



**CORFU2022**

9<sup>th</sup> International Conference  
on Sustainable Solid Waste  
Management

15-18 JUNE 2022



# Integration of bioactive compounds rich pomegranate wastes into biorefinery

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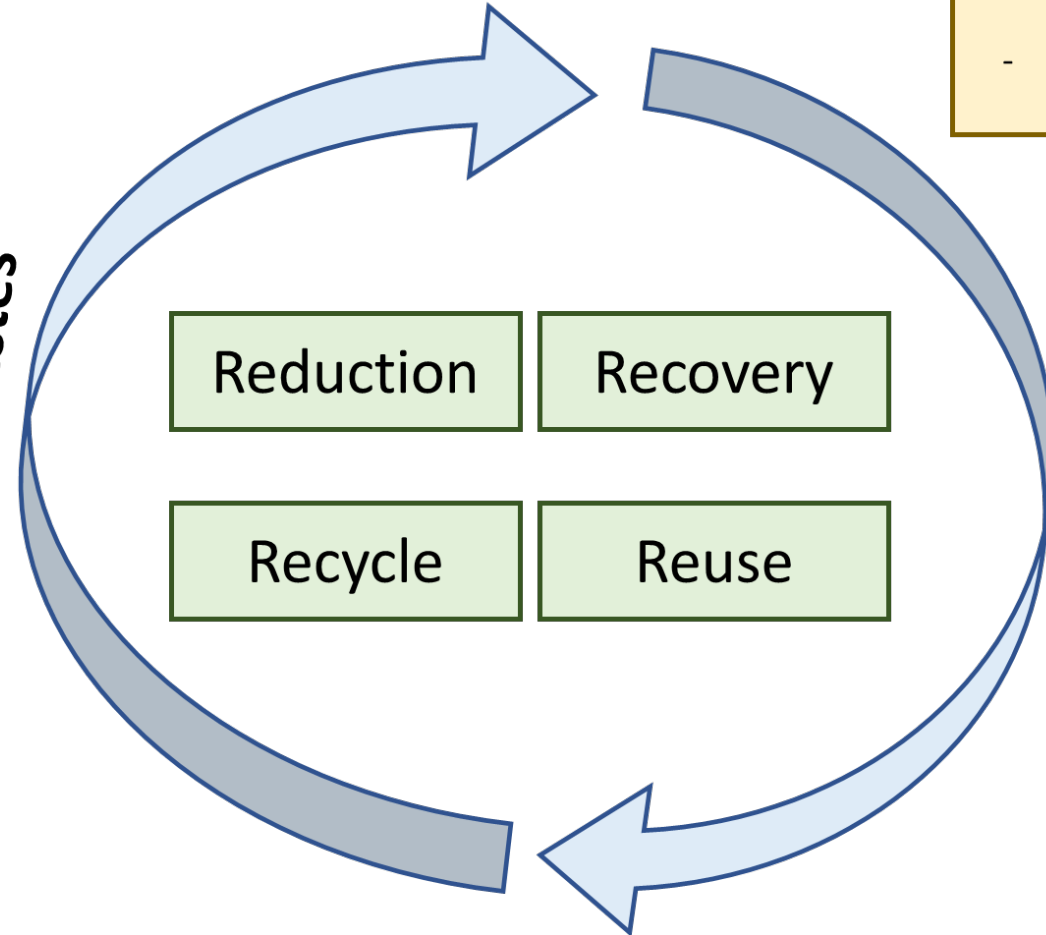
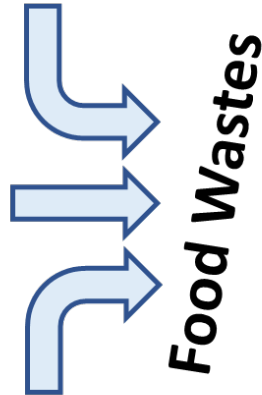
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- Food Industry Wastes**
- Dairy Industry
  - Vegetable Oil Industry
  - Sugar Industry
  - Fruit Juice Industry

- Solid Food Wastes**
- Fruits
  - Vegetables

- Wasted Food**
- Bakery products
  - Butcher/Poultry/Fishery products
  - Dairy products
  - Vegetables & Fruits
  - Ready meals



- Extraction**
- Recovery of oils, fatty acids, pectin, phenolic compounds etc.

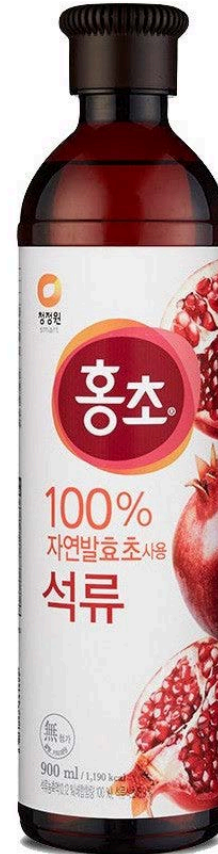
- Microbial Products**
- Biogas
  - Bioethanol
  - Enzymes & Organic acids
  - Single cell protein
  - Biopolymer

