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Development of sports electrolyte drink with prebiotic properties based on beetroot skin fractions

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Agri-food Waste Management for Sustainable bio-economy through Higher Education curricula and upskilling

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Goal

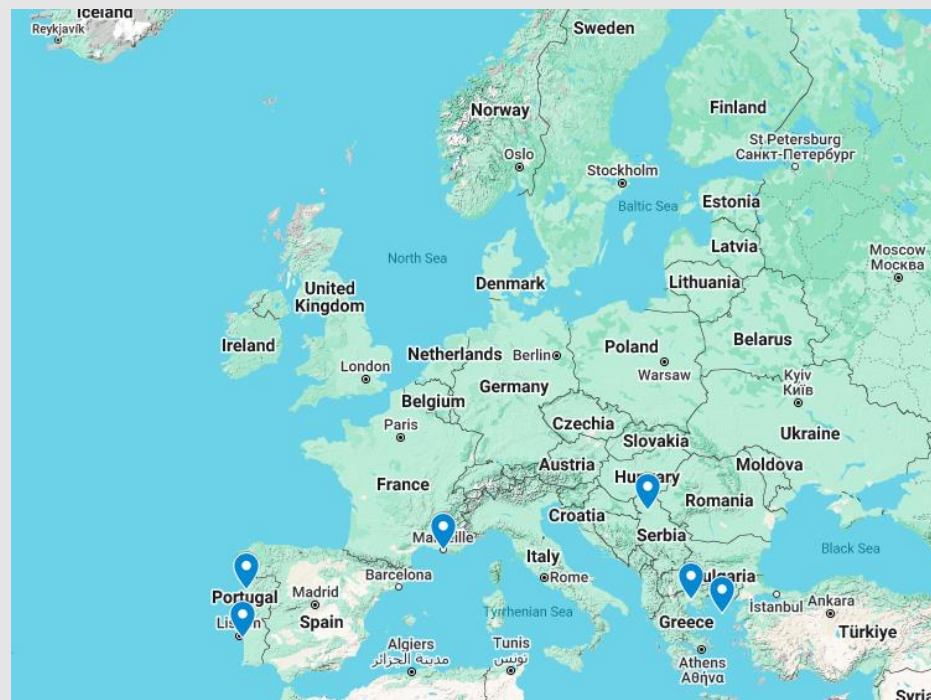
AGRIMA aims to foster universities' **capacity building** for the **green transition** through **innovative practices** and **higher education curricula updating** in **agri-food waste management** for the **circular bioeconomy**.

AGRIMA addresses:

- 1. Advancing pedagogical methods** for industrial agri-food waste valorisation **based on business-academia synergies**.
- 2. Integrating citizen science** in bio-economy-enhanced waste valorisation as a means of **civic engagement and environmental advocacy**.



Partners





BEETROOT

- Beetroot (*Beta vulgaris*) is a highly nutritious root vegetable known for its numerous health benefits.
- Rich in essential vitamins and minerals such as folate, vitamin C, potassium, and iron, beetroot also contains powerful antioxidants like betalains, which give it its deep red color.

BEETROOT

- It supports brain health by improving blood flow to the brain, and its high fiber content promotes healthy digestion and gut function.
- Evaluating its chemical composition will provide more information about its advantage and usage thereby promoting its application.

PROJECT PLAN

- Extract dried, ground beet peel with subcritical water under the following conditions:
Drug : solvent ratio = 1:20 , T = 130°C , t = 40 min , P(N₂) = 20 bar , Convection ~ 600 rpm
- Determine antiradical activity: DPPH and ABTS radicals , total antioxidant activity (Phosphomolybdenum method)
- Determine total phenols, total flavonoids
- Determine total anthocyanins , betalains (betaxanthin and betacyanin)
- Determine soluble fiber - quantification of pectin
- Determine total reducing sugars- Fehling`s test
- Formulate a functional drink for athletes enriched with Mg and prebiotics

New methodology for extraction

This project stands out due to the innovative application of subcritical water, harnessing precise temperature and pressure protocols. $T = 130^{\circ}\text{C}$, $t = 40$ min, $P(\text{N}_2) = 20$ bar, Convection ~ 600 rpm
For this part of the experiment we used 400ml of distilled water and 20g of dried beetroot peel, since the drug solvent ration was 1:20.





