

**Lakan siemenkuoren
ellagitanniinien teho
antibioottiresistenttejä
bakteereita vastaan**

VTT

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8.11.2022

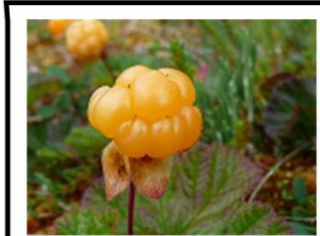


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Luonnontuotealan tutkimusseminaari, Mikkeli

Background

- Excessive use of antimicrobials has led to emergence and spread of multidrug-resistant (MDR) bacteria, which cause significant risks to routine medical procedures, and The World Health Organization (WHO) has declared antimicrobial resistance (AMR) among the top 10 global health threats.
- Of particular concern are pathogenic bacteria showing MDR phenotype to all classical antibiotics. Also, in food chain resistance towards classical biocides and disinfectants have increased.
- According to the Farm to Fork strategy the Commission will reduce by 50% the sales of antimicrobials for farmed animals and in aquaculture by 2030.
- Antimicrobial resistance linked to the use of antimicrobials in animal and human health leads to an estimated 33,000 human deaths in the EU each year.
- Superbug infections could cost the lives of around 2.4 million people in Europe, North America and Australia over the next 30 years if no action is taken (OECD).
- WHO recommends that future R&D strategies should focus on the discovery and development of new antibiotics specifically active against multidrug- and extensively drug-resistant *Staphylococcus aureus* and Gram-negative bacteria, like *Pseudomonas aeruginosa* and *Escherichia coli*.



