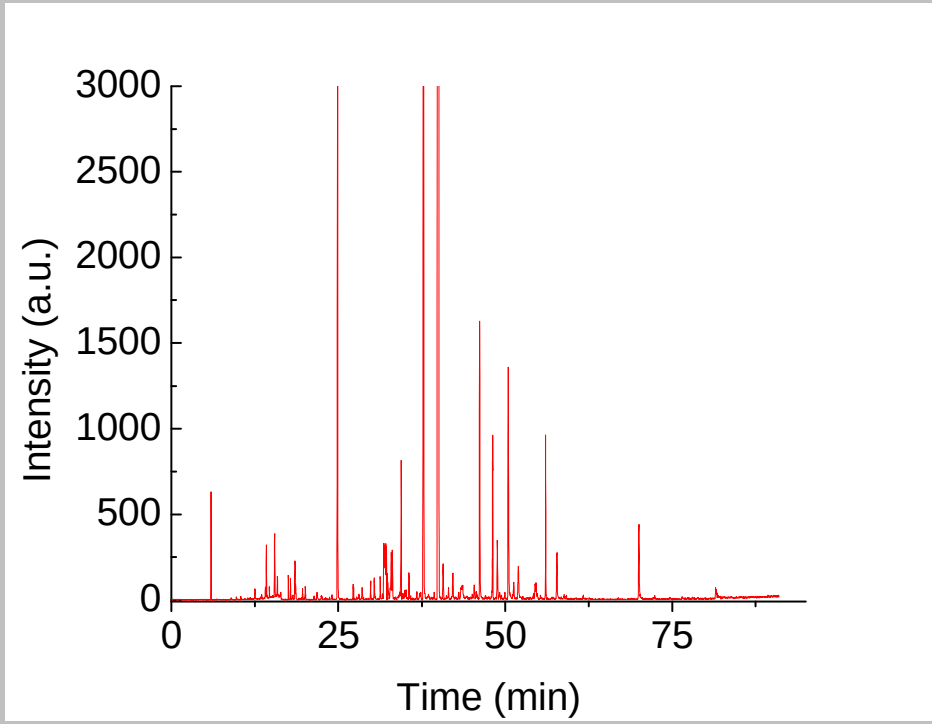
Analysis: volatile compounds

- The “best” extracts were tested for volatile compounds.
- SPME/GC-MS analysis.
  - Divinylbenzene/carboxene/PDMS fibre.
- Semi-quantitative analysis.
- Comparison between extracts made from different sources and/or with different solvents.
- Correlation between the extracts composition and their properties.

Analysis: volatile compounds

Examples of chromatograms.





Leaves extracts in water.

Stems extracts in ethyl acetate.

Analysis: volatile compounds

- Terpenes (hydrocarbons and oxygenated), ketones, esthers, alcohols, acids, hydrocarbons.
- Stems extracts in ethyl acetate richer in volatile compounds (36).
- In the other extracts less compounds were detected and/or at a lower concentration.

Analysis: volatile compounds

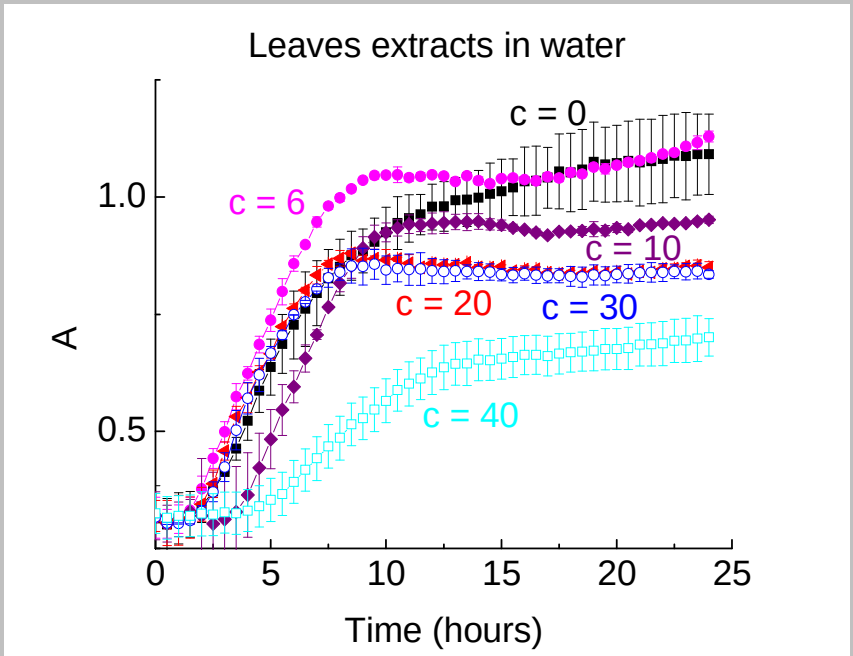
- Compounds only detected for ethyl acetate stem extracts:
  - α-pinene, methyl salicilate.
- Compounds detected in other extracts but at a much smaller concentration (between 10 and 200 times lower):
  - eugenol, α-terpineol, linalool.

