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University of Applied Sciences
Groningen

Dutch Delight – health opportunities of tulip pigments

Maaike Smelt, Hanze University of Applied Sciences, 21st of June 2022

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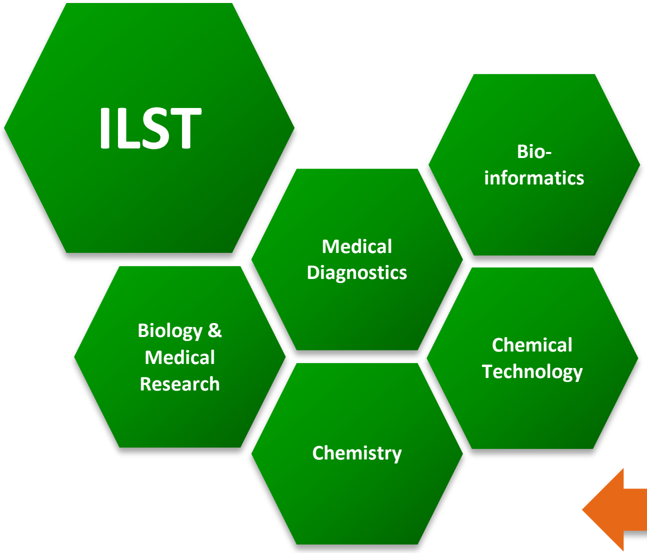
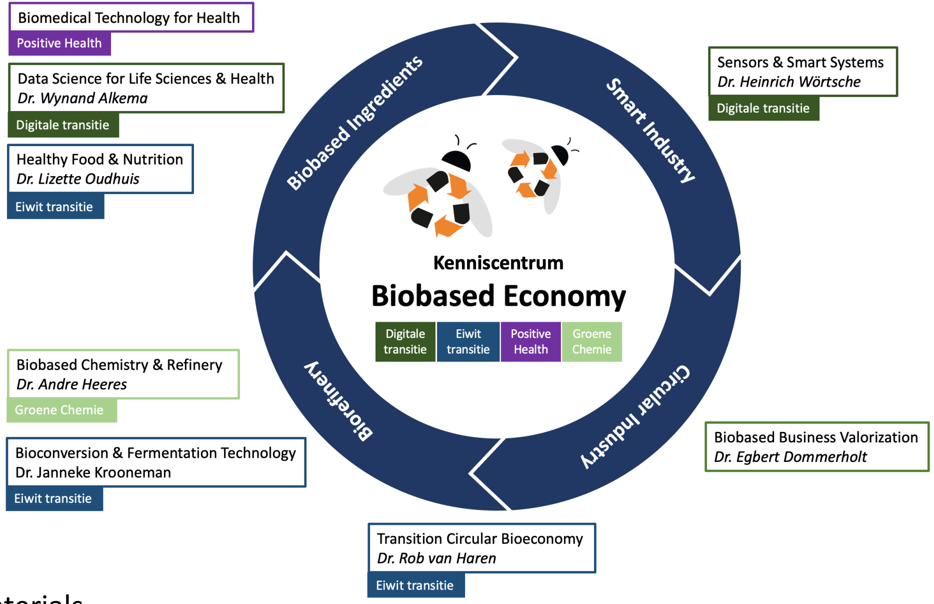
Knowledge Center Biobased Economy - Institute for Life Science & Technology
Hanze University of Applied Science Groningen





Biorefinery

- Conversion of biomass to bioactive (fine) chemicals and materials
- Potential use as building blocks, commodity chemicals, food, or pharmaceuticals

Lutein from Tagetes

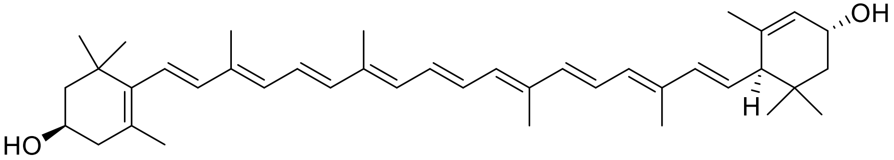
Agronomy Journal

Integrated Pest Management

Crop Rotation with *Tagetes* sp. is an Alternative to Chemical Fumigation for Control of Root-Lesion Nematodes