Cloudberry
Rubus chamaemorus



Raspberry (cultivated)
Rubus idaeus, var.
Ottawa



Bilberry
Vaccinium myrtillus



Black currant
Ribes nigrum



Chokeberry
Aronia melanocarpa



Arctic bramble
Rubus arcticus



Blue raspberry
Rubus caesius



Blueberry
Vaccinium corymbosum



Crowberry
Empetrum nigrum



Rowanberry
Sorbus aucuparia



Raspberry
Rubus idaeus



Lingonberry
Vaccinium vitis-idaea



Cranberry
Vaccinium oxycoccus

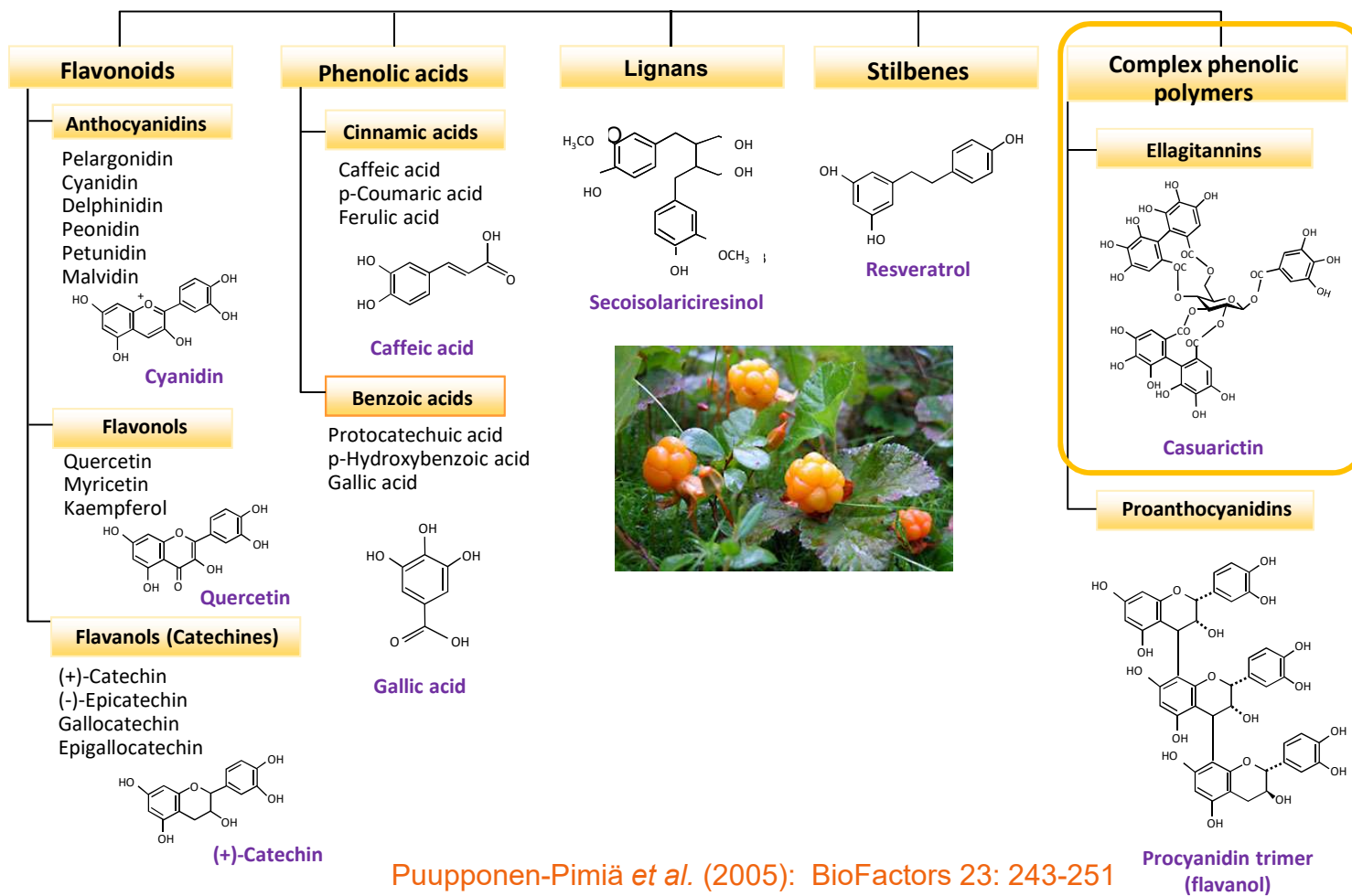


Sea buckthorn
Hippophae rhamnoides



Strawberry
Fragaria ananassa