Lyophilization

- Lyophilization is the process of water removal typically used to preserve perishable materials.



extraction yield
(%)

34.00 ± 1.68

YIELD BY DRYING

- The 3 cups with the extract are placed in a drying oven (about 60° C) and dried to a constant mass .
- The extraction yield was determined using two different methods.

Extraction yield - dryer, 60° C (%)	27.73 ± 0.70
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Total antioxidant activity

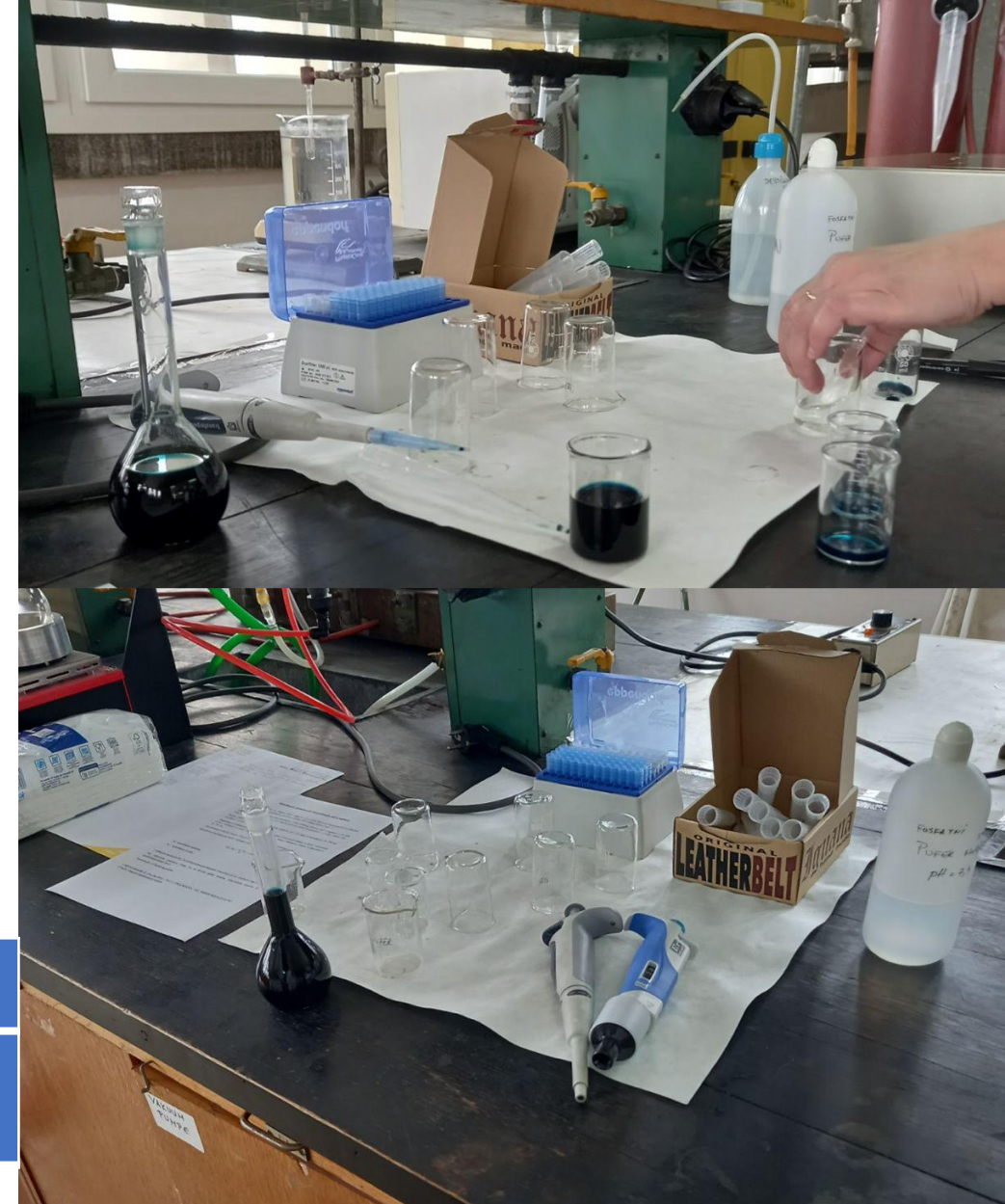
Phosphomolybdenum method:

- For this analysis we used 300 μ L of extract and diluted it in a 1:50 and a 1:100 ratio.
- Added 3 ml of the mixture of reagents (1:1:1) and incubated at 95° C for 90 min.
- After cooling, the absorbance is read at 695 nm.
- Ascorbic acid was used as a reference substance.

	mg/l extract	mg/100 g drug	mg/g dry extract
Total antioxidant activity (AAE)	898.88 \pm 9.02	1797.75 \pm 18.03	52.88 \pm 0.53

DPPH

- DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical and a common reagent used to measure antioxidant activity.
- Ascorbic acid was used as the reference substance in this test.



	mg/l extract	mg/100 g drug	mg/g dry extract
DPPH test (AAE)	289.03 ± 3.57	578.06 ± 7.14	17.00 ± 0.21

Total betalains (betaxanthin) and total betacyanin

- Measure betaxanthin content at 480 nm and betacyanin at 528 nm
- Distilled water is used as a blank test.
- The Lambert-Beer law is used for the determination of betalains, as well as for the determination of anthocyanins:

$$A = \varepsilon \cdot l \cdot c$$

A = Without dilution x f

f – dilution factor (how many times the extract is diluted)

ε (betaxanthin) = 62,000 l/mol·cm

ε (betacyanine) = 48,000 l/mol·cm

	mg/l extract	mg/100 g drug	mg/g dry extract
Total betalains (betaxanthin)	6.35±0.31	12.70±0.6	0.37±0.22
Total betalains (Betacians)	6.60±0.36	13.20±0.63	0.39±0.23



ABTS TEST

$\gamma_1=6\text{mg/ml}$
 $V=25\text{ ml}$
 $m=0.1900\text{g}$ of dry extract was mixed with 25ml of H_2O
The solution we used as a basis for further analysis was obtained by dissolving 0.1900g of dry extract in 25ml of distilled water. To obtain 5mg/ml, it was necessary to pour 8.333ml into a normal 10ml container and it was filled up with distilled water.
-ABTS+ solution was diluted with 5mM phosphorus buffer (pH=7.4) to have obtained $A = 0.7 \pm 0.002$ at 734nm