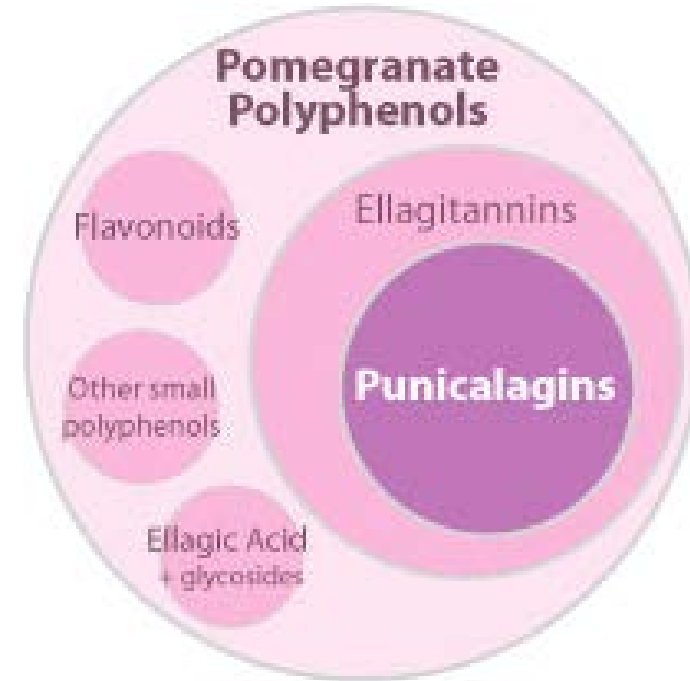
**Biofertilizer**

- Biomaterials**
- Biofilm
  - 3D edible foods



<https://www.kew.org/plants/pomegranate>





**Pomegranate peel** accounts for about **30–40% of the total fruit** weight and contains a high proportion of **ellagic acid** and its derivatives, **ellagitannins** such as **punicalagin** and **punicalin**, and proanthocyanidins and flavonoids.

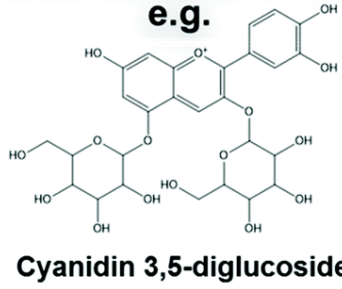
# Flavonoids

e.g.



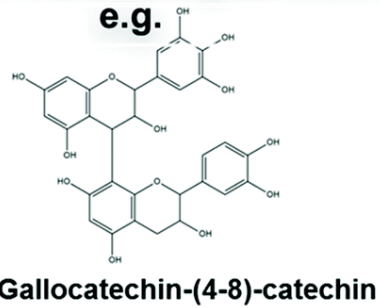
# Anthocyanins

e.g.



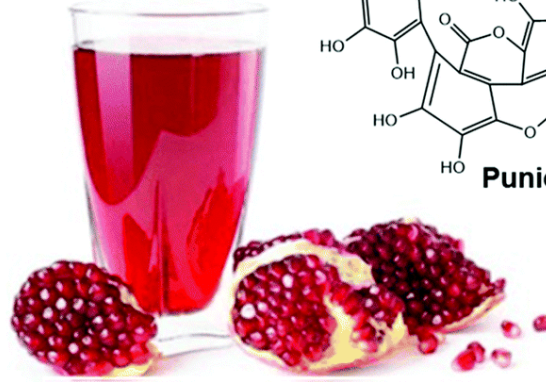
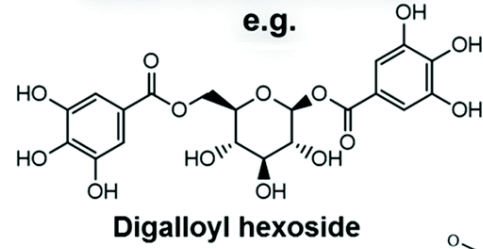
# Proanthcyanidins

e.g.



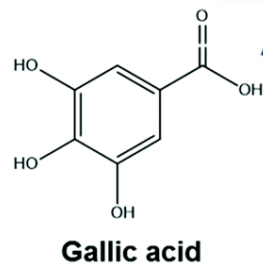
# Gallotannins

e.g.



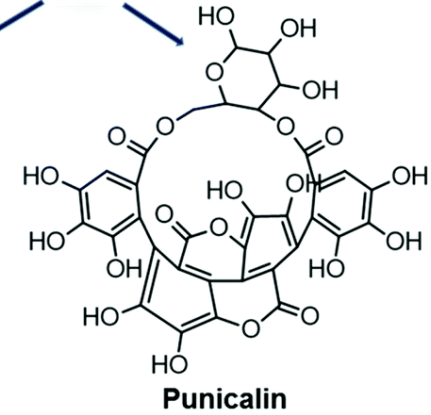
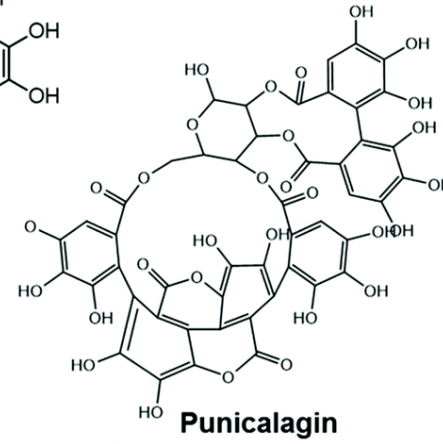
# Phenolic acids

e.g.



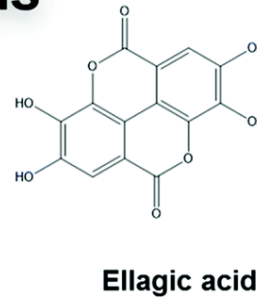
# Ellagitannins

e.g.