Berry phenolics have strong biological activities

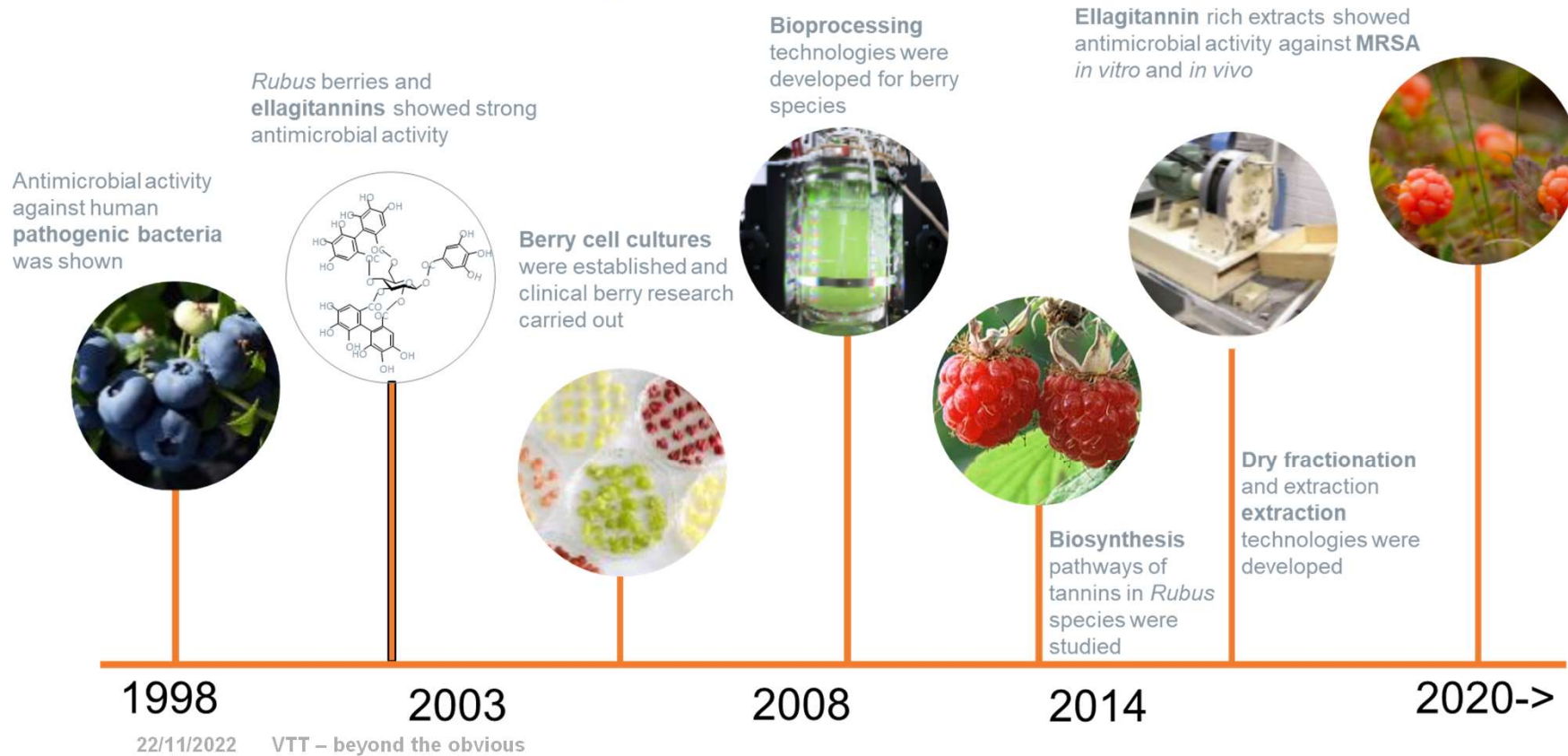


20 years of berry innovations

BUSINESS
FINLAND

ACADEMY
OF FINLAND

VTT



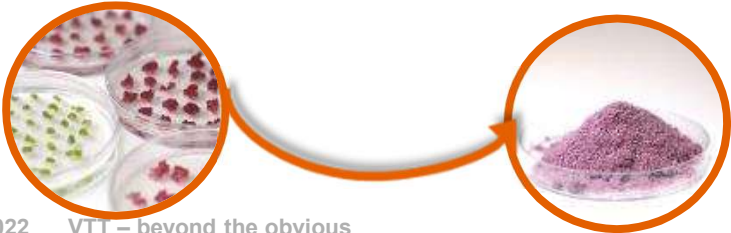
Approach and application



Berry processing side stream as raw-material

VTT has several patents for the technologies

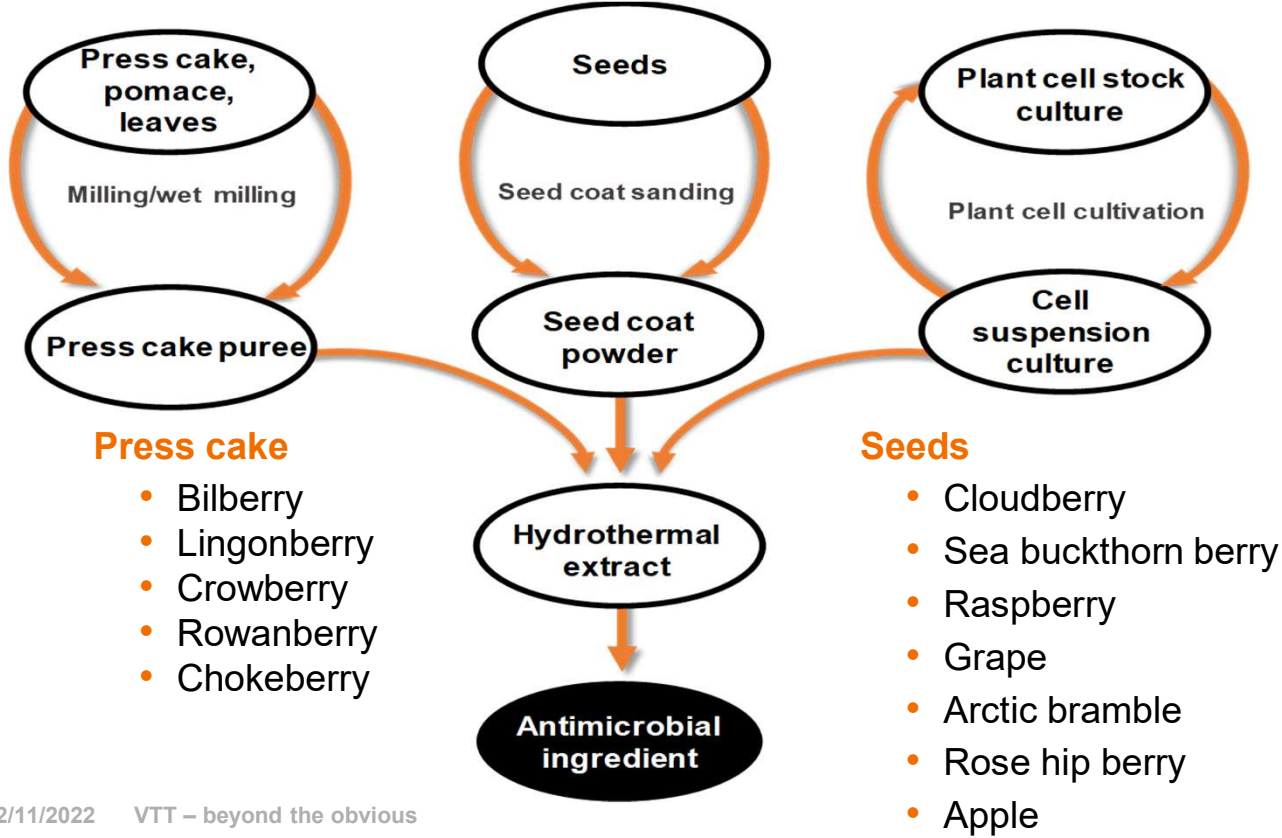
Berry cell cultures as raw-material for antimicrobial active ingredients



Targeted elimination of most critical antibiotic resistant microbes in the skin.