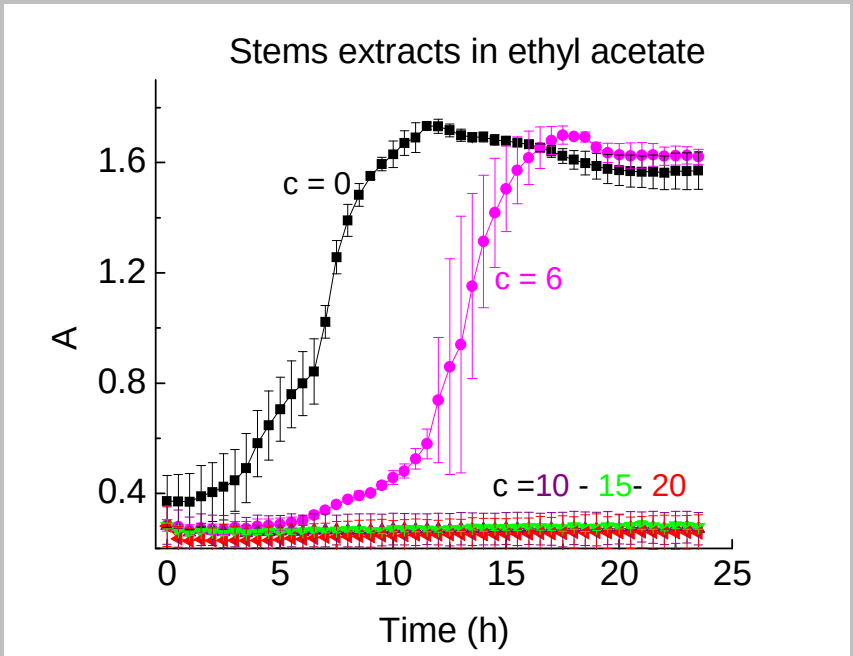
Antibacterial activity

- Inhibition in the growth of bacteria.
- Monitoring the bacterial growth in the presence of the extract.
  - Reading of Absorbance at λ = 610 nm at 37 ºC.
- Several bacterial strains were tested (contaminants in food, water, soil, etc.).
- Different extracts concentrations.
- Determination of Minimum Inhibitory Concentration (MIC).

Antibacterial activity

Examples of growth curves for Methycillin Resistant *Staphylococcus aureus* (MRSA).





Note: all concentrations are expressed in mg/ml.

Antibacterial activity: MIC values for stems extracts (mg/ml)

Bacterial strains	Ethyl acetate	Ethanol	Acetone
Staphylococcus aureus MSSA	10	10	20
Staph. aureus MRSA	10	10	20
Bacillus subtilis	10	15	10
Pseudomonas sp.	20	30	40
Pseudomonas aeruginosa	25	100	100
Flavobacterium	15	10	20
Salmonella	30	100	100
E. coli	30	100	100

Antibacterial activity: MIC values for leaves extracts (mg/ml)

Bacterial strain	Ethanol	Acetone
Staphylococcus aureus MSSA	20	15
Staph. aureus MRSA	30	15
Bacillus subtilis	30	40
Pseudomonas sp.	30	40
Pseudomonas aeruginosa	100	100
Flavobacterium	20	15
Salmonella	100	100
E. coli	100	100

Bactericidal activity

- Test if bacteria were killed by the extracts
- Bacterial solution in contact with the extracts for 24 h.
  - Concentration of the extract ≥ MIC.
- Plating on Mueller Hinton agar medium of 50 μl of solution.
- Incubation at 37 ^{o}C for 24 h.
- Minimum Bactericidal Concentration (MBC): minimum concentration for which bacteria were killed (no growth AFTER the extract was removed).

Bactericidal activity

A: extract concentration < MBC → bacteria is still alive.

B: extract concentration ≥ MBC → bacteria is dead.

Bactericidal activity: MBC values for stems extracts (mg/ml)

Bacterial strains	Ethyl acetate	Ethanol	Acetone
Staphylococcus aureus MSSA	15	40	20
Staph. aureus MRSA	20	30	20
Bacillus subtilis	>100	60	10
Pseudomonas sp.	30	40	60
Pseudomonas aeruginosa	30	>100	>100
Flavobacterium	30	30	30
Salmonella	40	>100	>100
E. coli	60	>100	>100

Bactericidal activity: MBC values for leaves extracts (mg/ml)

Bacterial strain	Ethanol	Acetone
Staphylococcus aureus MSSA	60	20
Staph. aureus MRSA	100	20
Bacillus subtilis	100	80
Pseudomonas sp.	60	40
Pseudomonas aeruginosa	>100	100
Flavobacterium	40	20
Salmonella	>100	100
E. coli	>100	100

Conclusions

- Extracts from Ginja cherry by-products were rich in volatile compounds.
- Stems > leaves.
  - Ethyl acetate > ethanol ≈ acetone.
  - Ethanol ≈ acetone > water.
- Stems extracts in ethyl acetate have highest antibacterial activity.
- Higher concentration of volatile antibacterial compounds.

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Future work

- Use of the extracts.
- Additive for food packaging.
  - The antibacterial properties help in food preservation.

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