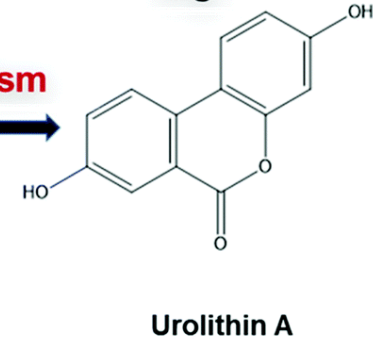


# Urolithins

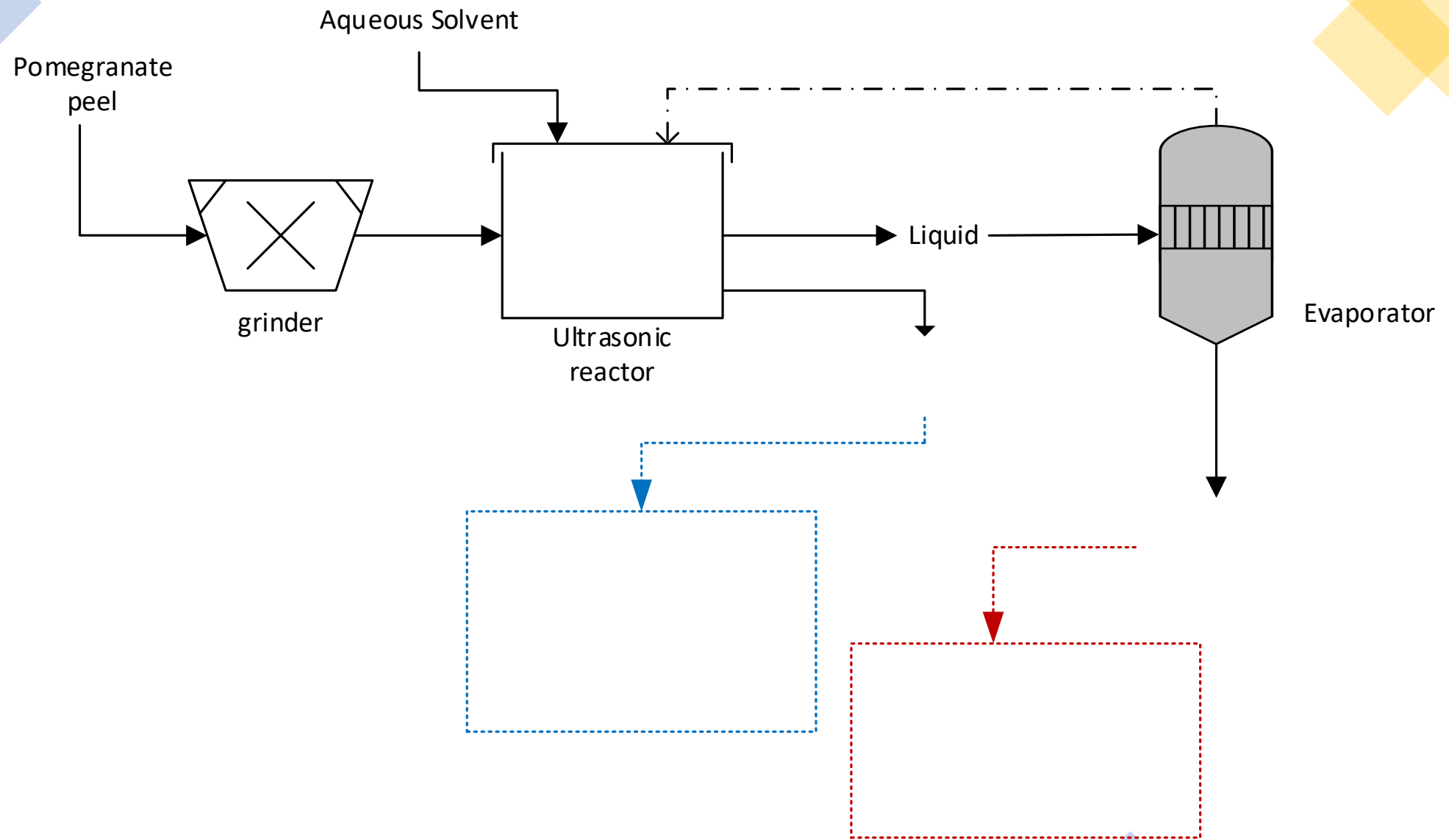
e.g.