L.Bruce Reynolds, John W. Potter, Bonnie R. Ball-Coelho

- Biological pesticide
- Improves soil quality
- Insect population
- Source of luteine



- Pharma, food supplement, cosmetics, anti-oxidant

Project proces & status

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- Tagetes sp selection – plant breeding programme
- Mechanical Harvest (17ha)
- Drying & Milling (>5ktons)
- Extraction (from 50 to 500L)
- Hydrolysis & Purification – optimised
- Stabilisation & Modification

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HLB research and consultancy in agriculture

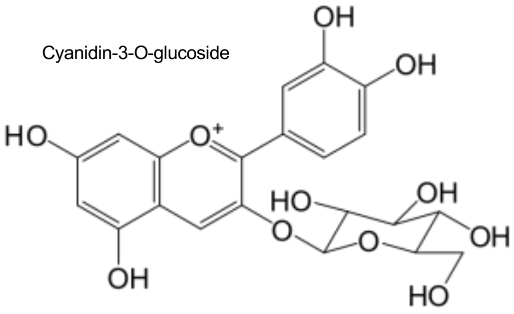
SYNCOM

Kompetenzzentrum Niedersachsen • Netzwerk Nachwachsende Rohstoffe und Bioökonomie e.V. 3N

New Businesses Agrifood

Anthocyanins

- Water-soluble pigments found in flowers and fruits
- Family of 15-carbon molecules containing phenolic rings and attached sugars
- Macromolecules stored in plant vacuoles, chloroplasts, and nucleus of epidermal cells in leaves, bark, flower petals or fruit skin



Anthocyanidin	R3	R5	R6	R7	R3'	R4'	R5'
Delphinidin	OH	OH	H	OH	OH	OH	OH
Cyanidin	OH	OH	H	OH	OH	OH	H
Petunidin	OH	OH	H	OH	OMe	OH	OH
Peonidin	OH	OH	H	OH	OMe	OH	H
Malvinidin	OH	OH	H	OH	OMe	OH	OMe
Pelargonidin	OH	OH	H	OH	H	OH	H

<u>Physiological function of the pigments</u>:
- Attraction of insect pollinators
- Protection by ROS scavenging
- In leaves: support photosynthesis? Gould *et al* 2000

<u>Many health benefits described/suggested</u>: anti-oxidant effects, anti-cardiovascular diseases, anti-inflammatory effects normalization of metabolic disorders

