

EVALUATION AND PURIFICATION OF BETALAIN PIGMENTS FROM RED-PURPLE VARIETY OF *ATRIPLEX HORTENSIS* “*RUBRA*” THAT EXHIBIT ANTIOXIDANT AND CARDIOPROTECTIVE ACTIVITY



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INTRODUCTION

- Atriplex hortensis* is an edible plant that is characterized by high resistance to unfavorable growing conditions such as drought, high salinity, and extreme temperature.
- A. hortensis* var. *rubra* is a red-purple variety rich in betalain pigments which are responsible for its coloration.
- Betalains cover the class of remarkable natural red-violet and yellow-orange plant pigments with prospective chemical and biological properties for wide-ranging applications in food industry.
- Betalains are considered as bioactive compounds with nutraceutical and therapeutic potential, including antioxidants, chemopreventive, and anti-inflammatory properties.

HYPOTHESIS

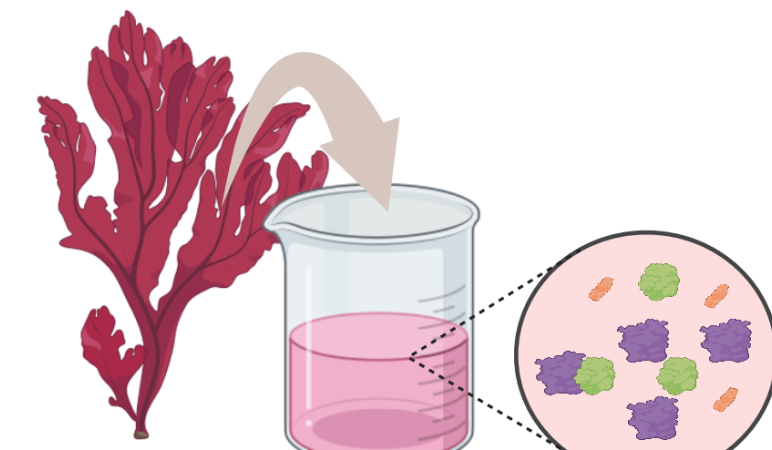
Searching for edible cultivars derived from agricultural by-products has been intensified since drought, high salinity, extreme temperature, and flooding, are a major cause of crop loss worldwide. In the coming years increased climatic variation and frequency of extreme weather events are also expected, requiring the cultivation of reliable, weather-resistant plants. Spinach-like red-violet *A. hortensis* var. *rubra* may become an attractive food-plant rich in betalains that can not only supplement daily diets but also contribute to world food security.

AIMS

- To develop a method of chromatographic purification of acylated betalains
- To determine betalain (betacyanins and betaxanthins) content in crude extracts of *A. hortensis* var. *rubra*
- To study antioxidant and cytotoxic and cardioprotective activity of *A. hortensis* var. *rubra* extracts and purified pigments