- Topical applications:
- skin infections
 - wound healing
 - surgery pretreatment

Technical description of the solution



**Output:
Water
soluble
compounds**

Further processing of berries

Enzyme treatment

- Modification of berry material by plant cell wall-degrading enzymes
- Release and modification of bioactive compounds

Increased
antimicrobial
activity

More
bioactive
compounds

Increased
antioxidant
activity

Viljanen et al. (2014): Food Chem. 157: 148-156

Fermentation

- Modification of berry material, its structure, taste, colour and shelf life
- Microbes used: LAB, yeasts
- Release of bioactive compounds

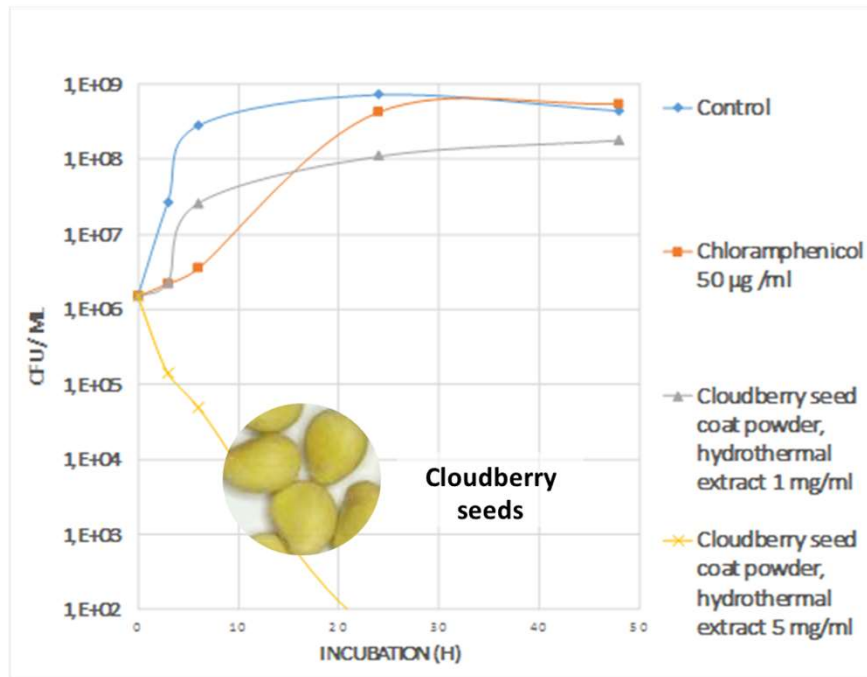
New
flavour

More
bioactive
compounds

New
colour

Puupponen-Pimiä et al. (2016): Food Chem. 197: 950-958

Cloudberry seed coat extract against *Staphylococcus aureus* MRSA*



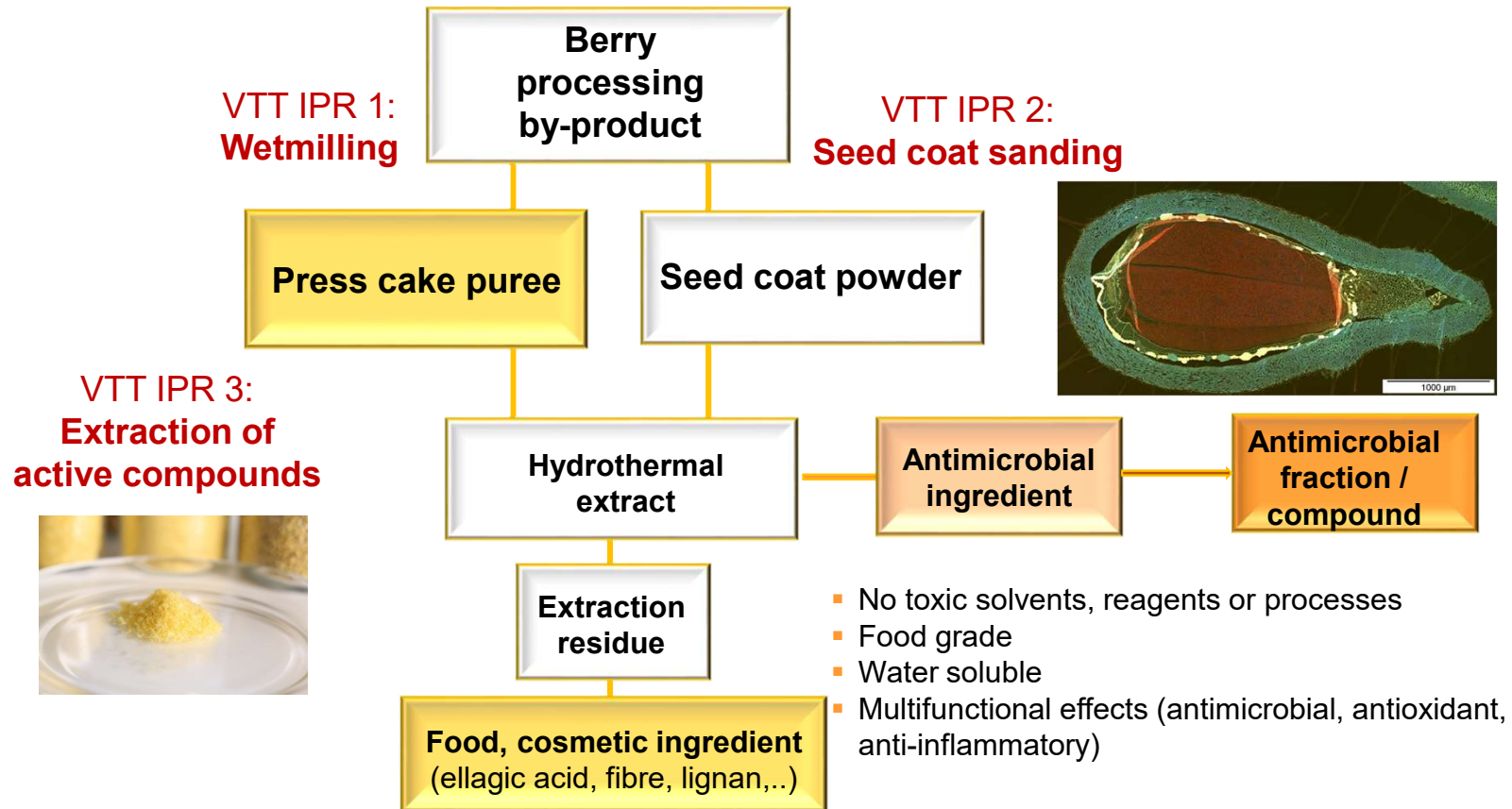
*Methicillin resistant *Staphylococcus aureus* (MRSA) collection strain

