SCREENING FOR NEW NATURAL FOOD ADDITIVES WITH ANTIBACTERIAL PROPERTIES AGAINST PATHOGENIC BACTERIA

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- Due to increasing customer awareness, there is a trend to seek new natural food additives that can be used as antibacterial and/or antioxidative agents in food products [1]
- Plant-based extracts consist of compounds that can possess antibacterial properties and may extend the shelf-life and enhance food safety [2]

Aim

- Find potential natural antimicrobials for food industry by testing antimicrobial properties of plant extracts against pathogenic bacteria

Conclusions

- Rhubarb root had the most broad-spectrum antimicrobial activity (Fig 1, Table 1), especially the extract prepared with 80% ethanol
- Rosemary and meadowsweet 80% ethanol extracts were the second and third most potent extracts (Table 1)
- Results clearly show that roots of rhubarb and extracts prepared with 80% ethanol may have the potential use in the food industry
- Other plant materials did not have antibacterial activity in the preliminary screening

- Collected from Estonia or commercial products (garlic and rosemary)
- Oven or freeze-dried
- Milled to ø 0.25-1.00 m

PLANT MATERIAL

Black currant (*Ribes nigrum* L.) fruit
Garlic (*Allium sativum* L.) bulb
Goutweed (*Aegopodium podagraria* L.) leaves
Meadowsweet (*Filipendula ulmaria* Maxim.) leaves
Rosemary (*Salvia rosmarinus* Spenn.) leaves
Rhubarb (*Rheum* spp.) roots and petioles
Scotch pine (*Pinus sylvestris* L.) needles

EXTRACTION

- Overnight shaking at RT (black currant, rosemary, rhubarb, Scotch pine)
- 45 min ultrasonification at RT (garlic, meadowsweet)
- 30 min in water bath at 80°C (goutweed)
- Extracted with 30% and 80% ethanol (v/v)
- Extracts filtered, condensed and freeze-dried

- Broth microdilution assays in 96-well plates following CLSI [3]
- 24h absorbance values and visual assessment

PRELIMINARY SCREENING

Bacillus cereus ATCC 11778
Campylobacter jejuni ATCC 33560
enteropathogenic *Escherichia coli* CB9615
Listeria monocytogenes DSM 112142, DSM 101804
Pseudomonas aeruginosa ATCC 27853
Salmonella enterica ser. Typhimurium ATCC 19585
S. enterica ser. Enteritidis ATCC 13076
Staphylococcus aureus ATCC 29212

MIC-DETERMINATION

- For extracts displaying ≥ 50% bacterial growth inhibition in the screening assay
- Determined in a 2-fold manner
- Assays performed at least twice, in triplicate