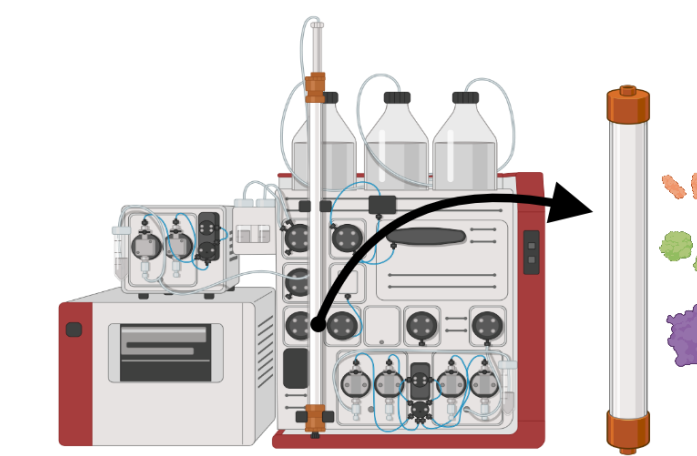
METHODS

1 Extraction of betalains



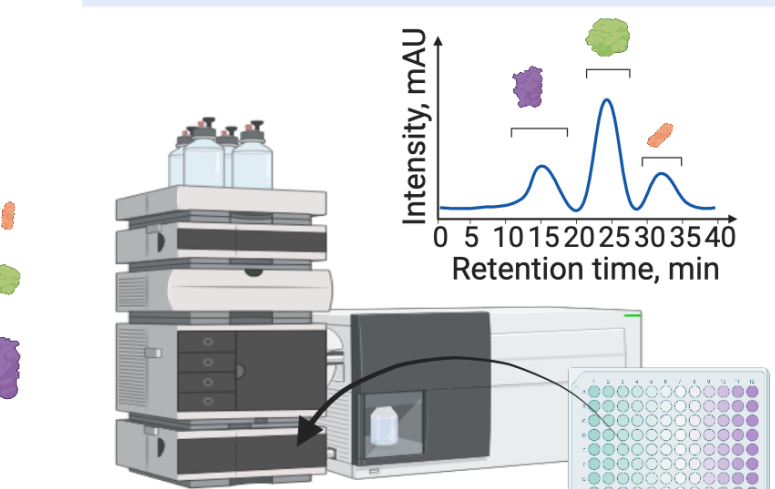
- Maceration with 20% acetone acidified with 1% formic acid (v/v)

2 Purification and isolation of pigments



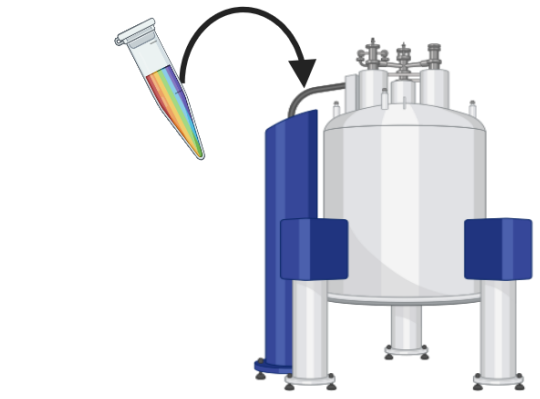
- Weak anion exchanger (WAX)
- Semipreparative RP-HPLC

