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RUTGERS

Antimicrobial phytochemicals from edible and medicinal plants

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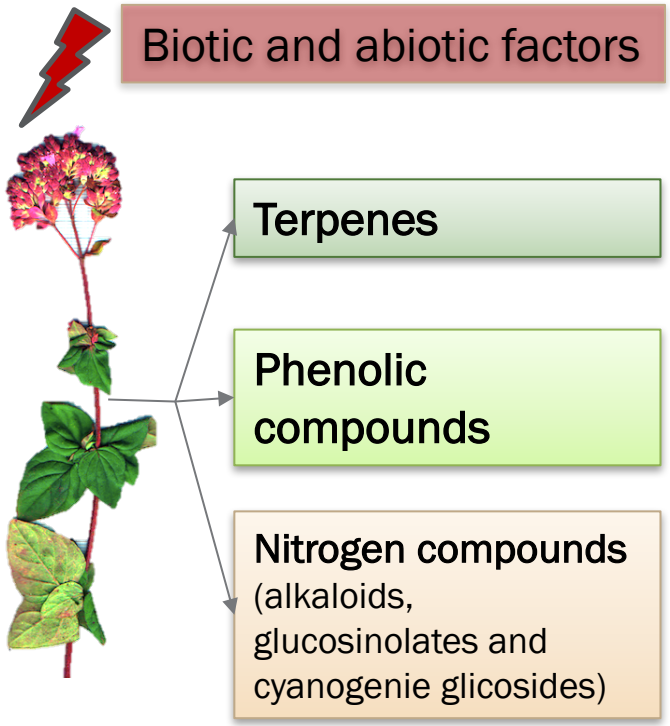
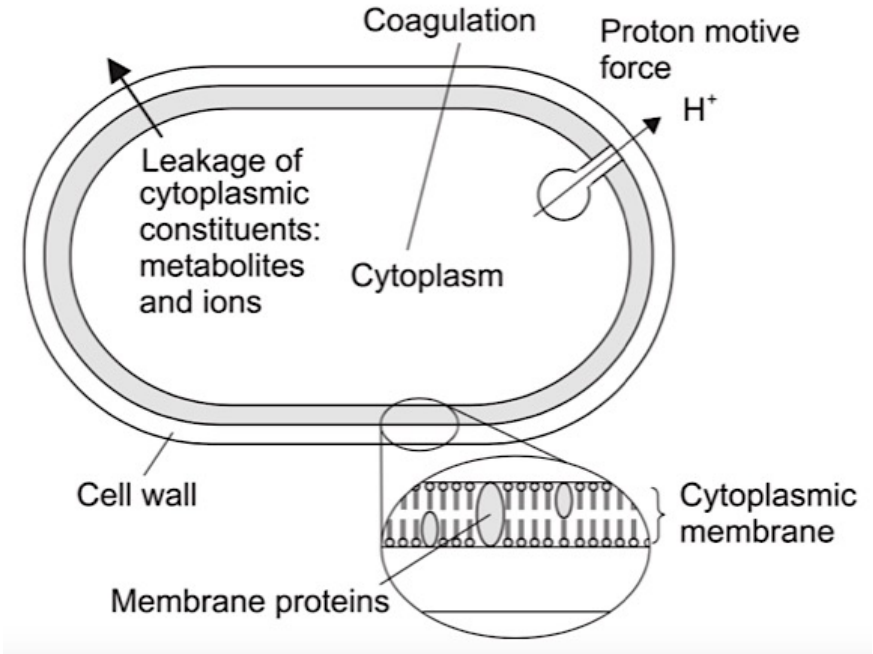
Plant secondary metabolites

Each plant species has unique profile of secondary metabolites and use them to:

- Attract pollinators or symbiotic organisms,
- protect against pests, diseases, and abiotic stresses
- acquire, transport and store nutrients



Mechanisms of action in the bacterial cell through the different plant components

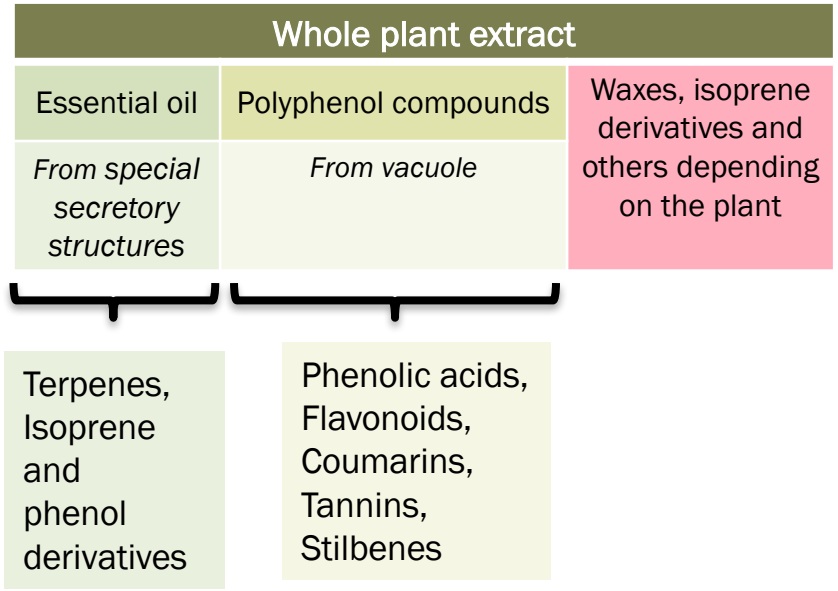


Picture from "Essential oils: their antibacterial properties and potential applications in foods" S. Burt / International Journal of Food Microbiology 94 (2004)

1. degradation of the cell wall
2. damage to cytoplasmic membrane
3. damage to membrane proteins
4. leakage of cell contents
5. coagulation of cytoplasm
6. depletion of the proton motive force

Goal

Study antimicrobial properties of edible and medicinal plants with high antimicrobial activity against bacterial and fungal pathogens.