<u>Putative antimicrobial effects</u>

- Potential as antibiotic or natural preservative

Source	Extract & Concentration	Strains*	Effectivity	Ref.
Raspberry, Cranberry, Elderberry, Strawberry, Bilberry, Blueberry, Optiberry	Whole berry extract (0,25-1%)	HP	All berries inhibited growth at all concentrations. Optiberry, Blueberry and Bilberry most effective	9
Cranberry	Whole cranberry concentrate (0-7,5% w/w)	EC	Cranberry inhibits E. coli growth in ground meet and induced downregulation of membrane protein expression	10
Bilberry, Lingonberry, Cranberry, Red Raspberry, Cloudberry, Strawberry, Black currant, Sea buckthorn berry, Chokeberry, Highbush bilberry, Crowberry	Phenolic extract (1 mg/mL)	BC, CJ, CP, HP, SA, SE, CA	Antimicrobial action depends on berry and micro-organism. B. cereus growth was inhibited by all berry extracts, whereas cloudberry appeared to inhibit all tested micro-organisms	11
Cranberry	Anthocyanin extract (MIC value 14,80-29,15 mg/L)	EC	Cranberry anthocyanins effectively inhibit E. coli growth at low concentrations. Effect is independent on pH and anthocyanins appear to disrupt the outer bacterial membrane within 20 minutes.	12
Bilberry, Blueberry	Anthocyanin extract (50uL)	LM, SA, BS, EF, EC, PA, CF, ST, & yeast	Anthocyanin extracts each contained 5 different anthocyanins. All bacterial strains were … to anthocyanins depended on the … sitive and gram-negative strains.	13
Pomegranate		… SS, SFC	… ns demonstrated growth inhibition … be more sensitive.	14
Blueberry, Raspberry, Lingonberry, Blackcurrant, Cloudberry, Cranberry, Sea buckthornberry, Strawberry	Phenolic extract (0,8-7 mg/well), and cyanidin-3-glucoside (28 ug/well)	BL, EF, EC, LC, LJ, LP, LP, LR, LRS, SE	Cyanidin-3-glucoside inhibited only E coli. Berry phenolic extracts strongly inhibited growth of E. coli, S. enterica. Gram-positive lactobacilli were not inhibited.	15
Zelen, Sauvignon Blanc, Rebula, Cabernet Sauvignon, Merlot and Pinot Noir grapes	Whole phenolic extract in 1:1 H_2O:EtOH (MIC value 6.4g/L pinot noir against L. monocytogenes)	SA, EC, BC, LM, SI, CJ	Pinot Noir gave the highes yield of phenolics, including anthocyanins and the lowest MIC-value. Gram-positive strains appeared to be most sensitive to phenolic compounds than gram-negative strains.	16
Cloudberry	Phenolic extract (1mg/mL) (MIC-value to E. coli adhesion to red blood cells approx. 5 ug/mL)	SA, EC, PA, LRS, SC, CA	Strong inhibition of S. aureus growth, medium inhibition of E. coli and P. aeruginosa growth. S. cerevisiae, C. albicans, L. rhamnosus growth were not affected.	17
Blueberry	Anthocyanin extract (50-1000 ug/mL)	Multidrug resistant PA, EC, SA, PM, AB	Extract contained 7 different anthocyanin moieties. The mixture strongly inhibited S. aureus growth. In all strains either the lag-phase of growth was delayed or the final OD was moderately reduced. Biofilm production was inhibited in all strains.	18
Tulips	Phenolic and anthocyanin extracts	AH, BC, BS, ECL, EC, KP, LM, PV, PA, ST, SA, YE, SC, CA	Extracts were most effective against S. aureus, L. monocytogenes and Y. enterocolitica	19
Cranberry	Anthocyanin extract	LM, BC, BS, ML, EF, SA, EC, EA, ST, SAG	Extracts were most effective against B. cereus and M. luteus, but all tested strains demonstrated growth inhibition	20

Difficult to interpret due to use of crude (phenolic) extracts and lack of standardisation and resolution in microbial assays

Research aim

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Antimicrobial efficacy of anthocyanins from tulips

- **Optimization of anthocyanin extraction and fractionation at lab-scale**
- **Chemical characterization**
- **Determination of antimicrobial efficacy of crude extracts and single anthocyanin molecules**