3 Phytochemical analysis



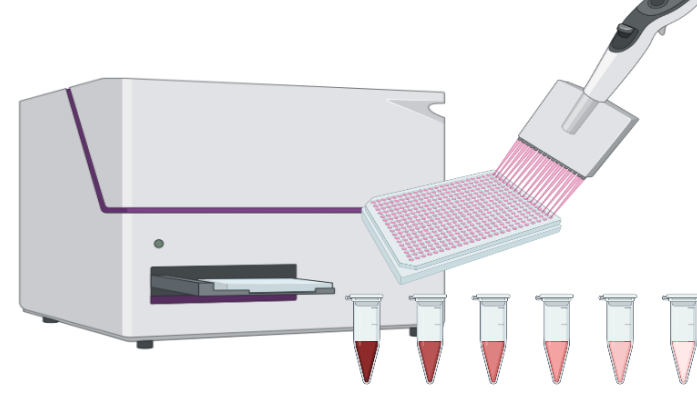
- LC-DAD-ESI-MS/MS

4 Structural analysis



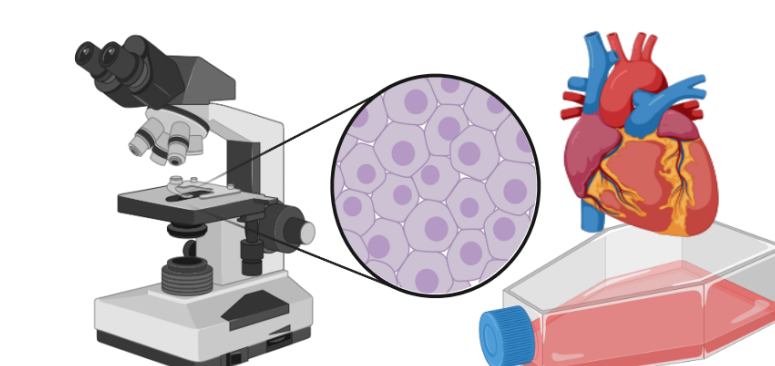
- NMR spectroscopy

5 Antioxidant activity



- ABTS, FRAP, ORAC assays

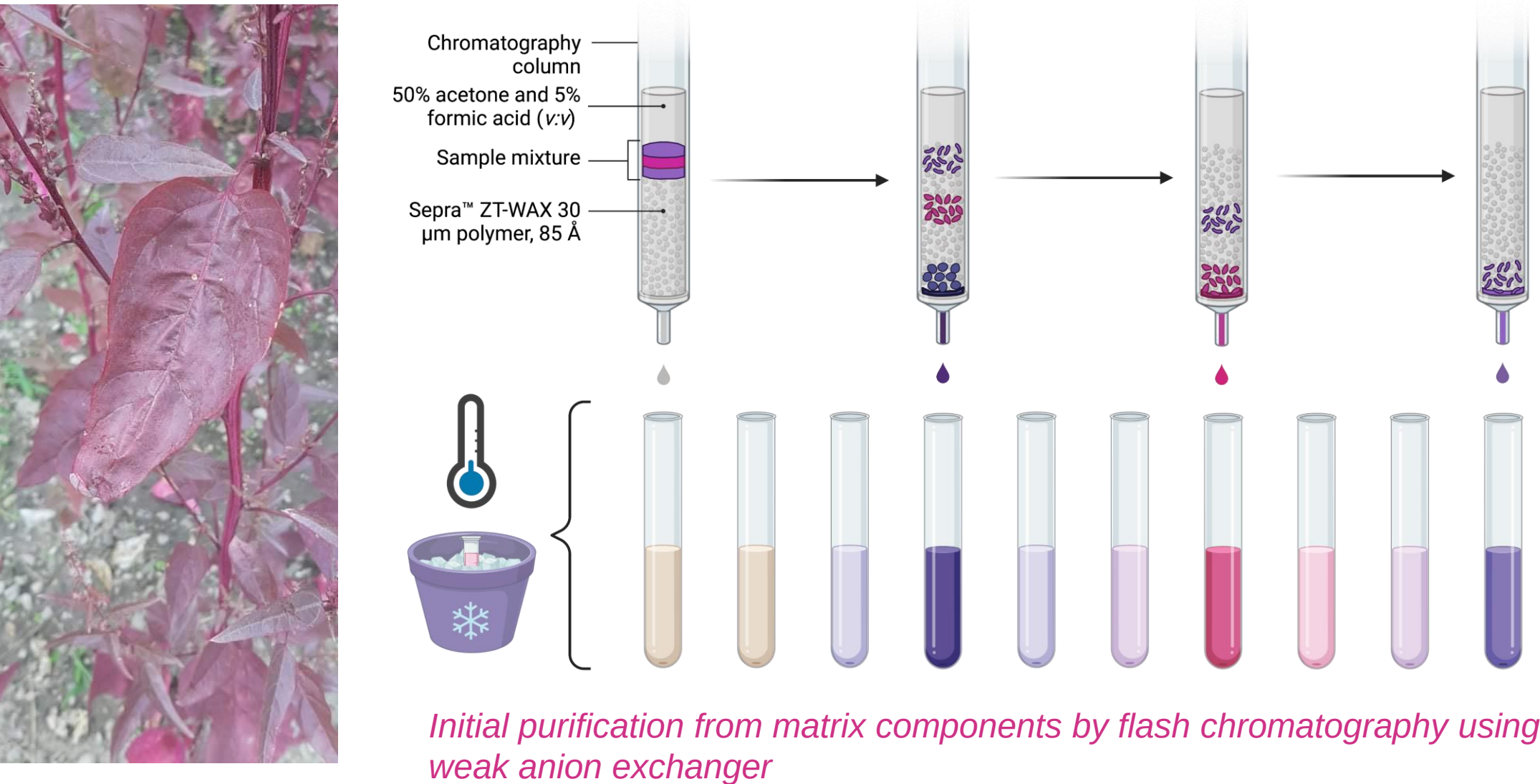
6 Cytotoxicity and cardioprotective activity