ABTS test

$\text{IC}_{50} = 7.19 \pm 0.51 \text{ mg dry extract /ml}$



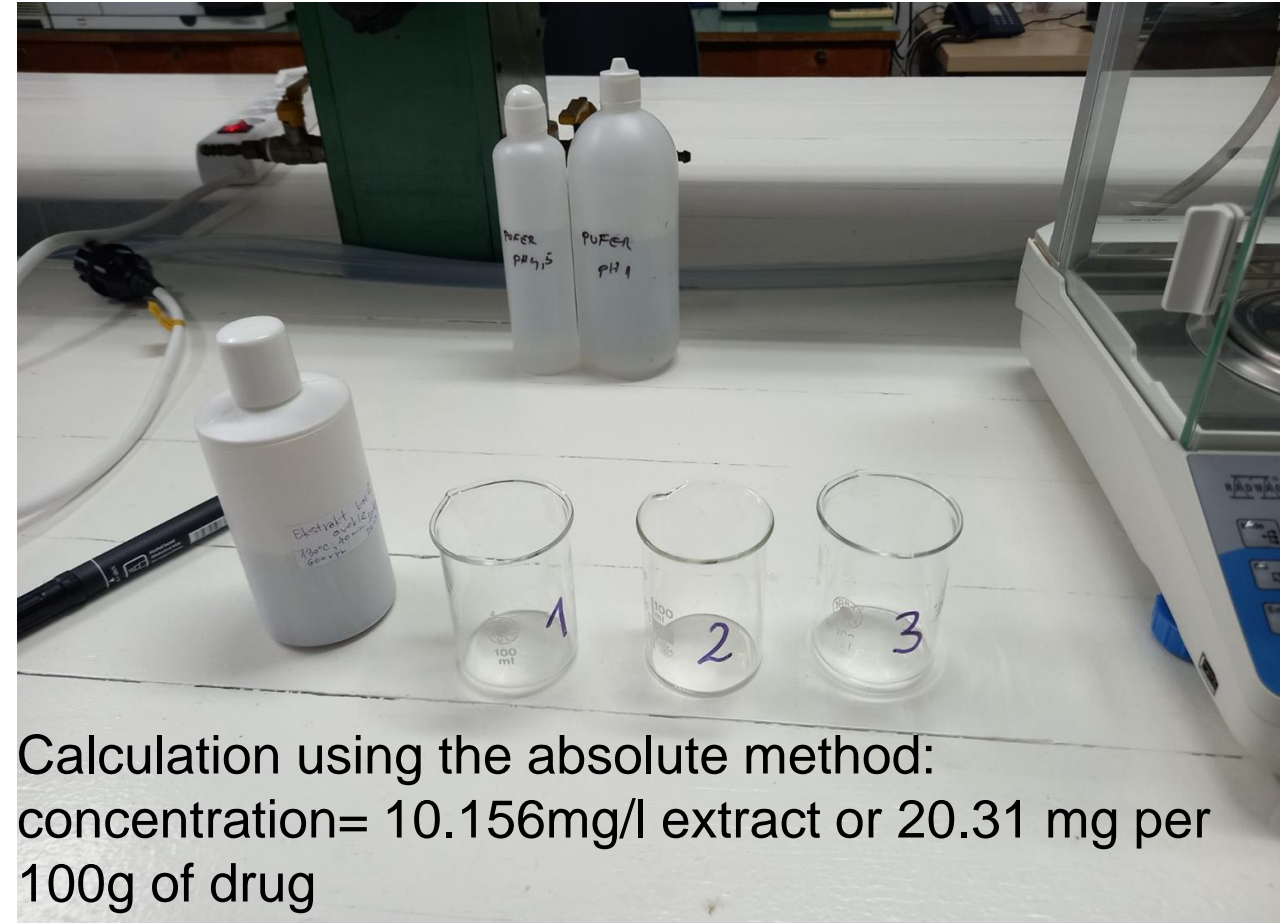
Determination of anthocyanin content

- To quantify total anthocyanins, we used two buffer solutions: pH 1.0 (0.025 mol/L KCl) and pH 4.5 (Na-acetate buffer) to dilute the extract.