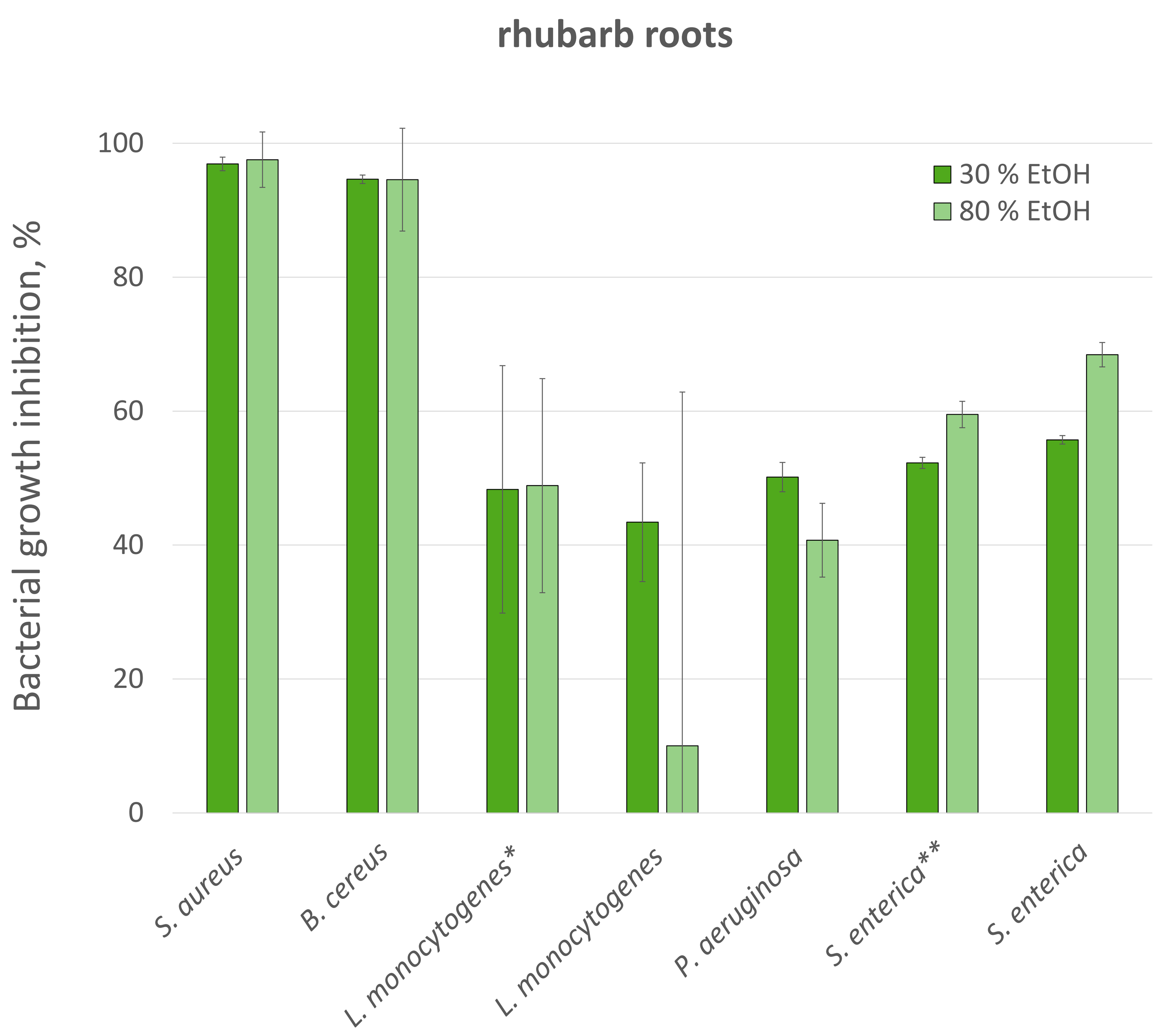

Fig 1. Antibacterial activity against clinical isolates of rhubarb root at the concentration of 1 mg/mL. The bars represent mean value of triplicate wells ± SD (n=1). * isolated from minced meat, ** serovar Typhimurium

Table 1. Minimum inhibitory concentration (MIC, mg/mL) determinations for plant extracts for (**A**) Gram- positive and (**B**) Gram-negative bacteria

A Plant material	Extraction (% EtOH)	MIC* (mg/mL) *S. aureus*	*B. cereus*	*L. monocytogenes**	*L. monocytogenes*
Rhubarb roots	30	0.5	0.5	1	1
50°C	80	0.25	0.25	1	1
Rhubarb	30	ND	>4[a]	ND	ND
petioles 45 °C	80	ND	3	ND	ND
Rhubarb petioles freeze-dried	80	4	ND	ND	ND
Rosemary	30	1	ND	ND	ND
commercial	80	0.5	0.02	ND	ND
Meadowsweet	30	2	ND	ND	ND
freeze-dried	80	1	1	ND	ND

B Plant material	Extraction (% EtOH)	MIC* (mg/mL) *S. enterica***	*S. enterica*	*P. aeruginosa*
Rhubarb roots	30	ND	4	>4[a]
50°C	80	4	3	3-4
Meadowsweet	30	ND	ND	>4
freeze-dried	80	ND	ND	3

ND: minimum threshold for inhibition not achieved in the screening assays, *isolated from minced meat, **serovar Typhimurium, [a]MIC not achieved at the highest concentration tested

[1] P. Raudsepp, J. Koskar, D, Anton, et al. Journal of the Science of Food and Agriculture, 99:5, 2311–2320, (2019).
[2] N.A. Santiesteban-López, J.A. Gómez-Salazar, E.M. Santos, et al. Foods 11, 2613, (2022).
[3] CLSI. CLSI standard document M07–A10. 10th ed. Wayne, PA: Clinical and Laboratory Standards Institute, 35:1–87, (2015).

Eesti Teadusagentuur Estonian Research Council
Eesti Maaülikool Estonian University of Life Sciences
This work was supported by the Estonian Research Council grant PRG 1441.
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