metabolism



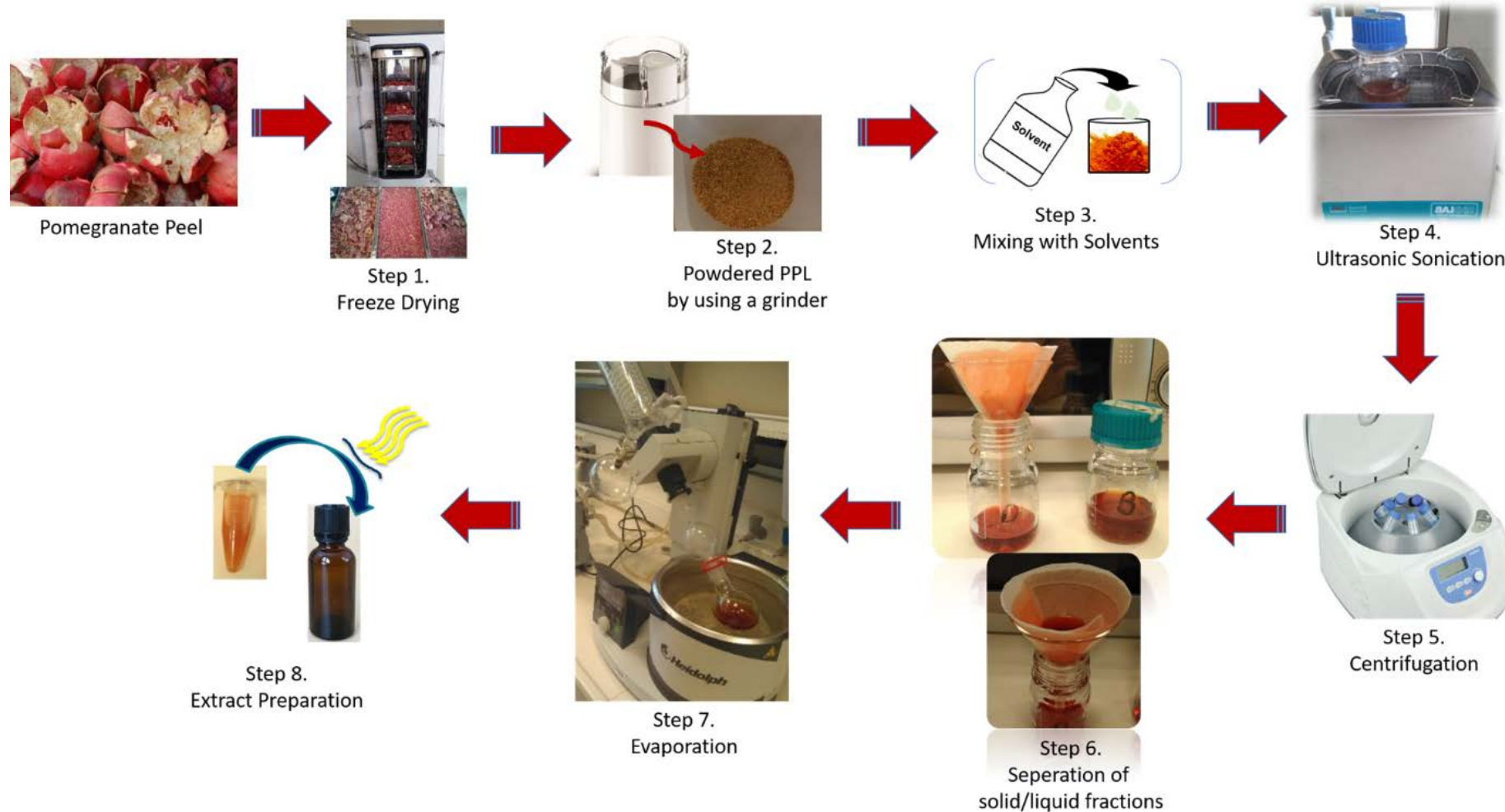
DOI: 10.3390/foods9111606





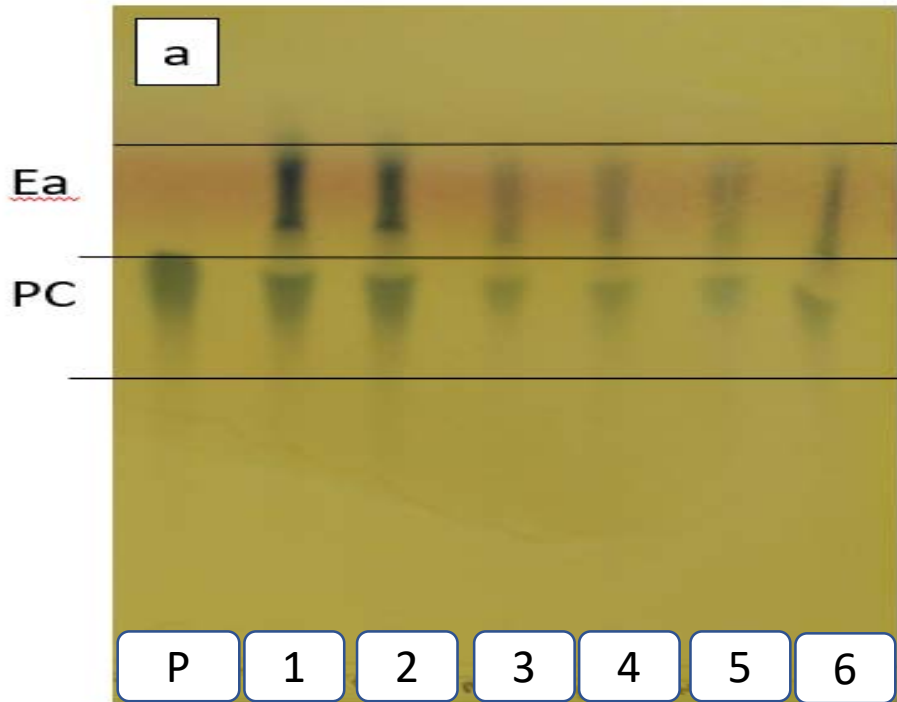
# Pomegranate peel extraction

## As Natural Antibiofilm Agents

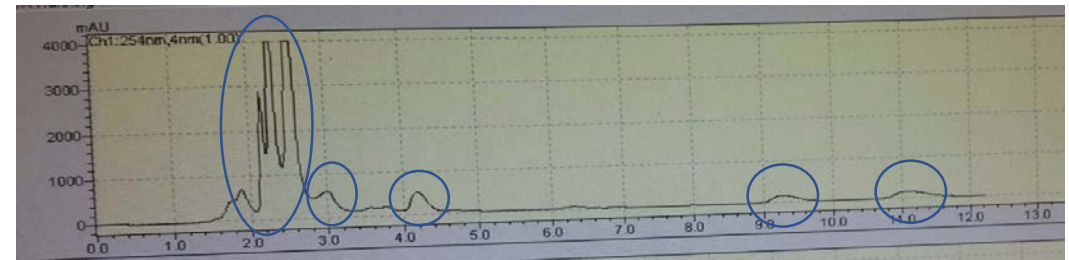


# Determination of phenolics compounds (punicalagins and ellagic acid)

## TLC – Punicalagin & Elagic acid



## HPLC – Punicalagin & Elagic acid

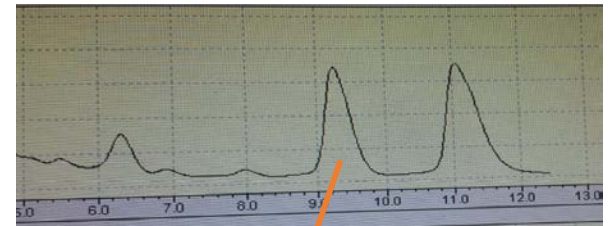
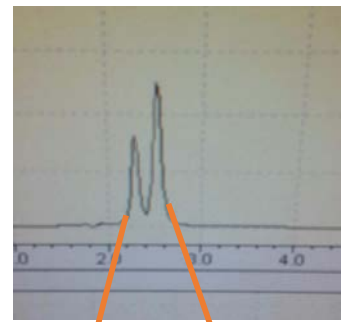