By the differential method

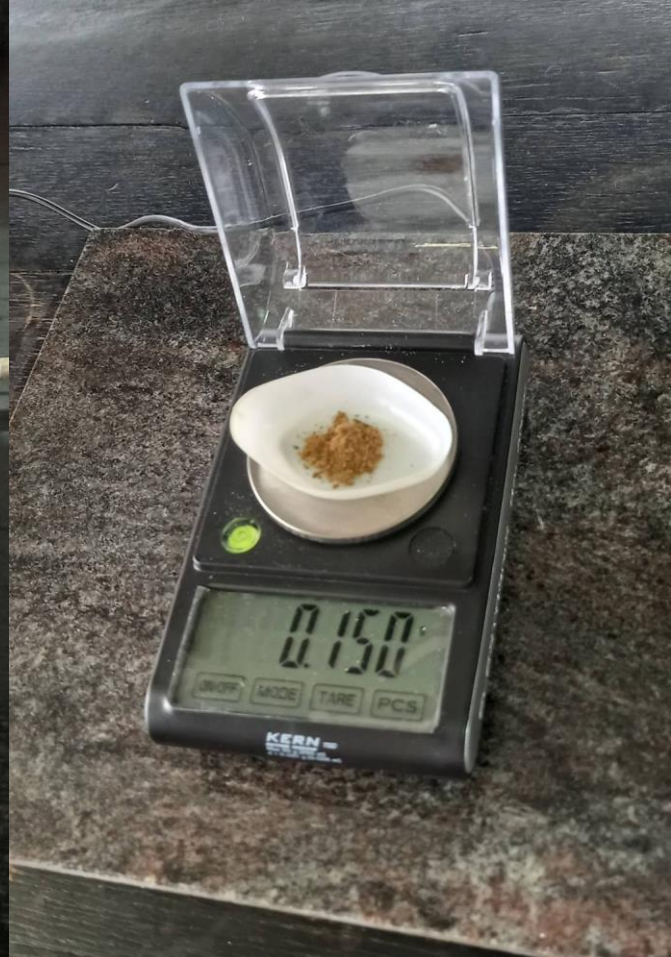
BUFFER PH=1 BUFFER PH=4.5

- dilution 1:3 - dilution 1:3
- on the spectrometer - on the spectrometer
- blind test: - blind test: at 520nm
- A= 0.180 A=0.195
- at 700nm
- A=0.028 A=0.040



Calculation using the absolute method:
 concentration= 10.156mg/l extract or 20.31 mg per 100g of drug

	mg/l extract	mg/100 g drug	mg/g dry extract
total anthocyanins (C-3-G)	10.16 ± 0.90	20.31±1.10	0.60±0.10



PECTIN

- The pectin content was determined by alcohol precipitation.
- After 48 hours, gelatinous turbidity was noticeable.
- Pectins were separated by squeezing on previously weighed cheesecloth that was in a desiccator.
- Gauze with the retained pectin was dried at 45° C to a constant mass.



Pectin (%)

3.80 ± 0.15

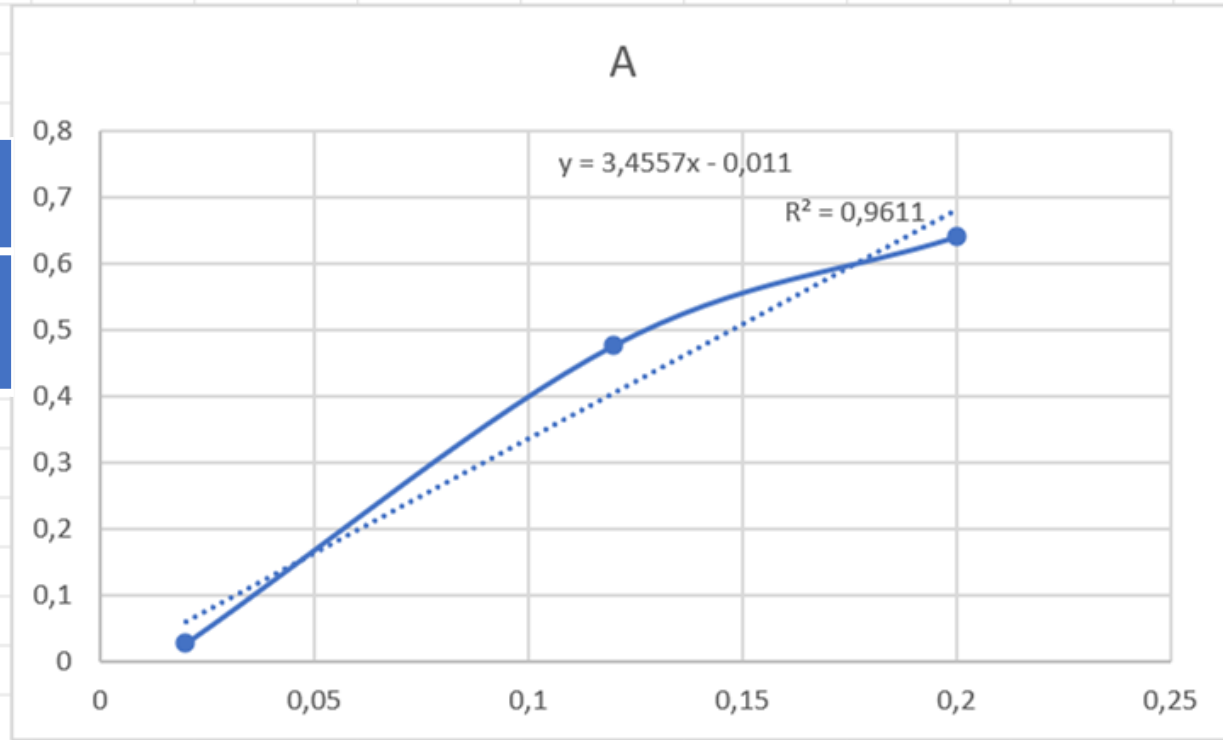
Total sugars (Fehling's test)