**Valorization of cloudberry by-products
by VTT's Innoberry technologies®**

VTT



Wetmilling and dry seed coat sanding










Puupponen-Pimiä *et al.* (2016): Food Chem. 197: 950-958

Puupponen-Pimiä *et al.* FI20146128 (WO2016/097488)

Article

Sanguiin H-6 Fractionated from Cloudberry (*Rubus chamaemorus*) Seeds Can Prevent the Methicillin-Resistant *Staphylococcus aureus* Biofilm Development during Wound Infection

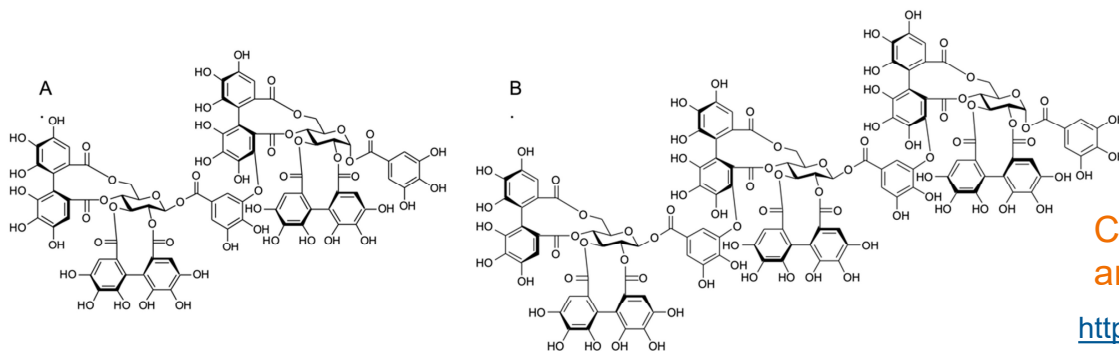
John Jairo Aguilera-Correa ^{1,2}, Sara Fernández-López ³, Iskra Dennisse Cuñas-Figueroa ³ , Sandra Pérez-Rial ⁴, Hanna-Leena Alakomi ⁵ , Liisa Nohynek ⁵, Kirsi-Marja Oksman-Caldentey ⁵ , Juha-Pekka Salminen ⁶, Jaime Esteban ^{1,2} , Juan Cuadros ^{7,8}, Riitta Puupponen-Pimiä ^{5,*} , Ramon Perez-Tanoira ^{7,8,*}  and Teemu J. Kinnari ^{9,*} 

Antibiotics 2021, 10, 1481.

<https://doi.org/10.3390/antibiotics10121481>

Antimicrobial activity in focus

- Antimicrobial activity was measured in various crude extracts, more defined and purified fractions and finally in pure ellagitannin extract
- The most active ellagitannins were Sanguiin-H6 (**A**) and Lambertianin C (**B**)
- Synergistic effects of various phenolic compounds are also obvious
- The antimicrobial activity of cloudberry extracts is likely caused by multiple mechanisms, such as immobilization of bacterial cells to phenolic extracts, changes in cellular membranes, and inhibition of quorum sensing and biofilm formation.