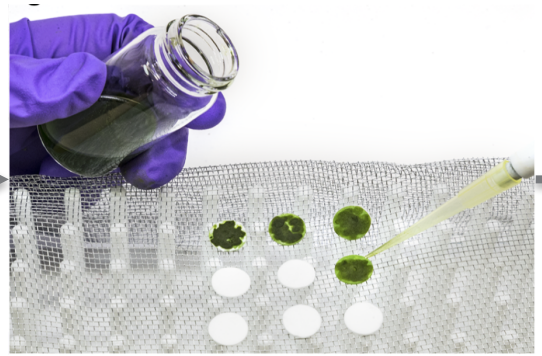


Methods

1. Screening of plant extracts for antibacterial and antifungal action:
   a) extraction of secondary metabolites from plant materials;
   b) conducting STN antimicrobial assays;
   c) measuring Minimum Inhibition Concentration (MIC);
   d) studying the influence of plant extract interactions on antimicrobial activity.
2. Determining extract component composition by UHPLC-MS.
3. Testing antimicrobial activity of plant extracts against *E. coli, B. subtilis* and *S. epidermidis* for plants screened with STN assays.

Using novel approaches

- In extraction with new RAMES (RApid Metabolome Extraction and Storage) technique.
- In measuring whole extract antimicrobial activity for each plant and for combinations of extracts in order to find synergism effect using GIBEX (Global Institute of Bioexploration) STN (Screen To Nature) assays.



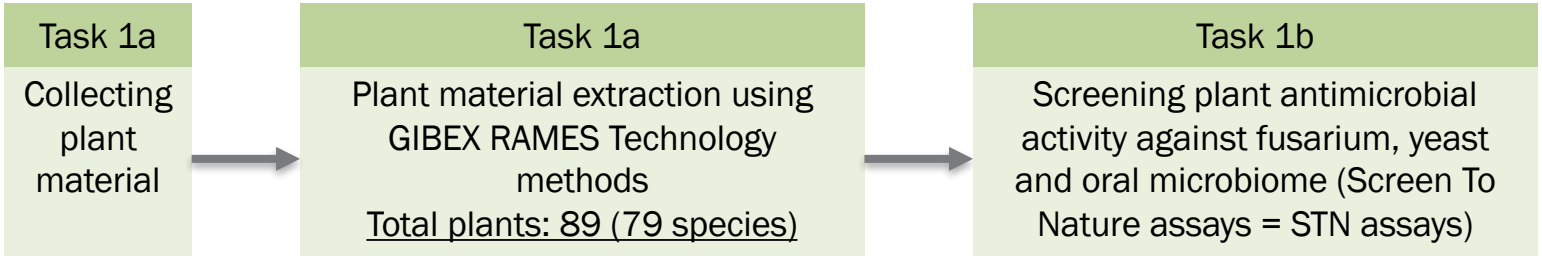
0.2g of dried tissue in 5ml 70% ethanol
Or 2 g of raw tissue in 5ml 95% ethanol

100 µl of extract per **glass fiber disc**

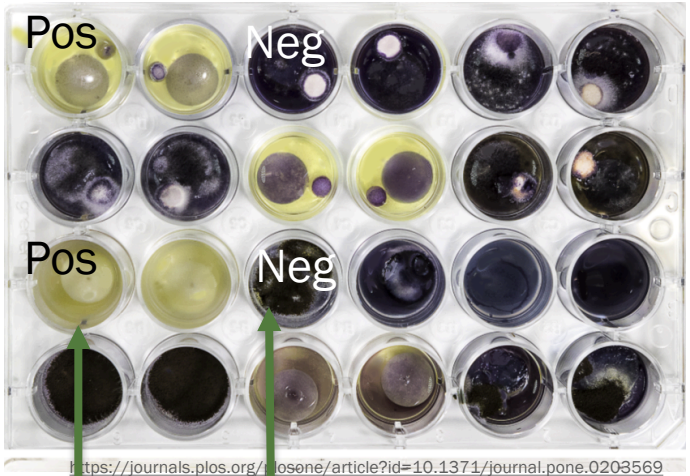
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0203569

RAMES method advantage

- Faster than regular extraction and compatible with most screens.
- Less component degradation during extraction.
- Greater stability during the storage compared to non-sorbed extracts.