Extraction and characterization procedure

Drying

Extraction

Concentration

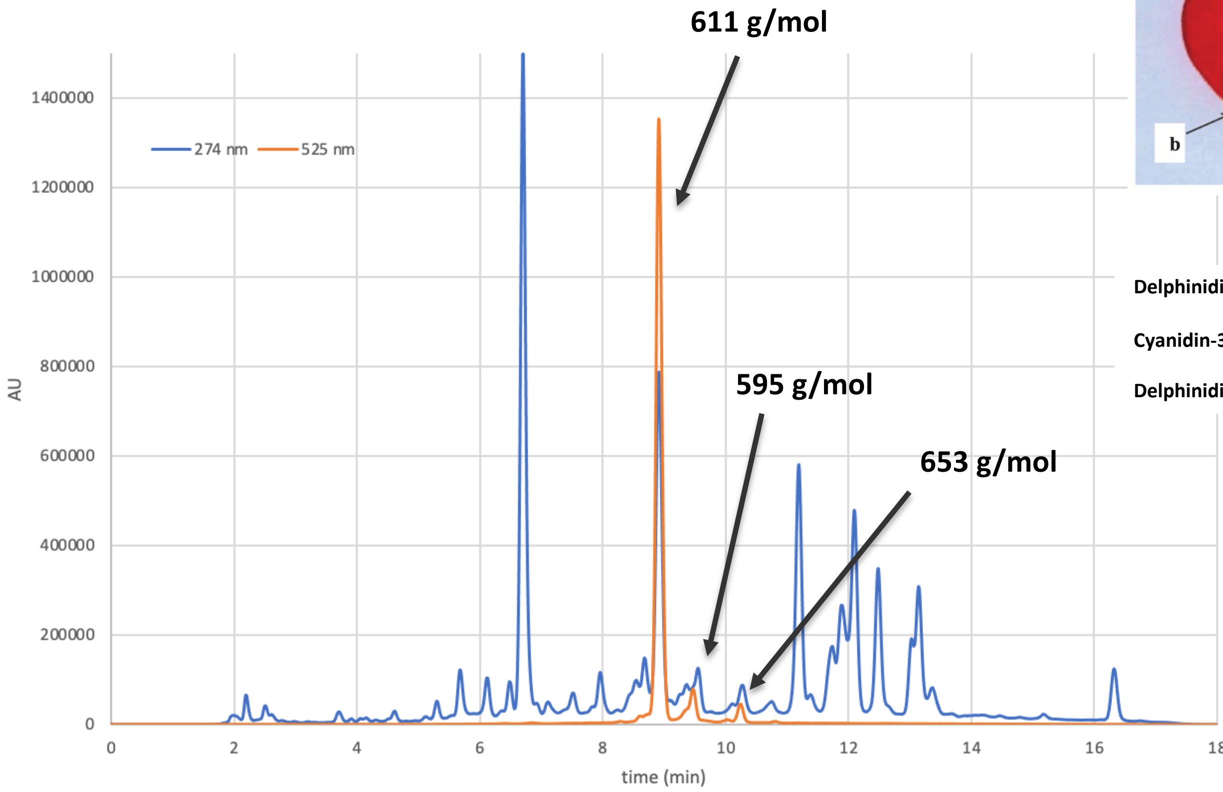
Crude anthocyanin extract

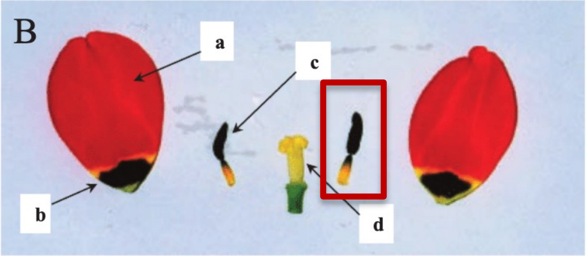
Isolation – preperative HPLC

ANALYSIS:

Liquid Chromatography - Mass Spectometry

Crude red tulip anther extract





Nakayama et al Japan Agricultural Research Quarterly · June 2004

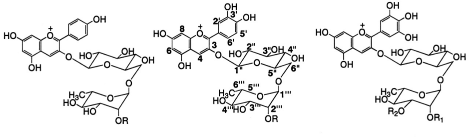
Delphinidin-3-O-rutinoside (611 g/mol)

Cyanidin-3-O-rutinoside (595 g/mol)

Delphinidin-3-O-(3'''-acetyl-rutinoside) (653 g/mol)

Table 1. Analytical data of anthocyanin 1–7

	Anthocyanins	M^+	λ max (nm)	ODS-HPLC Rt (min)
1	Pelargonidin 3-rutinoside	579	505, 430, 280, 265	20.8
2	Pelargonidin 3-(2'''-acetylrutinoside)	621	505, 430, 280, 265	27.0
3	Cyanidin 3-rutinoside	595	520, 280	18.4
4	Cyanidin 3-(2'''-acetylrutinoside)	637	520, 280	24.3
5	Delphinidin 3-rutinoside	611	525, 275	16.1
6	Delphinidin 3-(2'''-acetylrutinoside)	653	525, 275	21.6
7	Delphinidin 3-(3'''-acetylrutinoside)	653	525, 275	17.7