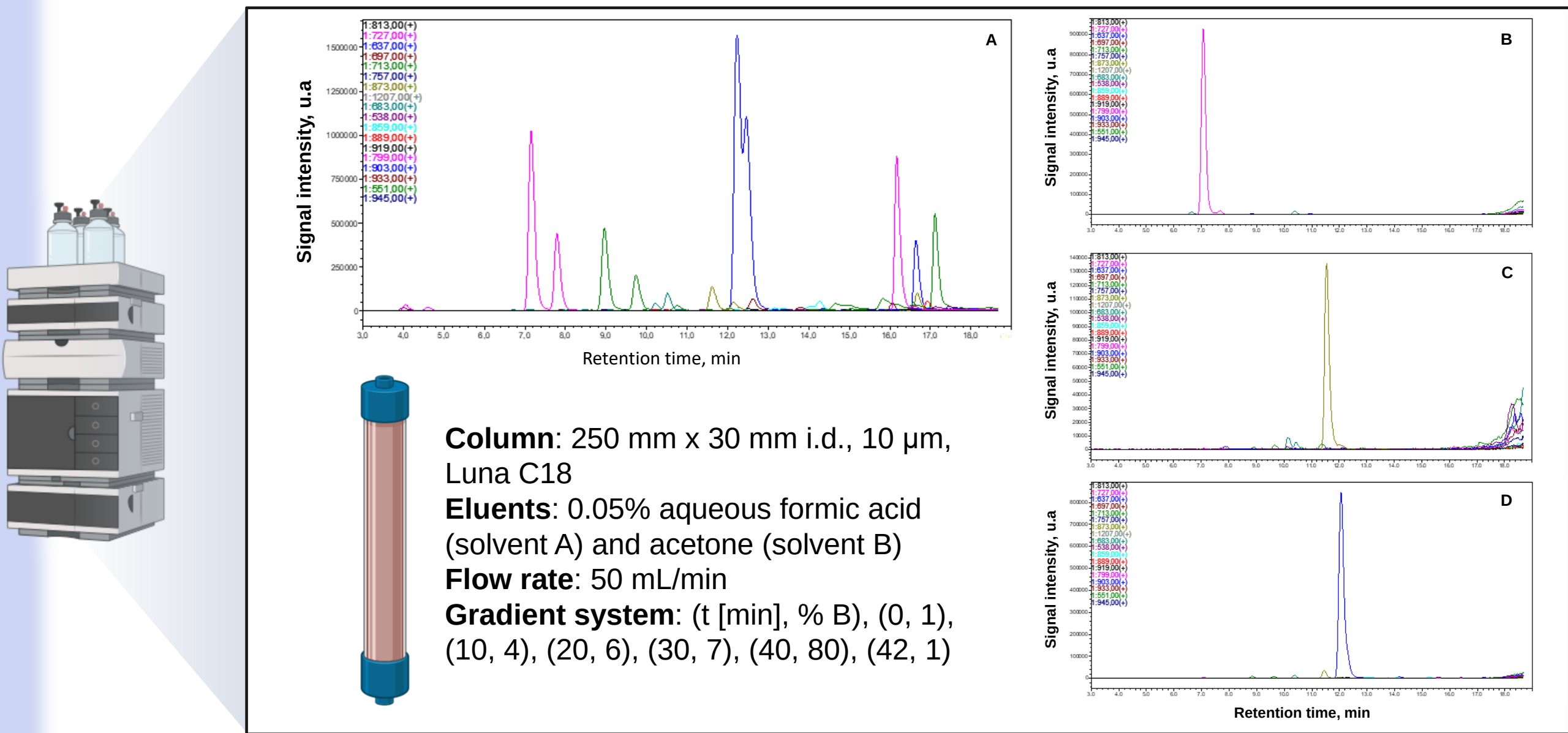
- Rat cardiomyocytes (H9c2)

RESULTS

Preparation and initial purification of betalain-rich crude extracts



Isolation of betalains by semipreparative reversed-phase liquid chromatography



LC-MS chromatograms of betalains present in *A. hortensis* var. *rubra* extract (A) and amaranthin (B), argentinian (C), and celosianin (D) purified using prep-HPLC technique. Chromatograms are registered in the selected positive ion monitoring (SIM).