STN assay with MTT



Positive control - disc with 100µl of

100x Spectinomycin – for bacteria
300 mg/ml Econazole nitrate – for fungi

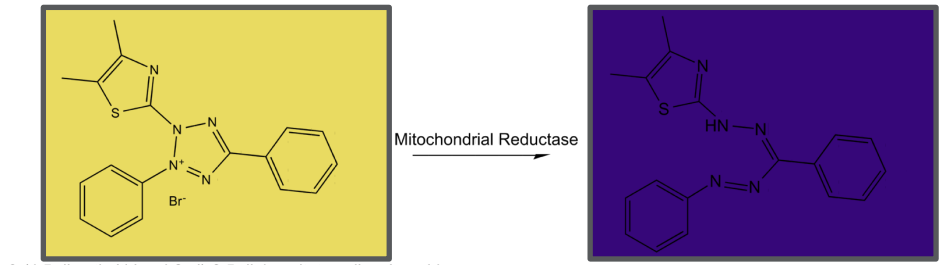
Plants with high antimicrobial activity

Task 1c

Measuring MIC with improved GIBEX STN assays

Task 3

Making UHPLC-MS



MTT

Formazan

https://en.wikipedia.org/wiki/MTT_assay

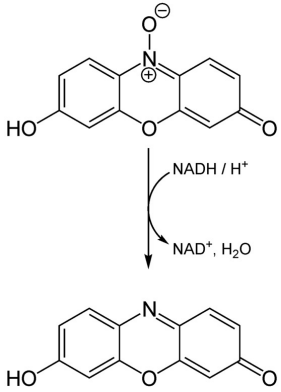
MIC determination with Resazurin

Task 1c

Measuring MIC with improved STN assays

Task 2

Identifying potential synergetic combinations



Resazurin

Rezorufin

https://en.wikipedia.org/wiki/Resazurin

MIC for the best samples

Sample number	Common name	*Scientific name*	First result			Average results for dilution								
						AFA			AYA			ABA		
			AFA	AYA	ABA	1x	2x	4x	1x	2x	4x	1x	2x	4x
00002	Boldo	*Peumus boldus*	0	3	0				3	3	2			
00004	Hops	*Humulus lupulus*	3	3	3	3	3	3	3	3	3	3	3	2
00005	Ginko	*Ginkgo Biloba*	0	3	2				3	3	3			
00006	Chamomile	*Matricaria recutita*	0	3	3				3	2	1	3	3	2
00011	Artichoke	*Cynara scolymus*	0	2	3							3	2	1
00013	Licorice	*Glycyrrhiza glabra*	2	3	2				3	3	2			
00014	Eucaliptus	*Eucaliptus globulus*	0	3	3				3	3	3	3	3	2
00024	Calamus	*Acorus calamus*	3	1	0	3	2	2						
00028	Calendula	*Calendula officinalis*	0	3	0				3	2	1			
00037	Elecampane	*Inula helenium*	2	3	0				3	1	0			
00041	Rosemary	*Rosmarinus officinalis*	0	3	1				3	3	3			
00042	Birch	*Betula pendula*	1	3	1				3	3	3			
00043	Cranberry	*Vaccinium macrocarpon*	2	3	3				3	2	1	3	2	0
00044	Pomegranate	*Punica granatum*	3	3	3	3	3	3	3	3	3	3	2	2
00046	Sage (ada tea)	*Salvia officinalis*	0	3	1				3	2	1			
00047	Pomegranate	*Punica granatum*	3	3	3	3	2	1	3	3	3	3	2	1
00067	Juniper berries	*Juniperus communis*	2	3	0				3	3	2			
00071	Lantana camara	*Lantana camara*	0	3	1				3	3	0			
00074	Upland cress	*Barbarea verna*	3	0	1	3	2	2						
00079	Cedar	*Pinus sibirica*	2	3	1				3	3	3			
00080	Pine tree	*Pinus palustris*	2	3	1				3	3	3			
00081	Labrador	*Ledum groenlandicum*	2	3	1				3	3	2			
00083	Lucuma	*Pouteria lucuma*	3	2	2	3	3	3						

12 samples with high antimicrobial activity

Task 3

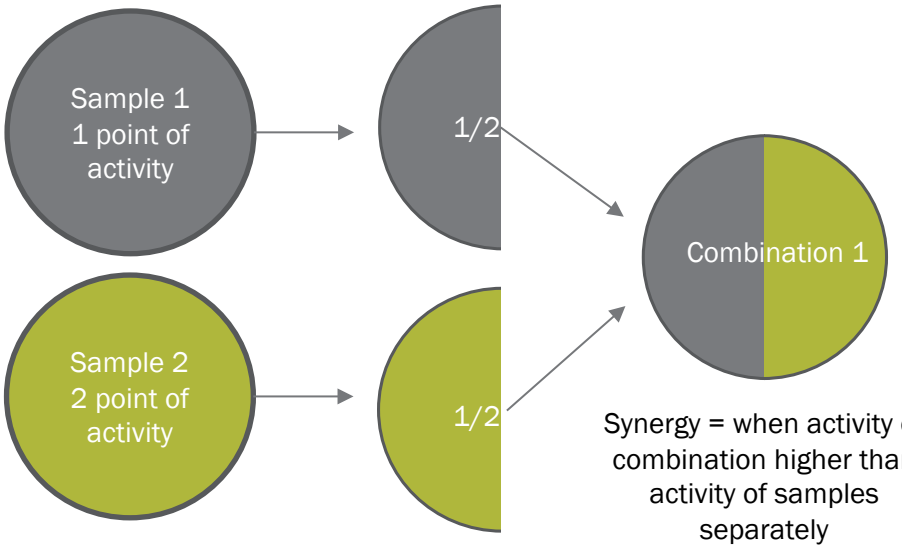
Making UHPLC-MS profile

Antimicrobial activity

0 1 2 3

AFA – anti-fusarium assay
AYA – anti-yeast assay
ABA – anti-bacterial assay

Synergy testing in ABA assay



12 plant extracts

80 combinations

3 synergy combinations associated with 5 plant extracts

Combination #	Combination of 2x diluted extract	Antimicrobial activity separately	
		Score for sample 1	Score for sample 2
1	3 points	1	0
2	3 points	1	1
3	2 points	1	1

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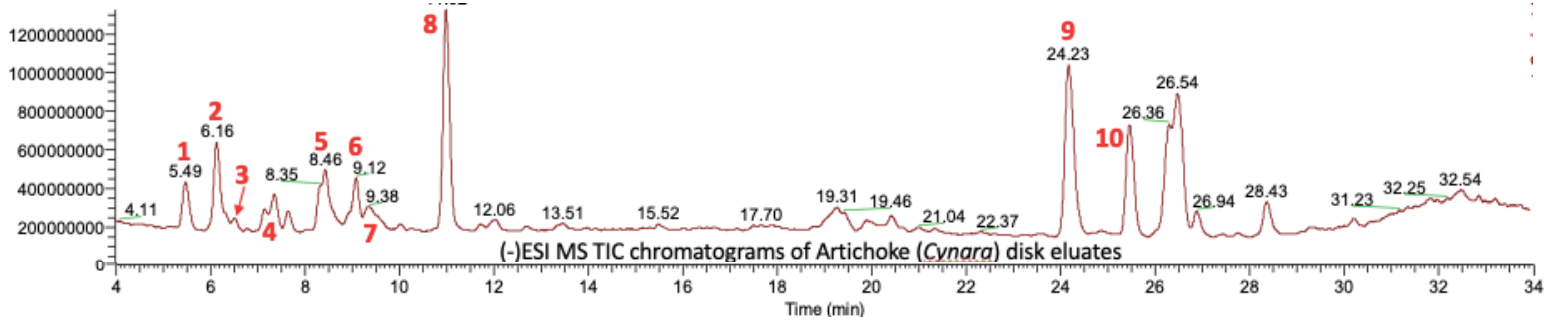
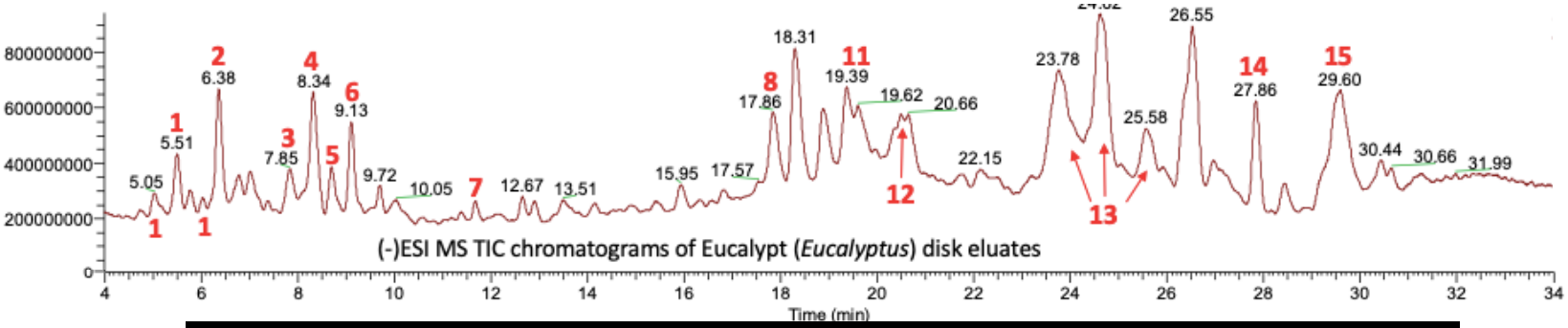
UHPLC-MS for Artichoke