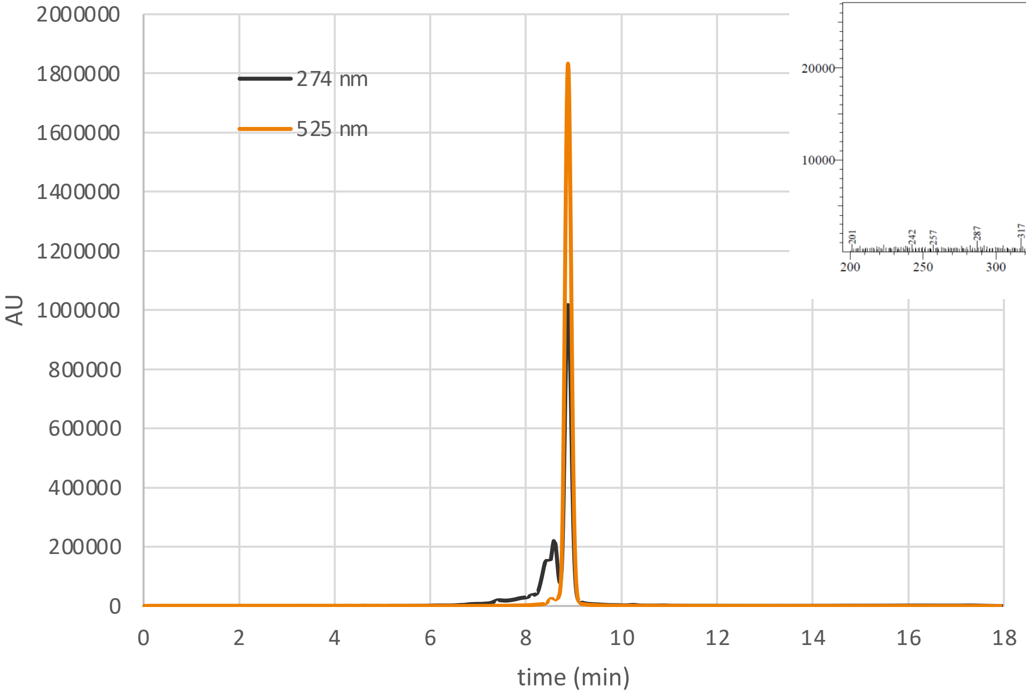
(1) R = H
(2) R = CH_3CO

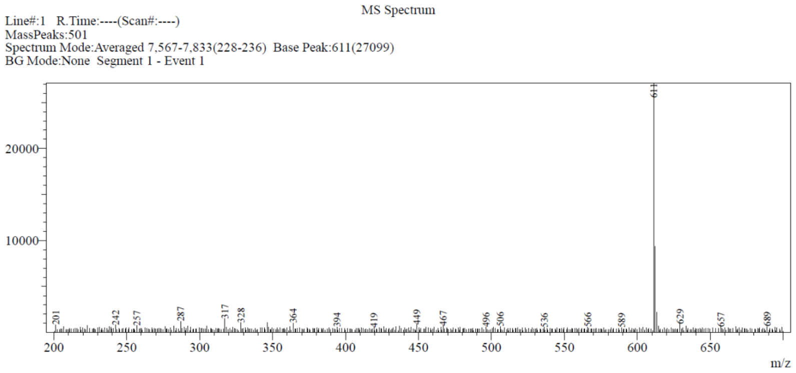
(3) R = H
(4) R = CH_3CO

(5) R_1 = H, R_2 = H
(6) R_1 = CH_3CO, R_2 = H
(7) R_1 = H, R_2 = CH_3CO

Fig. 1. Structures of anthocyanin 1–7

Isolation of Delphinidin-3-O-Rutinoside from tulip anthers





Tr: 8,9 min

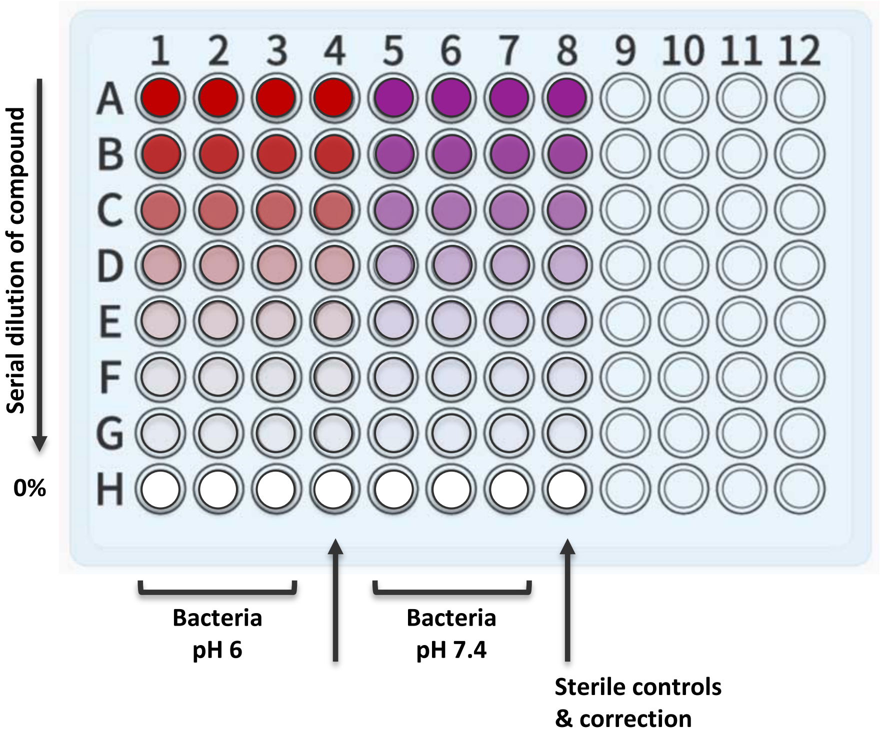
Mass: 611 g/mol

NMR in progress

Small impurity

Presence of D3R confirmed with standard

Determination of bio-activity



Bacterial species:

- *S. Aureus* ATCC259323
- *E. Coli* ATCC25922
- *K. Pneumoniae* ATCC13883