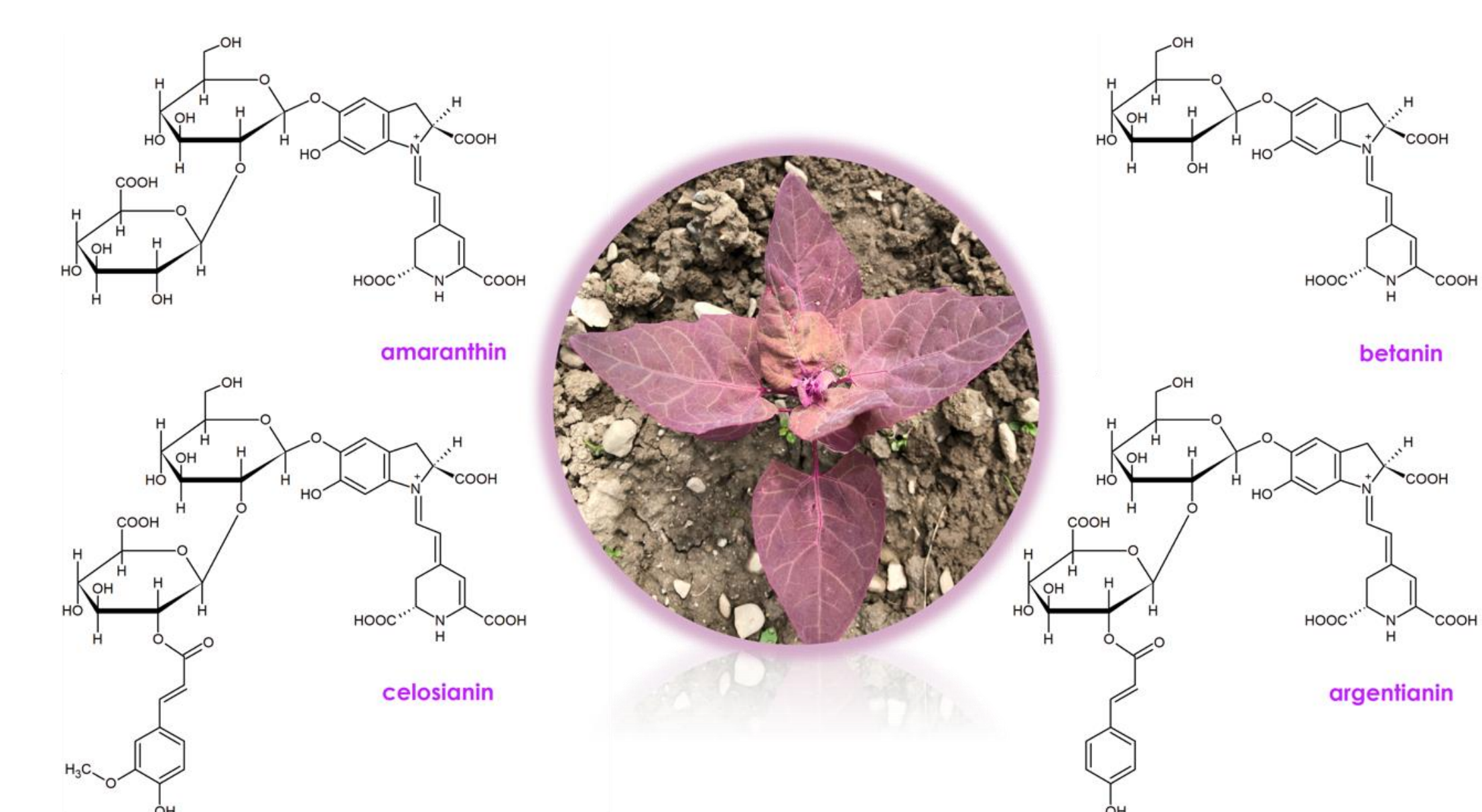
Identification and quantification of betalain pigments

Betacyanin content in *Atriplex hortensis* var. *rubra* extract prepared from leaves [mg/100g]

Compound	R _f [min]	λ[nm]	m/z [M+H] ⁺	betacyanin content, mg/100g FW
amaranthin	13.4	536	727	85.19 ± 0.99
isoamaranthin	15.0	536	727	61.31 ± 1.34
betanin	16.9	533	551	31.64 ± 0.54
isobetanin	17.7	533	551	21.34 ± 0.41
argentinian	19.1	542	873	16.79 ± 0.24
isoargentinian	19.4	542	873	5.90 ± 0.14
(iso)celosianin	19.5	542	903	214.93 ± 1.14