Peak(s) No.	Mol. formula	Putative compound identification
1	$C_{16}H_{18}O_9$	Chlorogenate
2	$C_{21}H_{30}O_9$	Cynarascoloside C (a Sesquiterpene lactone glycoside)
3	$C_{21}H_{32}O_9$	Cynarascoloside A
4	$C_{21}H_{30}O_9$ $C_{15}H_{22}O_5$	Mix of same as 2 and Cynaratriol
5	$C_{15}H_{20}O_5$ $C_{21}H_{20}O_{11}$	Mix of Luteolin-glycoside and a Sesquiterpene lactone
6	$C_{25}H_{24}O_{12}$	Dicaffeoyl quinic acid
7	$C_{24}H_{22}O_{14}$	Either Kaempferol-, or Luteolin malonyl glycoside (not reported from ***A***.)
8	$C_{19}H_{22}O_6$	Cynaropicrin
9	$C_{18}H_{30}O_2$	Linolenate (not reported from ***A***.)
10	$C_{18}H_{32}O_2$	Linoleate (not reported from ***A***.)

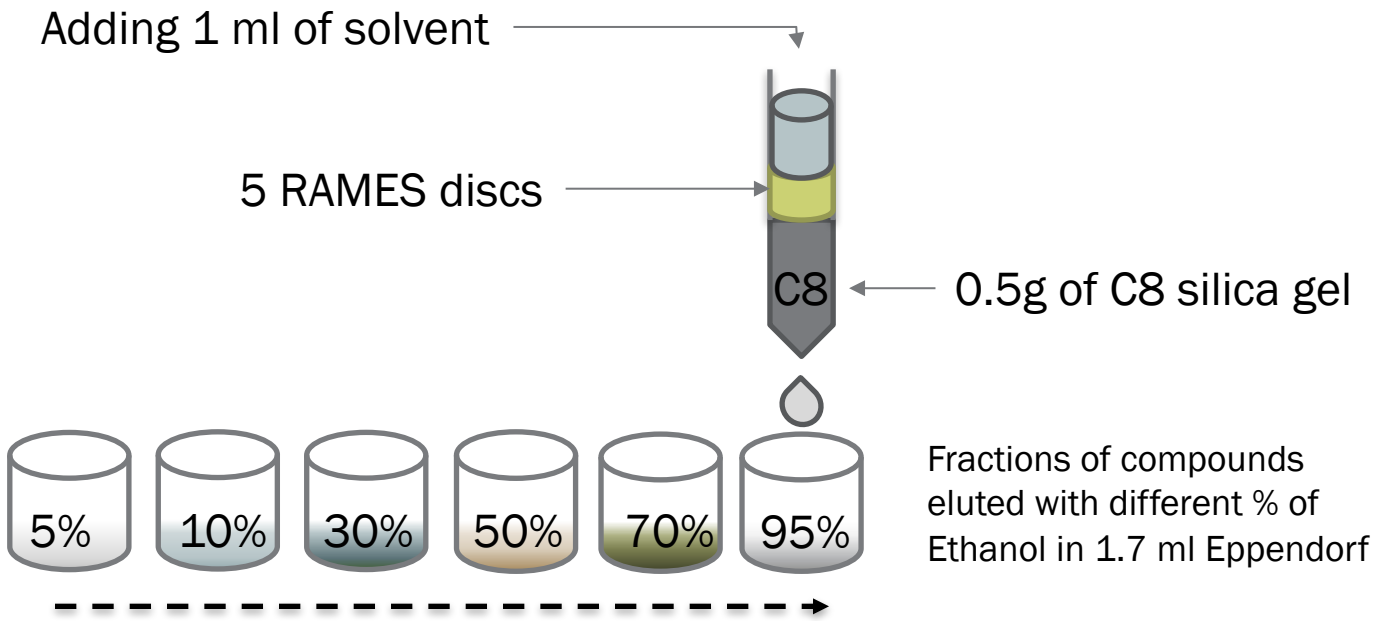
UHPLC-MS for Eucaliptus



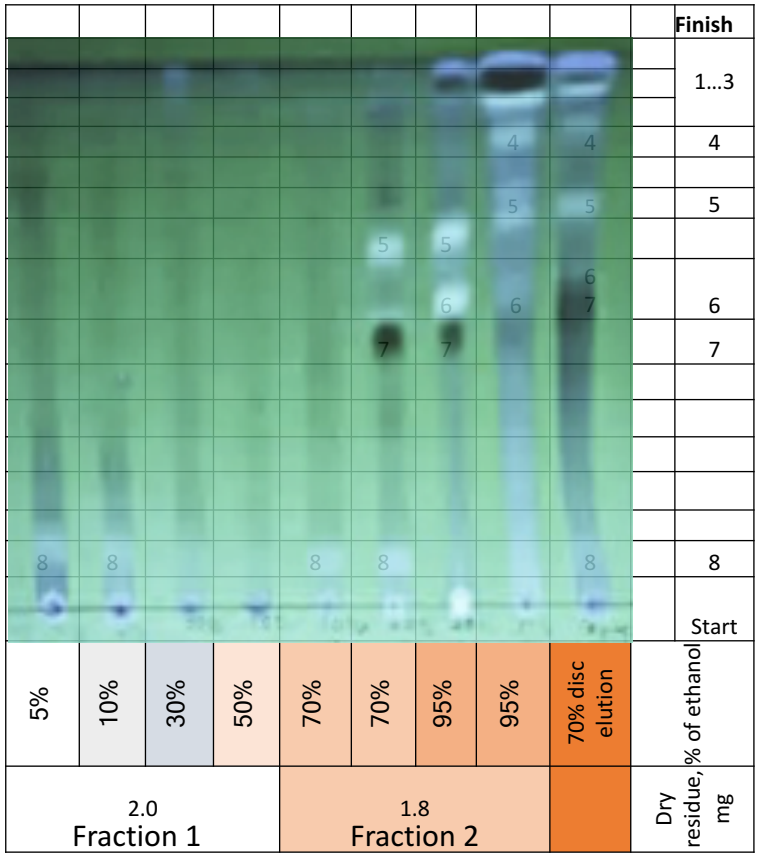
Peak(s) No.	Mol. formula	Putative compound identification
1	$C_{16}H_{18}O_9$	Chlorogenates
2	$C_{19}H_{30}O_8$	Reoseoside
3	$C_{26}H_{34}O_{12}$	Eucalmaidin derivative
4	$C_{21}H_{18}O_{13}$	Quercetin glycoside