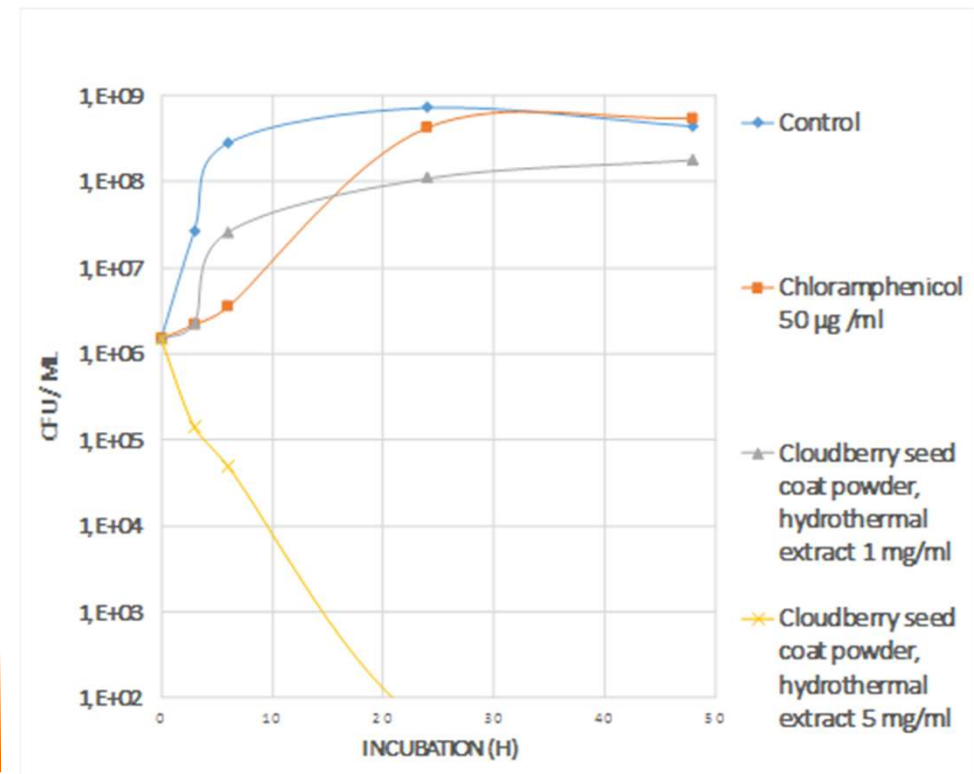


Chemical structure of sanguin H-6 (A) and lambertianin C (B)

<https://www.mdpi.com/2079-6382/10/12/1481/htm>

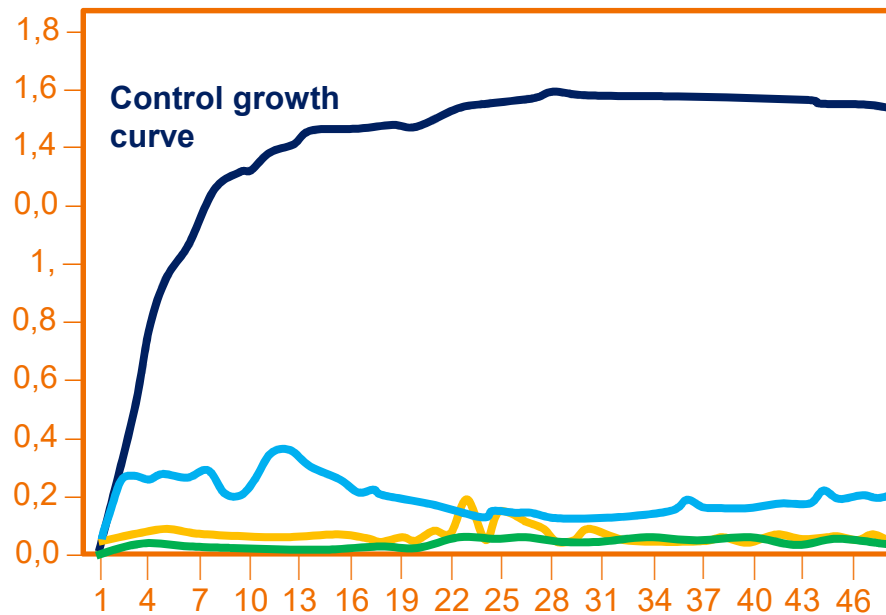
Antimicrobial activities of cloudberry extracts

- Antimicrobial activity against human pathogen microbes was tested, e.g. *S. aureus*, *P. aeruginosa*, *Campylobacteri jejuni*, *Candida albicans*,...
- No inhibition on human beneficial bacteria (LAB)
- Very strong activity against methicillin resistant *S. aureus* MRSA**



Puupponen-Pimiä *et al.* (2021): ACS Food Sci. Technol. 1, 917-927

Staphylococcus aureus MRSA*



Cloxacillin 512 mg/ml

Cloudberry seed coat HT extract 1 mg/ml

Cloudberry seed coat HT extract 0.25 mg/ml +
Cloxacillin 16 mg/ml

* Clinical methicillin resistant *Staphylococcus aureus* (MRSA) strain isolated from infected human skin wound