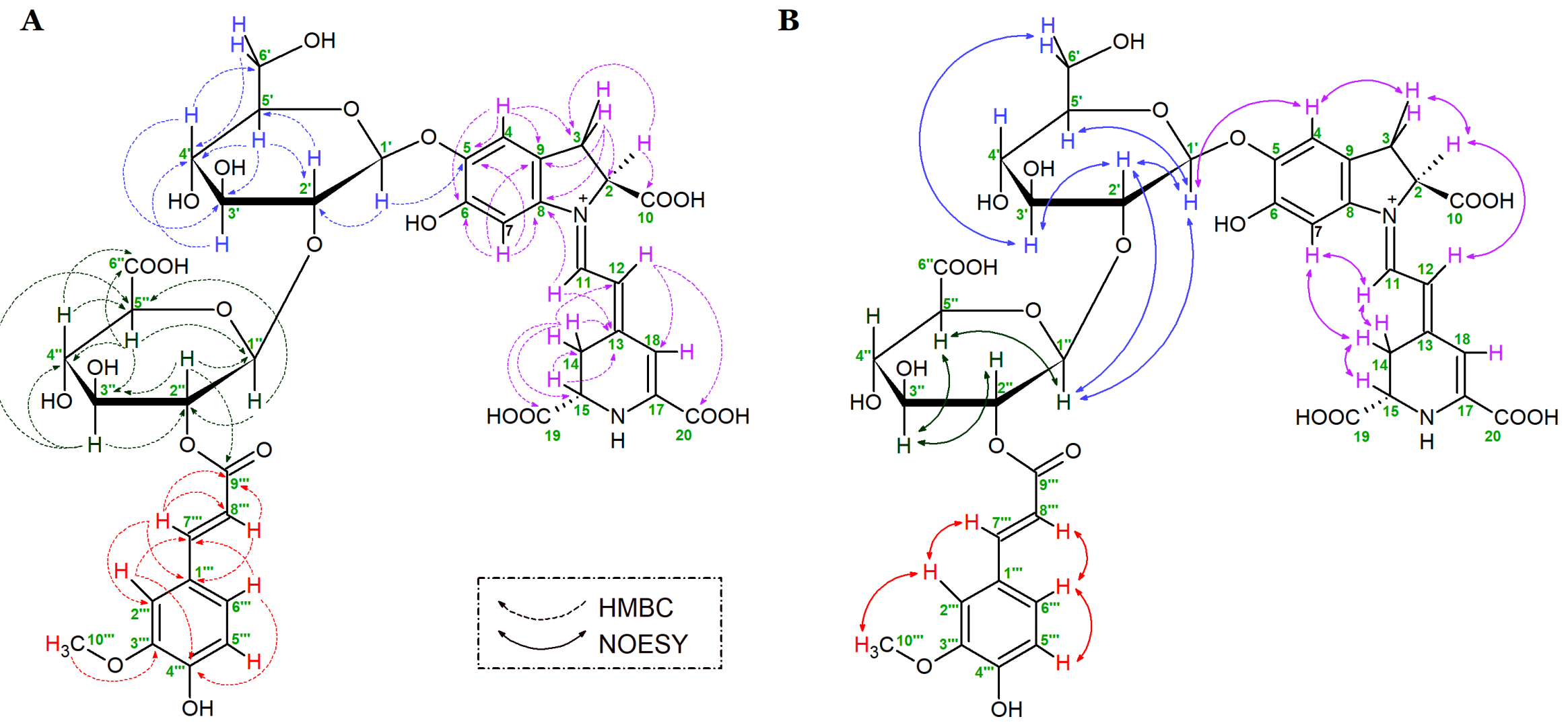
Betaxanthin content in *Atriplex hortensis* var. *rubra* extracts prepared from leaves [mg/100g]

Compound	R _f [min]	λ[nm]	m/z [M+H] ⁺	betaxanthin content, mg/100g FW
muscaurin VII	3.60	474	349	1.94 ± 0.14
portulacaxanthin III	4.65	470	269	5.65 ± 0.098
vulgaxanthin I	5.79	470	340	1.94 ± 0.11
isoidicaxanthin	13.00	470	309	0.54 ± 0.056
indicaxanthin	13.40	470	309	29.27 ± 0.30