5	$C_{19}H_{24}O_9$	An isobutylchromone derivative
6	$C_{21}H_{18}O_{12}$	Kaempferol glucuronide (or Me-Ellagic acid rhamnoside)
7	$C_{26}H_{32}O_{11}$	Eucalmaidin derivative
8	$C_{17}H_{14}O_5$	A dihydroxy-methoxy-methylflavanone
9	$C_{18}H_{16}O_5$	Dihydroxy-MeO-diMe-flavanone, or Hydroxy-Me-DiMeO-flavone
10, 11, 12	$C_{28}H_{42}O_7$	Macrocarpal I, or Macrocarpal J
13	$C_{28}H_{40}O_6$	23 compounds reported from ***E.***; macrocarpals and sesquiterpenes
14	$C_{25}H_{36}O_2$	A carotenoid? (Not reported from ***E.***)
15	$C_{28}H_{38}O_5$	32 compds reported from E.; macrocarpals, sesquiterpenes and others

Additional task

- Applying C8 column for plant extracts separation in case of searching fractions with antimicrobial active compounds



TLC for Hops fractions



Mobile phase

Solvent	Proportion
Hexane	60
Ethyl acetate	38
Acetic acid	2

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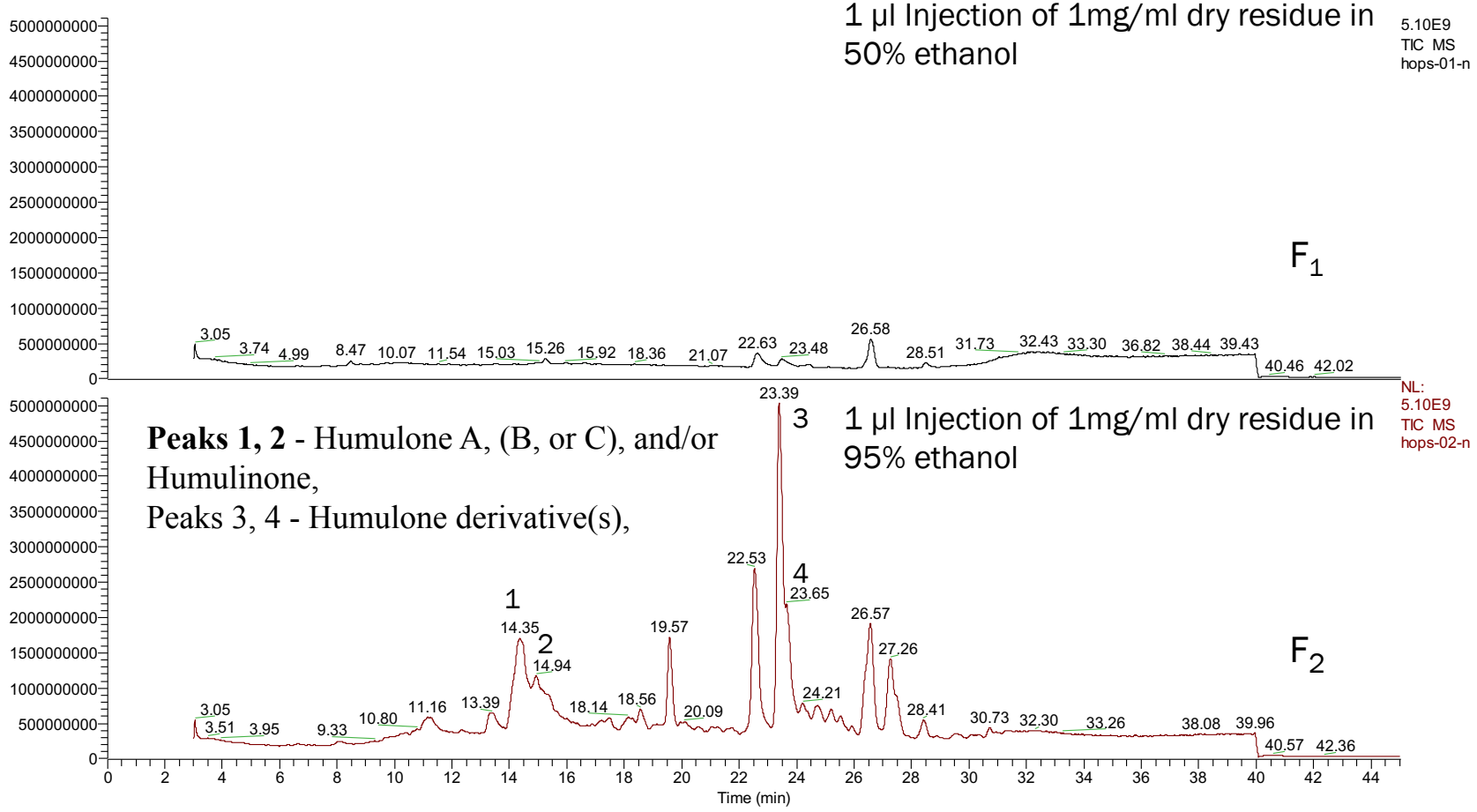
Hops C8 separation (-)ESI MS TIC chromatograms