### Instrument Parameters

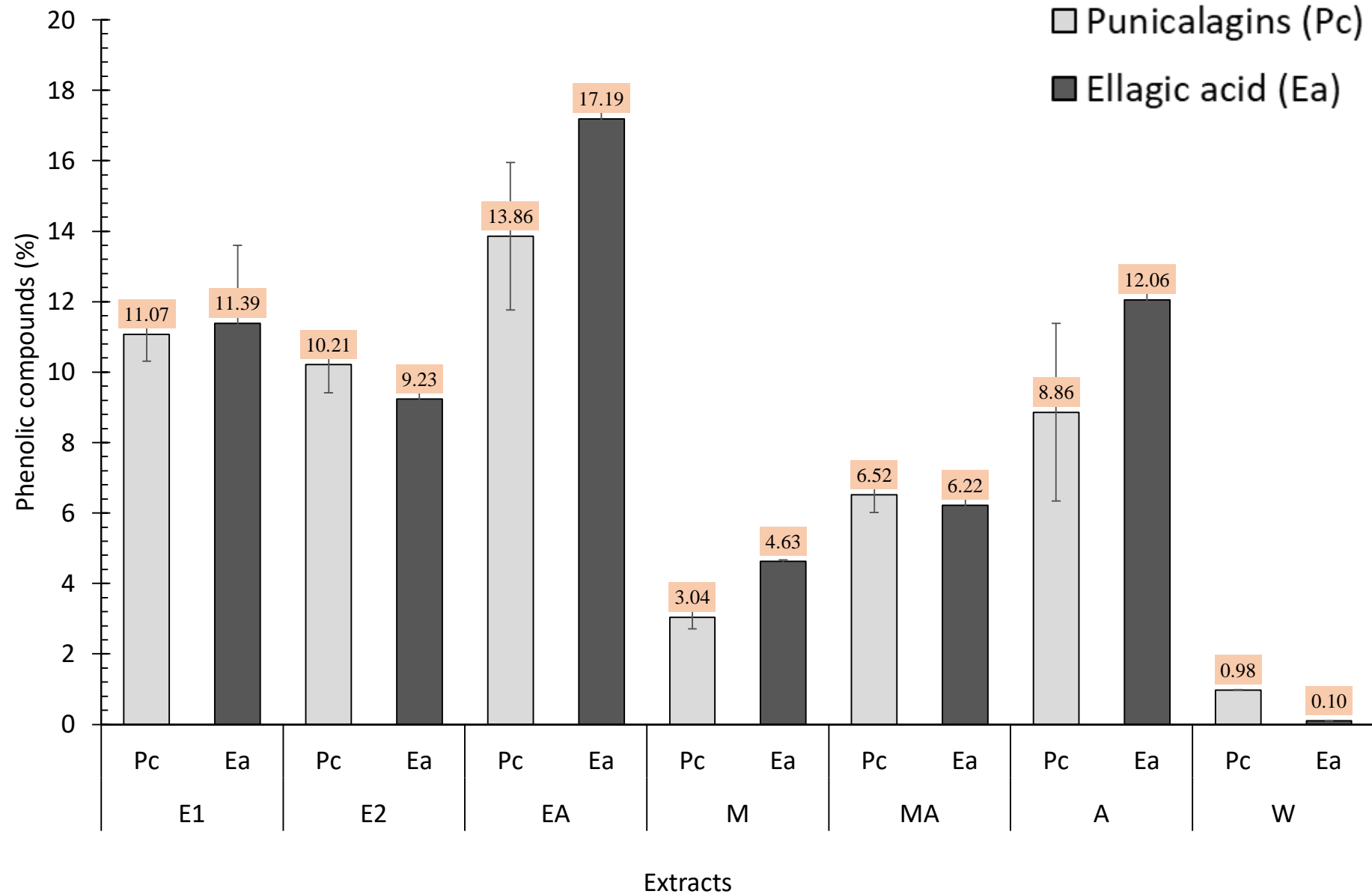
Instrument: Dionex HPLC System  
 Detection: UV-vis  
 Mobile Phase A: 1% Formic Acid in Milli-Q Water  
 Mobile Phase B: Acetonitrile  
 Gradient Program:

Time (min)	%A	%B
0	95	5
18	85	15
20	35	65
25	95	5
30	95	5

Column: Dionex Acclaim® Polar Advantage PA II  
 150 × 3.0 mm, 3 µm particle  
 Flow Rate: 1.0 mL/min  
 Temperature: 30 °C  
 Injection Volume: 10 µL  
 UV Detection: 260 nm



## 5 g pomegranate peel + 100 mL solvent



**5 g pomegranate peel + 100 mL solvent**

Extracts	pH	Malic acid (%)	Citric acid (%)	Total Sugar (%)
EtOH (30 min)	3.10 ± 0.05	0.01 ± 0.00	0.44 ± 0.03	3.72 ± 0.24
EtOH (60 min)	3.10 ± 0.04	0.03 ± 0.01	0.62 ± 0.07	4.98 ± 0.21
EtOH + acid	0.30 ± 0.02	<b>1.59 ± 0.12</b>	<b>1.13 ± 0.14</b>	4.93 ± 0.14
MeOH	3.40 ± 0.04	0.00 ± 0.00	0.69 ± 0.03	4.76 ± 0.29
MeOH + acid	0.26 ± 0.02	<b>3.19 ± 0.14</b>	<b>0.97 ± 0.04</b>	5.95 ± 0.07
Aceton	3.50 ± 0.05	0.37 ± 0.04	0.65 ± 0.13	4.00 ± 0.13
Water	3.55 ± 0.07	0.04 ± 0.01	0.10 ± 0.01	1.69 ± 0.25