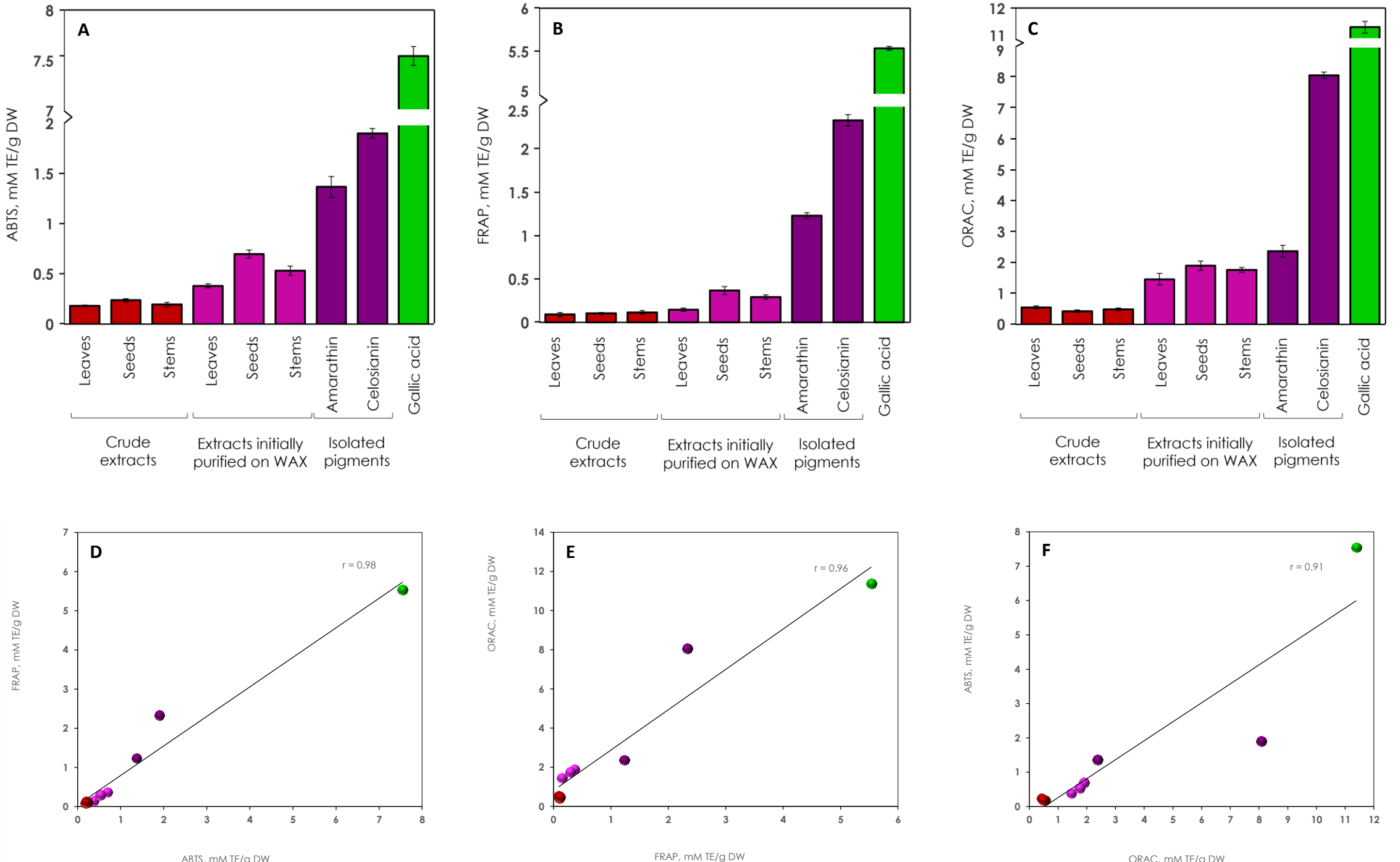
Chemical structure of main betacyanin pigments present in *Atriplex hortensis* var. *rubra*