Ability of the best samples to inhibit bacterial metabolic activity

Sample number	% of inhibition of bacterial metabolic activity for 6 mg of extracted plant tissue per ml of LB		
	E. coli	*B. subtilis*	*S. epidermidis*
1	90	99	99
2	75	99	99
3	50	99	99
4	50	99	90
5	75	99	99
6	50	99	50
7	50	99	50
8	75	90	90
9	90	99	99
10	90	99	99

Conclusions

- Extracts of seventy nine plant species were prepared and screened by STN antimicrobial assays;
- MIC values were determined for individual plant extracts and their combinations;
- Components of twelve plant extracts with antimicrobial activities were analyzed with UHPLC-MS;
- A method for pre-fractionation of plant extracts was developed and used to analyze four plant extracts;
- Three plant extracts with high inhibitory activity against *E. coli*, *B. subtilis*, and *S. epidermidis* were found;
- Three synergistic plant extracts combinations were discovered in a screen for inhibition of oral microbiota growth. These combination can be potentially used to create new food preservatives.


Thank you
for attention!

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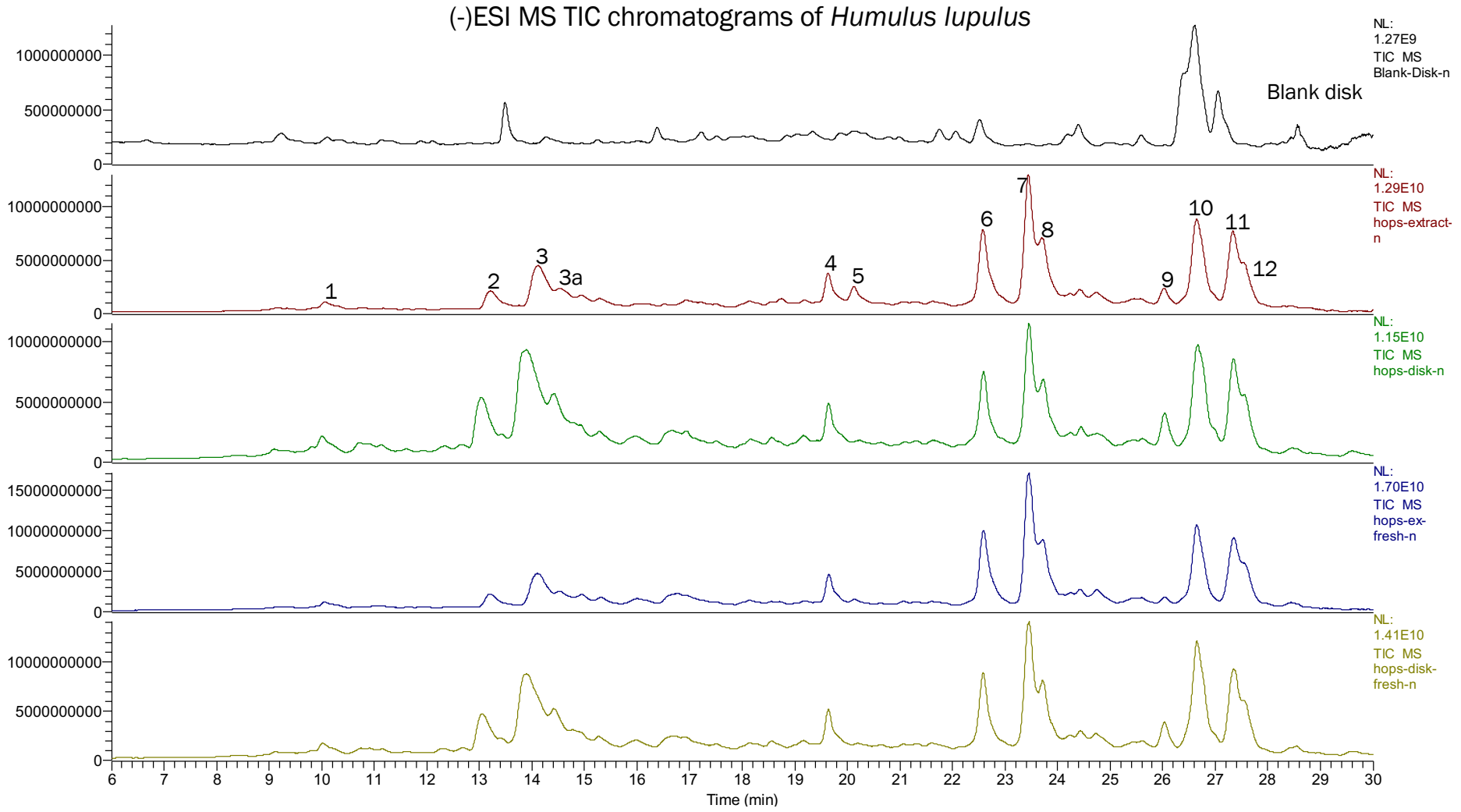