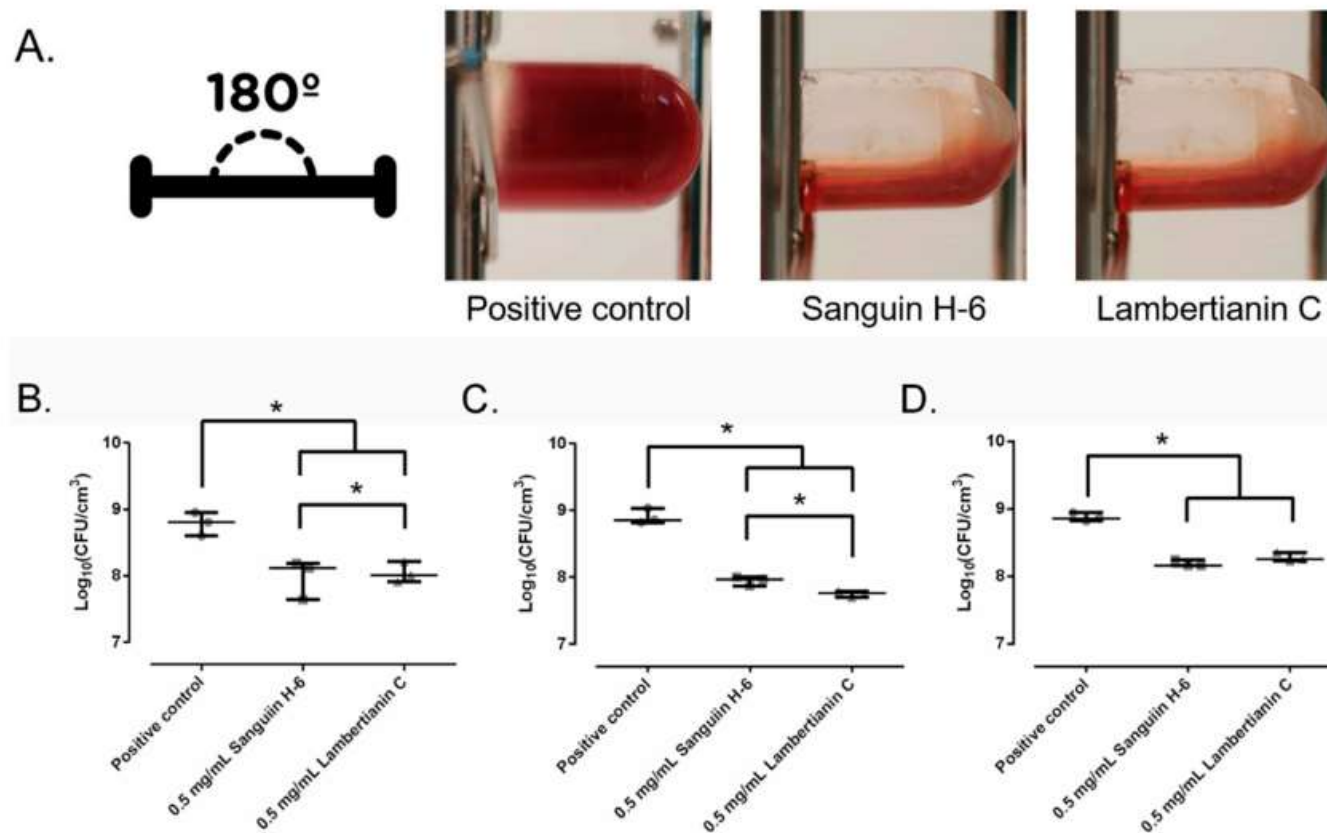
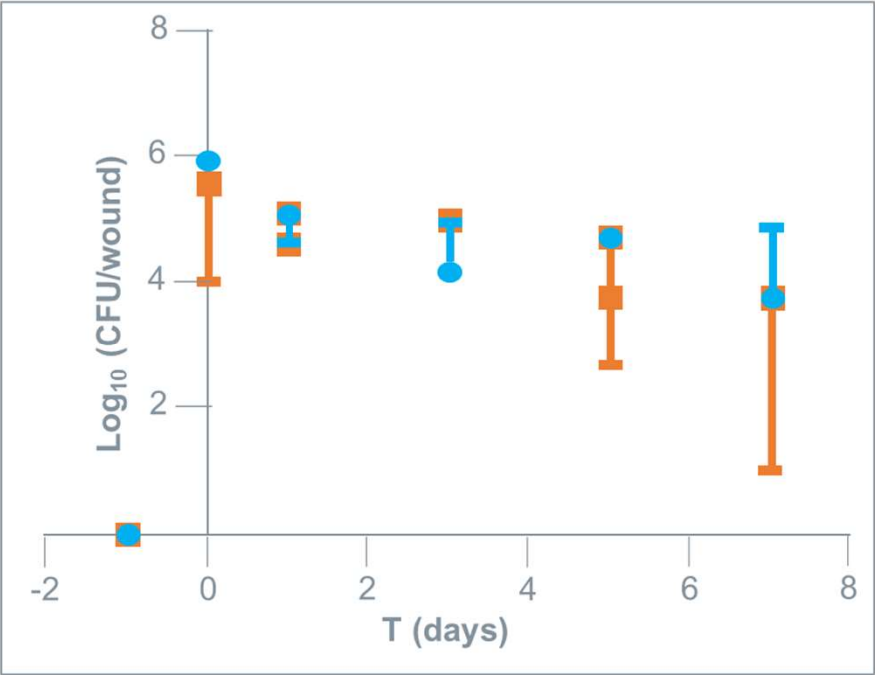


Figure 7. Macroscopic appearance of wound-like medium after the SAP231 biofilm development (A). The effect of sanguin H-6 and lambertianin C at different concentrations on SAP231 (B), MRSA1 (C), and MRSA2 (D) biofilm development in wound-like medium.: *: *p*-value < 0.05 for Wilcoxon test.