10 g pomegranate peel + 100 mL solvent

Extracts	pH	Total Sugars (%)	Organic acids (%)		Phenolic compounds (%)	
			Malic acid	Citric acid	Punicalagin	Ellagic acid
MeOH + acid	0.26 (0.03)	6.68 (0.23)	<b>0.92 (0.11)</b>	0.41 (0.07)	<b>4.51 (0.72)</b>	<b>10.94 (0.64)</b>
MeOH	3.28 (0.04)	8.20 (0.42)	0.07 (0.02)	0.72 (0.06)	4.28 (0.22)	7.56 (0.01)
EtOH + acid	0.20 (0.02)	7.04 (0.34)	0.60 (0.04)	<b>0.93 (0.08)</b>	4.76 (0.16)	4.42 (0.19)
EtOH	3.05 (0.05)	6.72 (0.19)	0.10 (0.03)	0.69 (0.05)	1.88 (0.23)	1.66 (0.47)
Water	3.45 (0.04)	2.13 (0.12)	0.04 (0.01)	0.10 (0.02)	0.62 (0.19)	0.09 (0.02)

5 g pomegranate peel + 100 mL solvent



Pomegranate



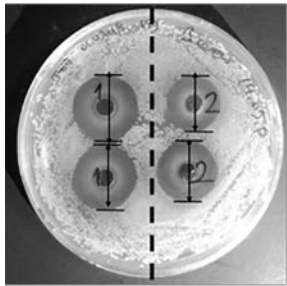
Pomegranate peel

Extracts

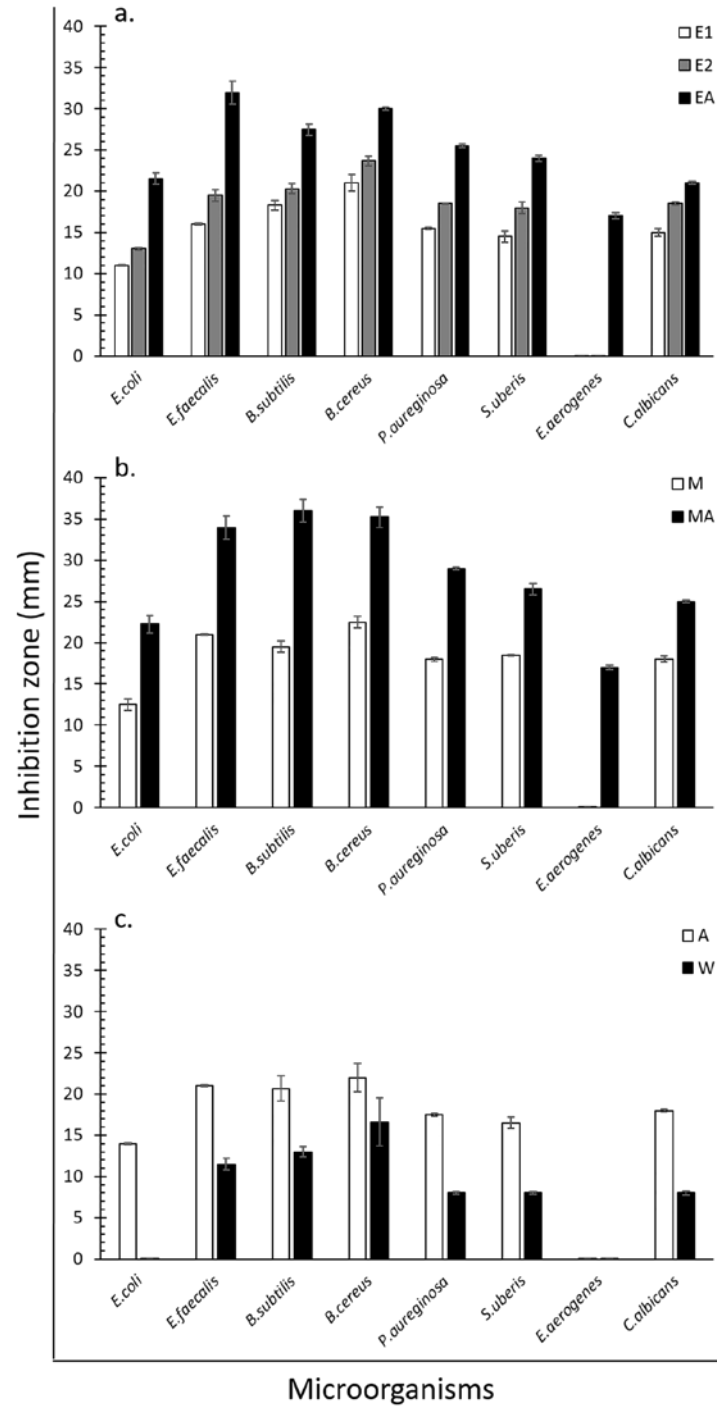


E2 E1 EA M MA A W

Bioactive compounds

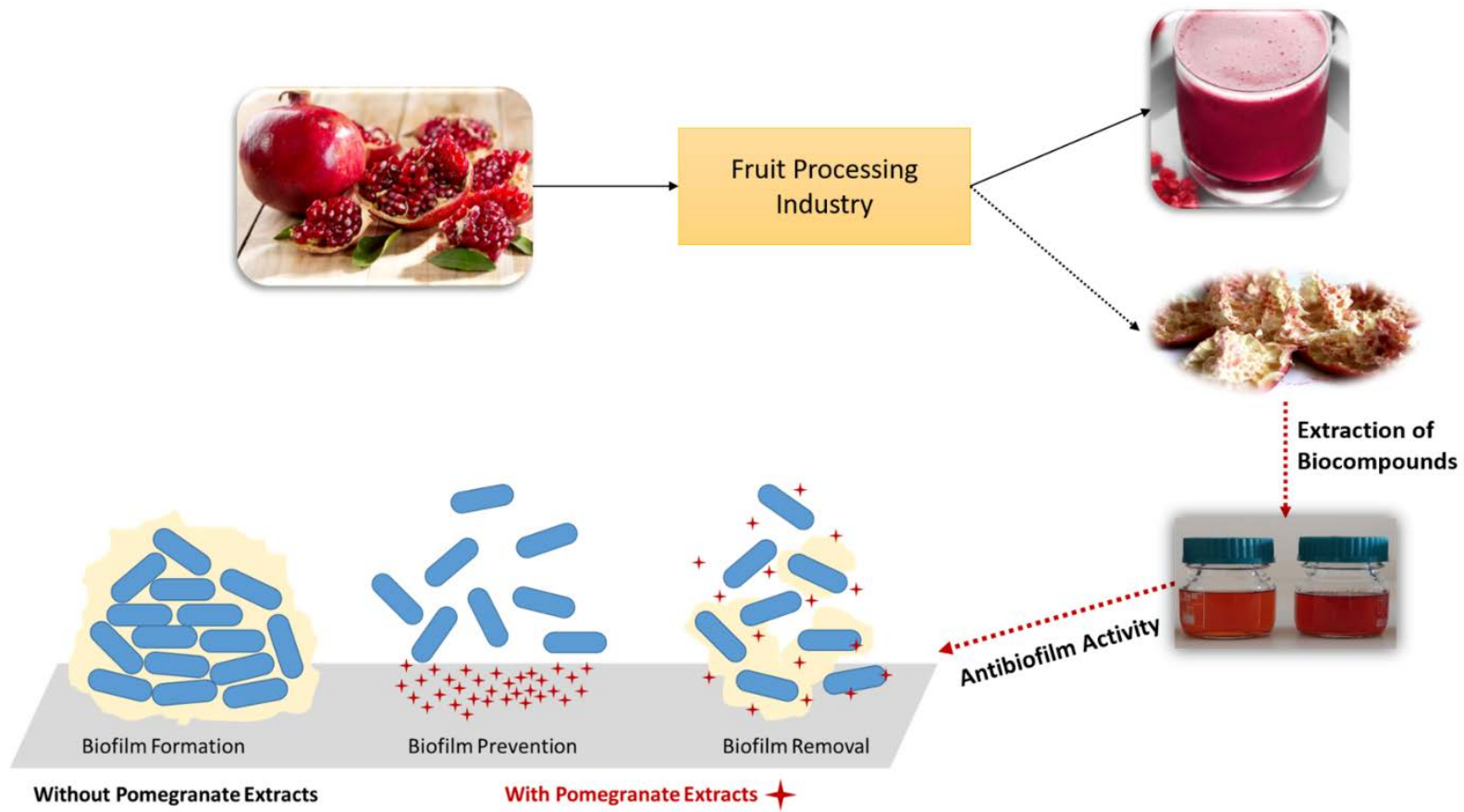


Antimicrobial Activity



**10 g pomegranate peel + 100 mL solvent**

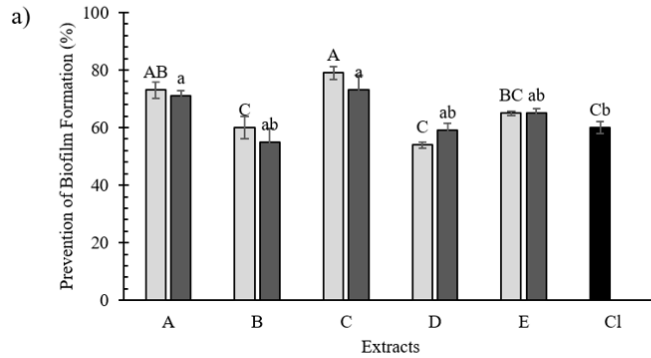
Strain	Extracts	Dilutions				
		1	1/2	1/5	1/10	1/25
<i>Bacillus cereus</i>	MeOH + acid	33 (2) <sup>Aa</sup>	30 (1) <sup>Aa</sup>	22 (2) <sup>Ba</sup>	19 (1) <sup>BCa</sup>	14 (1) <sup>Ca</sup>
	MeOH	25 (2) <sup>Abc</sup>	24 (1) <sup>Ab</sup>	21 (1) <sup>ABa</sup>	17 (1) <sup>Ba</sup>	13 (1) <sup>Ca</sup>