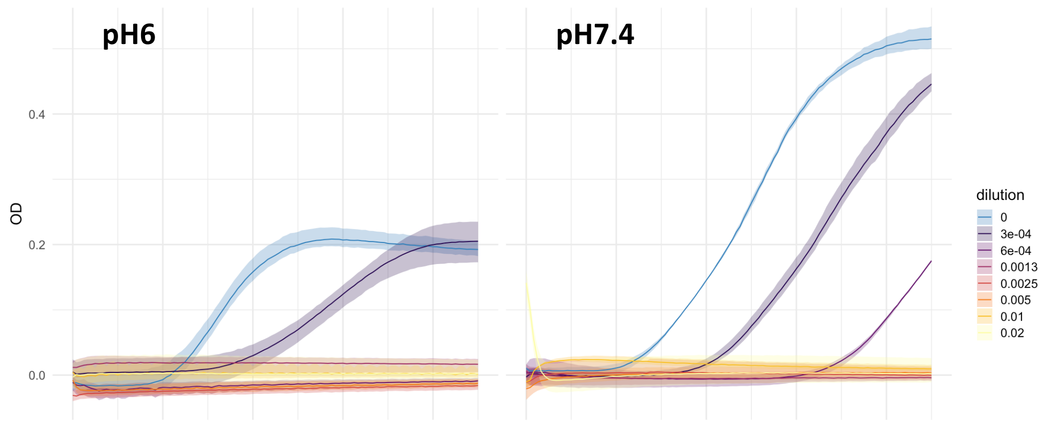
Procedure:

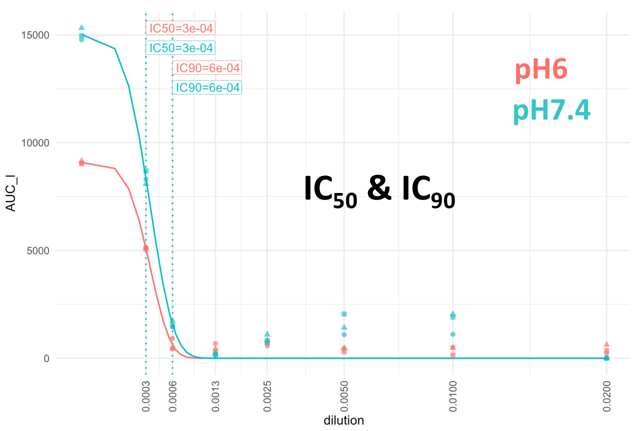
- Growth – 18 hrs, 37ºC
- Optical Density – 630nm every 10min

Analysis:

- R-package ’GrowThis’
- Visualisation,
- Growth curve analysis based on ‘Growthcurver’
- Concentration-response analysis

GROWTHIS
AN R PACKAGE FOR ANALYSIS AND VISUALIZATION OF VARIOSKAN DATA





Growth curve analysis:

- Initial population size (N_0)
- Maximum possible population size (k)
- Intrinsic growth rate (r)
- Generation Time (T_gen)
- Area Under Curve (AUC)

Minimal Inhibitory Concentration (MIC)