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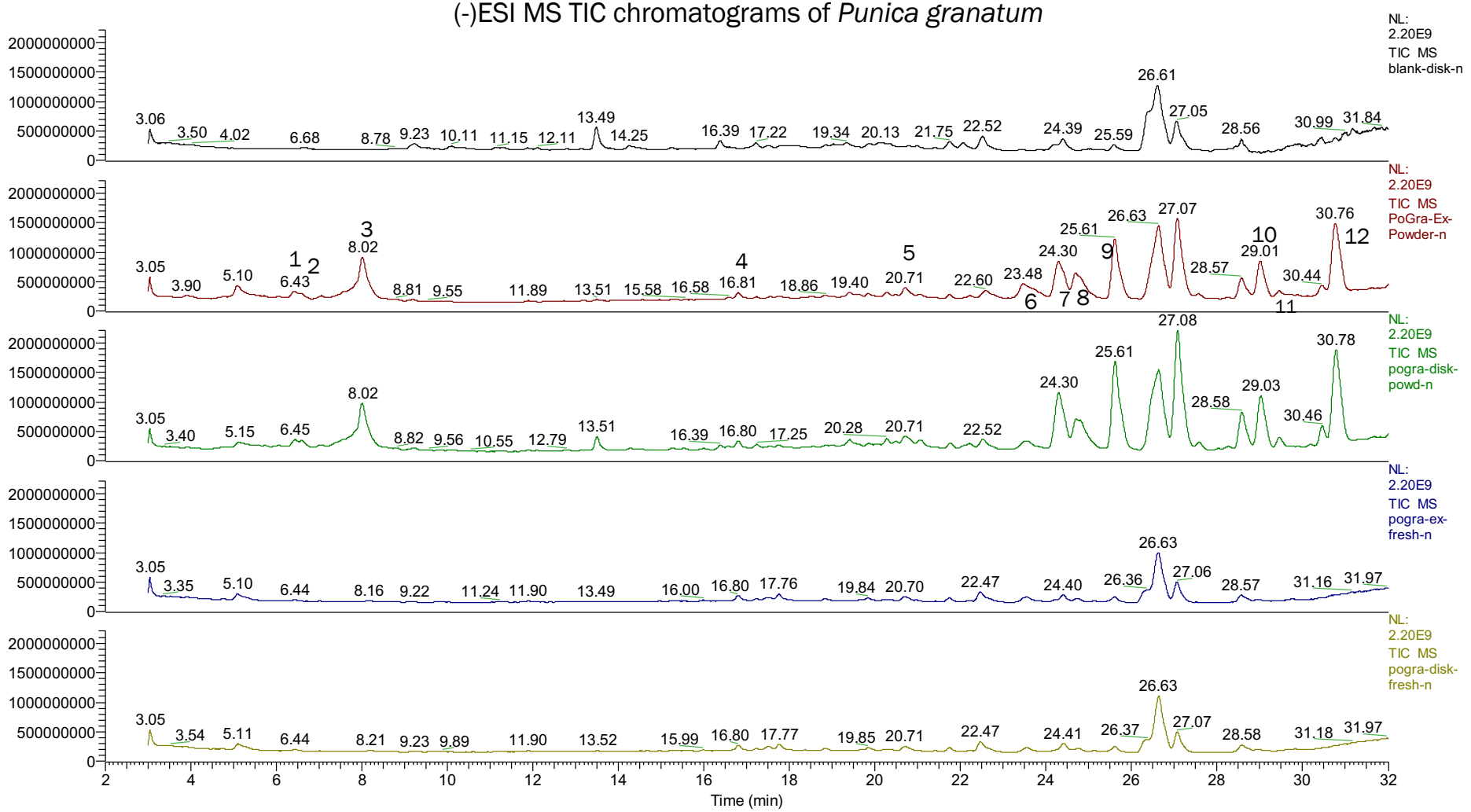

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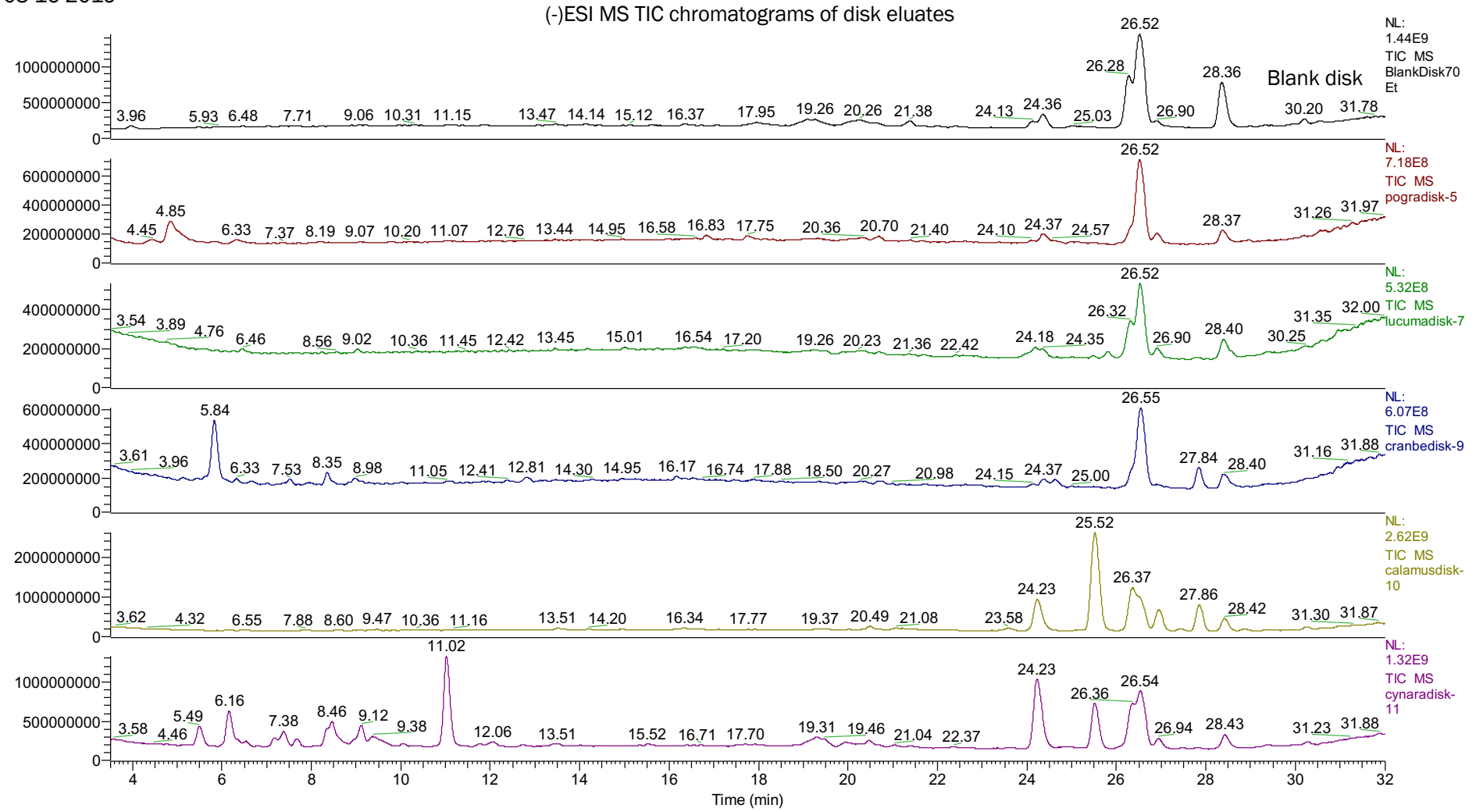
03-16-2019