NMR experiments



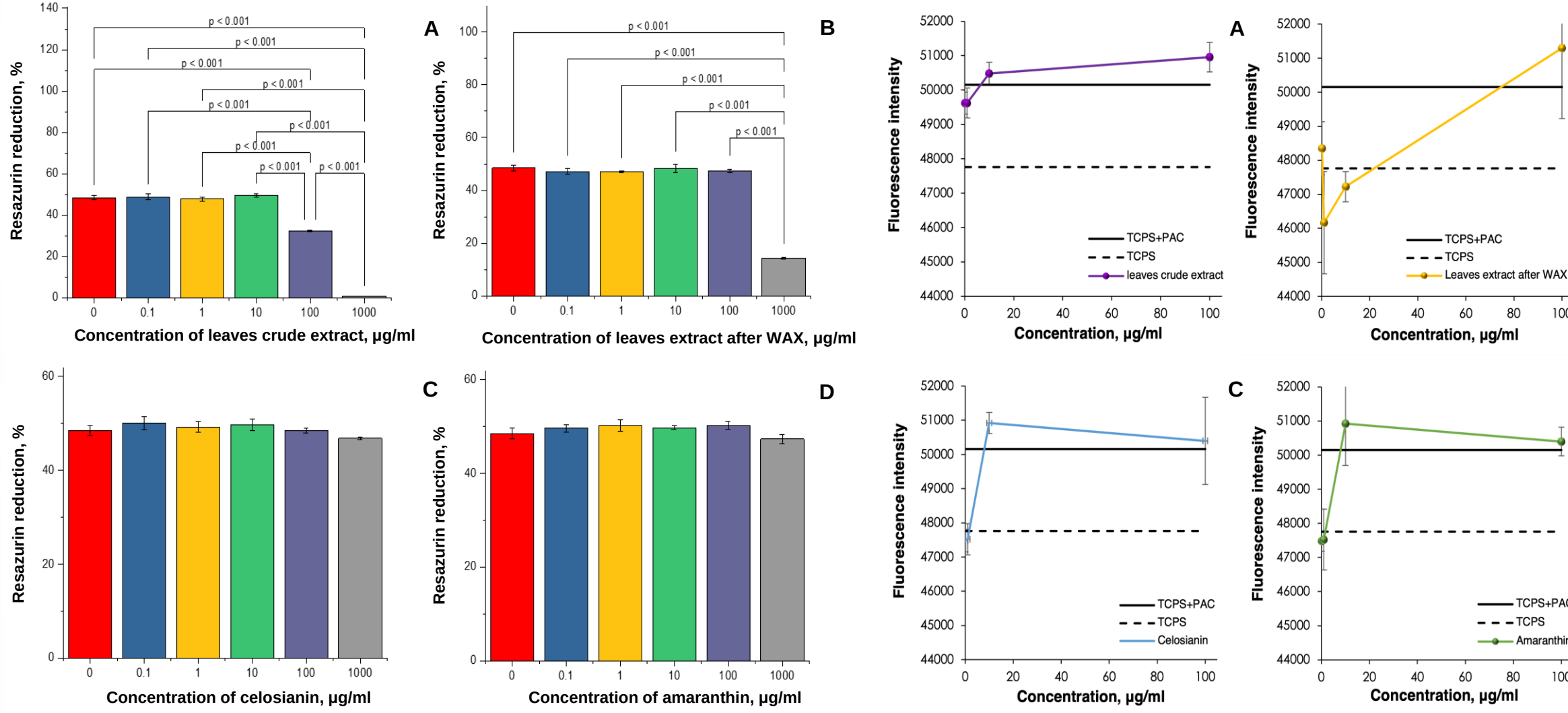
Important HMBC and NOESY NMR correlations in celosianin structure

Antioxidant activity measurements



TEAC values determined for *A. hortensis* var. *rubra* extracts, initially purified extracts, pure pigments and standard gallic acid under ABTS (A), FRAP (B), and ORAC (C) assays. Correlations between the TEAC values obtained in ABTS and FRAP (D), FRAP and ORAC (E), as well as ORAC and ABTS (F).

Cytotoxic and cardioprotective activity



Metabolic activity (A-D) of H9c2 cells incubated in the presence of leaves (A), extract initially purified on WAX (B), purified celosianin (C) and amaranthin (D)

Evaluation of the effectiveness of the leaves extract of *A. hortensis* var. *rubra* (A), leaves extract after WAX (B), celosianin (C), and amaranthin (D) in prevention of H9c2 cells from apoptosis

CONCLUSIONS

- Celosianin and amaranthin were the dominant pigments in *A. hortensis* var. *rubra* and the chemical structure of celosianin was confirmed by 1D and 2D NMR analysis for the first time.
- The presence of betalains in tested extracts was correlated with high antioxidant activity measured by ABTS, FRAP, and ORAC assays.
- Celosianin (IC₅₀ value of 21.5±1.85 µg/ml) was more potent scavenger than amaranthin (IC₅₀ value of 32±2.55 µg/ml).
- Resazurin assay shows that all samples do not induce cytotoxicity in a wide range of tested concentrations in rat cardiomyocytes.
- A. hortensis* var. *rubra* extracts and purified pigments (0.1 and 1 µg/ml) increase the resistance of H9c2 cells to factors causing apoptosis.