Minimal Bacteriocidal Concentration (MBC)

Tulip anthocyanins demonstrate selective and pH-dependent antimicrobial efficacy

S. aureus	MIC	IC_{50}	IC_{90}
Crude anther extract pH6 (*N=2*)	18 µg/mL	7,5±2,12 µg/mL	15±4,24 µg/mL
Crude anther extract pH7.4 (*N=2*)	36 µg/mL	10,5±2,12 µg/mL	19,5±2,12 µg/mL

K. pneumoniae	MIC	IC_{50}	IC_{90}
Crude anther extract pH6 (*N=4*)	150 µg/mL	61,5±3,87 µg/mL	81±4,9 µg/mL
Crude anther extract pH7.4 (*N=2*)	75 µg/mL	29,25±1,5 µg/mL	41,25±10,8 µg/mL

E. coli	MIC	IC_{50}	IC_{90}
Crude anther extract pH6 (*N=2*)	36 µg/mL	21 µg/mL	31,5±6,36 µg/mL
Crude anther extract pH7.4 (*N=2*)	75 µg/mL	40,5±2,12 µg/mL	36 µg/mL

Tulip anthocyanins demonstrate selective and pH-dependent antimicrobial efficacy

S. aureus	MIC	IC_{50}	IC_{90}
Crude anther extract pH6 (*N=2*)	18 µg/mL	7,5±2,12 µg/mL	15±4,24 µg/mL
Crude anther extract pH7.4 (*N=2*)	36 µg/mL	10,5±2,12 µg/mL	19,5±2,12 µg/mL
D3R fraction pH6 (*N=1*)	150 µg/mL	222 µg/mL	381 µg/mL
D3R fraction pH7.4 (*N=1*)	75 µg/mL	42 µg/mL	78 µg/mL
D3R pH6 (*N=1*)	300 µg/mL	195 µg/mL	297 µg/mL
D3R pH7.4 (*N=1*)	36 µg/mL	*To be determined*	*To be determined*

K. pneumoniae	MIC	IC_{50}	IC_{90}
Crude anther extract pH6 (*N=4*)	150 µg/mL	61,5±3,87 µg/mL	81±4,9 µg/mL
Crude anther extract pH7.4 (*N=2*)	75 µg/mL	29,25±1,5 µg/mL	41,25±10,8 µg/mL
D3R fraction pH6 (*N=1*)	300 µg/mL	210 µg/mL	291 µg/mL
D3R fraction pH7.4 (*N=1*)	75 µg/mL	48 µg/mL	69 µg/mL
D3R pH6 (*N=1*)	No inhibition	No inhibition	No inhibition
D3R pH7.4 (*N=1*)	75 µg/mL	57 µg/mL	72 µg/mL

E. coli	MIC	IC_{50}	IC_{90}
Crude anther extract pH6 (*N=2*)	36 µg/mL	21 µg/mL	31,5±6,36 µg/mL
Crude anther extract pH7.4 (*N=2*)	75 µg/mL	40,5±2,12 µg/mL	36 µg/mL

D3R and D3R fraction -> no inhibition

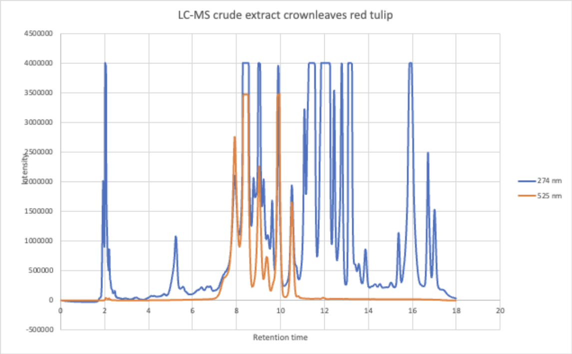
Conclusions and future perspectives

- **D3R demonstrates antimicrobial efficacy against *S. aureus* and *K. pneumoniae*, but not against *E. coli***
- **Efficacy appears to be pH dependent**

- Characterisation and isolation of anthocyanins in tulip leaves
- Determine efficacy of crude extracts and individual components
- Determine efficacy of anthocyanin-combinations

- Insight in structure-function relationship
- Insight in synergistic/ antagonistic effects

- Membrane stress responses in *S. aureus*



Acknowledgements

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