- 1 ml of the liquid sample was diluted with 2 ml of distilled water and 20 drops of Fehling's reagent were added.
- The appearance of a brick-red precipitate was considered a positive reaction.

TOTAL SUGARS

	B	C	D	E	F	G	H	I	J	K	L	M	N
g/l		A											
1	0,02	0,026											
2	0,12	0,476											
3	0,2	0,64											

	mg/l extract	mg/100 g drug	mg/g dry extract
Total sugars (GE)	6.6 ± 0.2 g/l	13.20 ± 0.40 g/100 g	0.39 ± 0.02 g/g dry extract



TOTAL PHENOLS

- The **Folin–Ciocalteu method** is used to determine the total phenolic content of a sample.
- A solution in the ratio of 1:20 and 1:50 was made.
- For the solution in the ratio of 1:20, 500 μL of the extract was added to a normal 10ml vessel.
- For the 1:50 solution, 1ml of extract was added to a normal 10ml container.

	mg/l extract	mg/100 g drug	mg/g dry extract
Total phenols (GAE)	429.66 \pm 2.02	859.31 \pm 4.03	25.27 \pm 0.13

TOTAL FLAVONOIDS

- Rapid method with aluminum chloride: 2 ml of 2% AlCl_3 is added to 2 ml of sample or standard solution.
- After 10 min of incubation, the absorbance is read at 430 nm.
- As a blank, distilled water is used instead of the sample.
- Flavonoids form a complex with aluminum chloride, producing a yellow color that can be measured spectrophotometrically .

	mg/l extract	mg/100 g drug	mg/g dry extract
Total flavonoids (RE)	203.94 ± 1.43	407.88 ± 2.86	12.00 ± 0.08

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ABTS test	IC ₅₀ = 7.19±0.51 mg dry extract /ml		
Pectin (%)	3.80 ± 0.15		
Extraction yield - lyophilization (%)	34.00 ± 1.68		
Extraction yield – in the dryer, 60° C (%)	27.73 ± 0.70		

Formulation of sports electrolyte drink with prebiotic properties

- No sugar added – calculated caloric value
- 0.5% of dry beetroot skin extract (antioxidants)
- 1 g/l of L-ascorbate 2-monophosphate
- 85 mg/l of added Mg^{2+}
- Prebiotics from partially hydrolysed cellulose fraction (cello-oligosaccharides by subcritical water)

	mg/l extract	mg/100 g drug	mg/g dry extract
Total sugars (GE)	6.6 ± 0.2 g/l	13.20 ± 0.40 g/100 g	0.39 ± 0.02 g/g dry extract

CONCLUSION:

- The results of this project highlights the potential value of beetroot peels, suggesting that they may contain beneficial compounds that are often overlooked.
- While the current findings provide a promising starting point, they also reveal the need for further research to fully understand the nutritional and functional properties of beetroot peels.
- Exploring these properties more deeply could lead to innovative applications in food, health, and even cosmetic products.