	EtOH + acid	32 (1) <sup>Aab</sup>	25 (1) <sup>Bb</sup>	21 (1) <sup>Ca</sup>	18 (1) <sup>Da</sup>	15 (0) <sup>Da</sup>
	EtOH	23 (1) <sup>Ac</sup>	21 (1) <sup>ABc</sup>	19 (0) <sup>BCa</sup>	17 (1) <sup>Ca</sup>	14 (1) <sup>Da</sup>
	Water	17 (1) <sup>Ac</sup>	16 (0) <sup>ABd</sup>	14 (1) <sup>Bb</sup>	11 (1) <sup>Cb</sup>	9 (1) <sup>Cb</sup>
<i>Bacillus subtilis</i>	MeOH + acid	34 (1) <sup>Aa</sup>	25 (0) <sup>Ba</sup>	20 (0) <sup>Ca</sup>	14 (1) <sup>Da</sup>	10 (0) <sup>Da</sup>
	MeOH	23 (1) <sup>Ab</sup>	19 (0) <sup>Bbc</sup>	14 (1) <sup>Cb</sup>	10 (0) <sup>Db</sup>	0
	EtOH + acid	32 (1) <sup>Aa</sup>	25 (2) <sup>Bab</sup>	20 (1) <sup>BCa</sup>	15 (0) <sup>CDa</sup>	10 (1) <sup>Da</sup>
	EtOH	23 (1) <sup>Ab</sup>	18 (1) <sup>Bc</sup>	15 (2) <sup>Bb</sup>	10 (1) <sup>Cb</sup>	0
	Water	15 (1) <sup>Ac</sup>	10 (0) <sup>Bd</sup>	0	0	0
<i>Enterococcus faecalis</i>	MeOH + acid	32 (2) <sup>Aa</sup>	28 (1) <sup>Aa</sup>	20 (0) <sup>Ba</sup>	15 (1) <sup>Ca</sup>	0
	MeOH	20 (1) <sup>Ab</sup>	18 (2) <sup>Ab</sup>	16 (1) <sup>Ab</sup>	11 (1) <sup>Bb</sup>	0
	EtOH + acid	31 (2) <sup>Aa</sup>	26 (0) <sup>Ba</sup>	19 (1) <sup>Ca</sup>	14 (0) <sup>Da</sup>	11 (1) <sup>Da</sup>
	EtOH	20 (0) <sup>Ab</sup>	18 (1) <sup>Ab</sup>	14 (1) <sup>Bb</sup>	13 (1) <sup>Bab</sup>	9 (1) <sup>Cb</sup>
	Water	13 (0) <sup>Ac</sup>	10 (0) <sup>Bc</sup>	0	0	0