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Proof of concept

– Mouse wound infection model with MRSA*



Control, no treatment

Berry extract 2 mg/ml

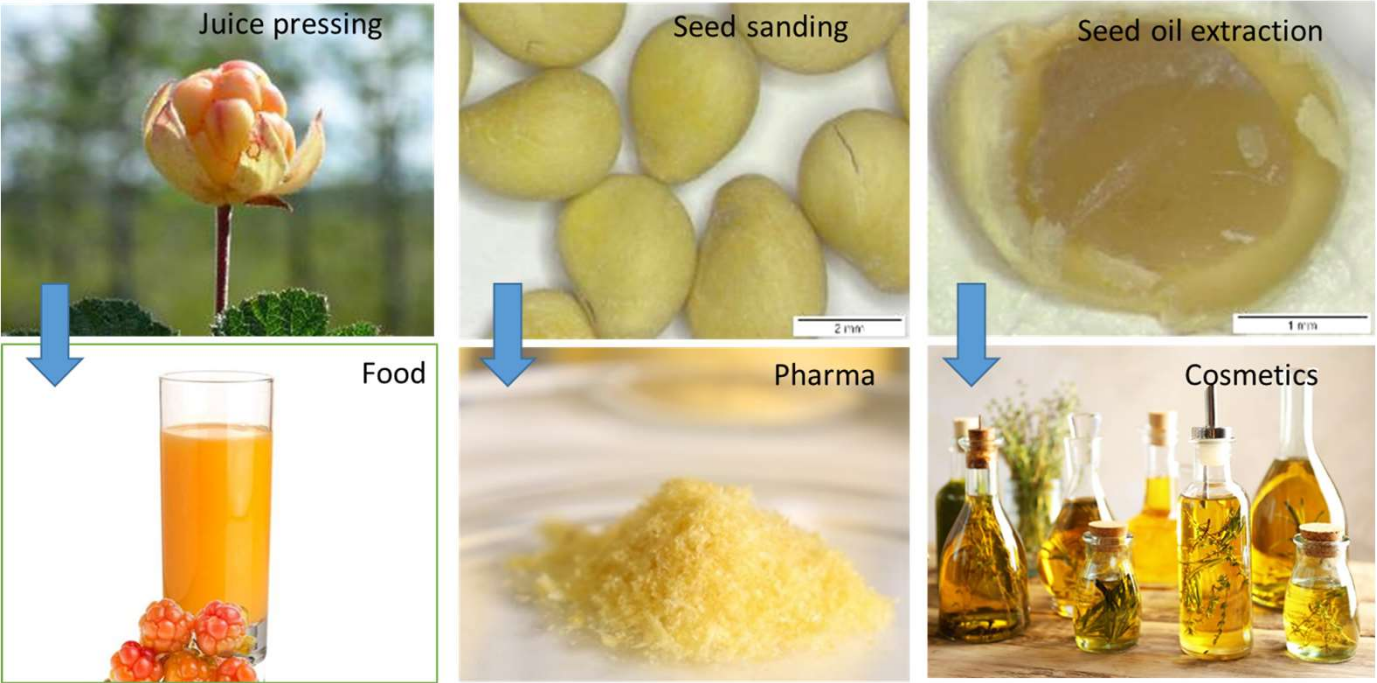
*Clinical methicillin resistant *Staphylococcus aureus* (MRSA) strain isolated from infected human skin wound

Johtopäätökset



- Lisäarvoa hyödyntämällä koko marja ja sivuvirrat.
- Lakan siemenen kuoresta eristetyissä yhdisteissä, **ellagitanniineissa (sanguiin H-6 ja lambertiaani C)**, on merkittävä antimikrobinen teho. Yhdisteet estivät tehokkaasti hiirissä sairaalabakteeri MRSA:n kasvua haavankaltaisissa olosuhteissa.
- Pinnoilla ja kudoksessa kasvaessaan mikrobit muodostavat biofilmiä, joka estää antimikrobisten yhdisteiden tunkeutumisen solujen sisään ja heikentää siten antimikrobisten yhdisteiden tehoa. Tutkimuksessamme ellagitanniinit estivät mikrobien kiinnittymisen pinnoille ja biofilmin muodostuksen.
- Tämän löydöksen mielenkiintoinen sovellus voisi olla esimerkiksi leikkausta edeltävä ihon käsittely MRSA-bakteeria vastaan, vaikeiden haavainfektiohoitojen lisäksi.
- Suomalaisista kasveista eristetyissä antimikrobisissa yhdisteissä on potentiaalia uusien merkittävien innovaatioiden pohjaksi ja kaupallistettaviksi tuotteiksi.

New berry value chain for cloudberry (Rubus chamaemorus)



Kiitokset

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