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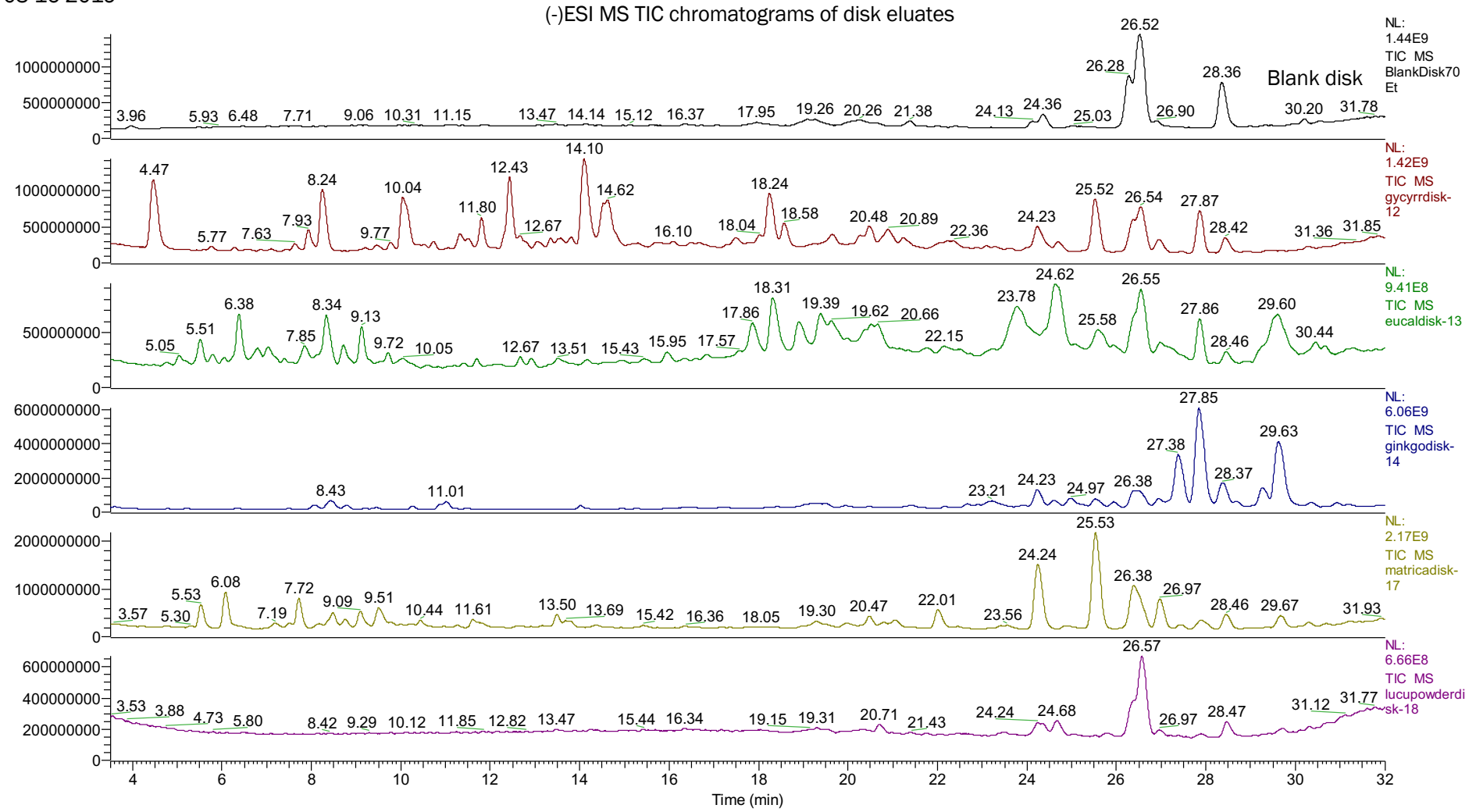
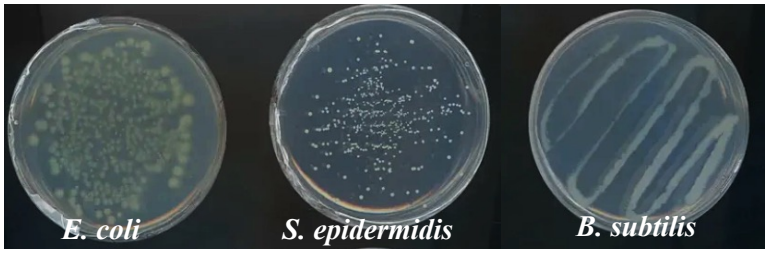


Method development



Making control plate with dilution from 1 to 0.0625 *10^7 CFU/ml

130 µl media with culture (1*10^7 CFU/ml)

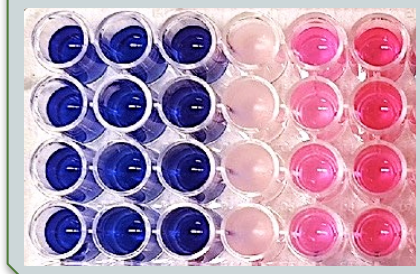
+

20 µl of extract to final concentration of dry extract 1 mg/ml

1) 18h incubation under +25*C

Adding 15 µl of Resazurin (3 mg/ml)

2) 2.5-4 hours Incubation under +37*C and 100 rpm



1 / 0.5 / 0.25/...–>

Reading fluorescence

(530-570nm excitation and 585-590nm emission)

https://www.google.ru/url?sa=i&source=images&cd=&cad=rja&uact=8&ved=2ahUKEwjpoPPyo0hAhWkOAKHYs_BGcQjB16BAgBEAQ&url=https%3A%2F%2Fwww.azerscientific.com%2F96-well-plate-ps-u-bottom-clear-non-sterile-300-l&psig=AOvVaw0H6_Z_g4Fb7QCZ48naroWy&ust=1556079346162318

Next steps

- Purify extract used for synergy combinations;
- Find synergy combinations between purified fractions;
- Check synergy combinations against pathogenic microorganisms;