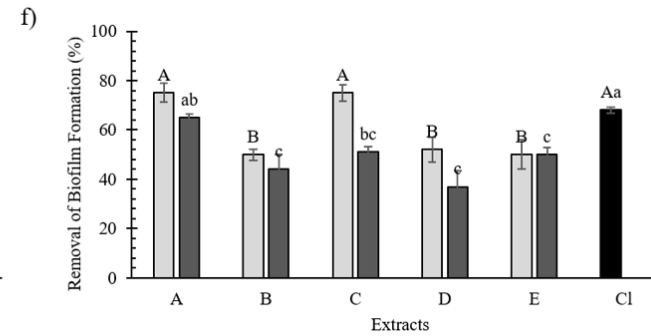
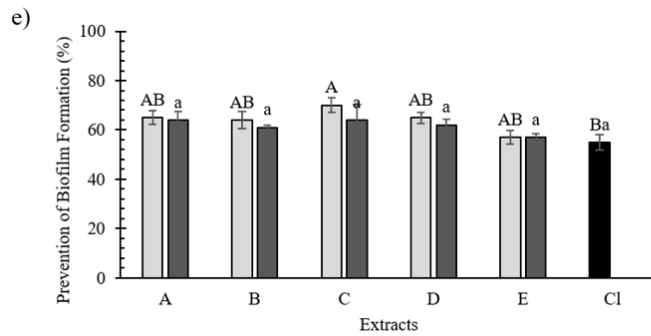
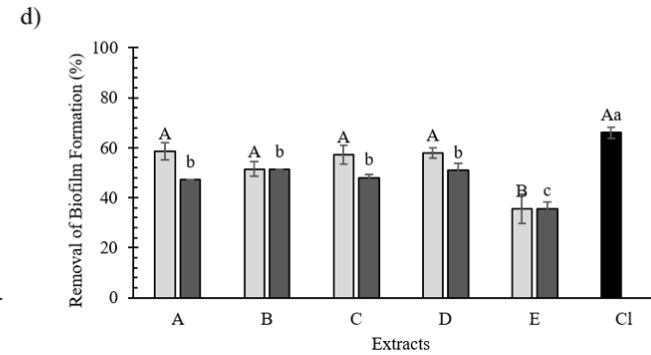
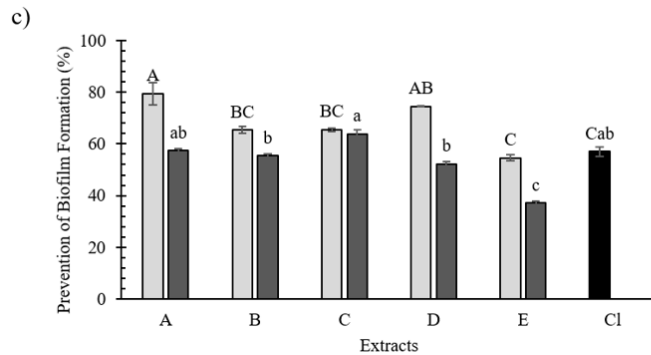
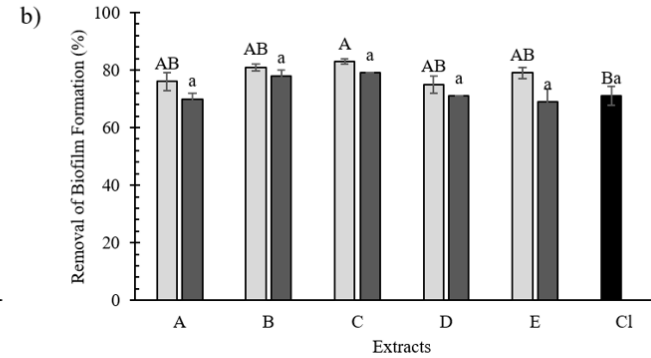


**5 g pomegranate peel + 100 mL solvent**

**Biofilm Prevention**

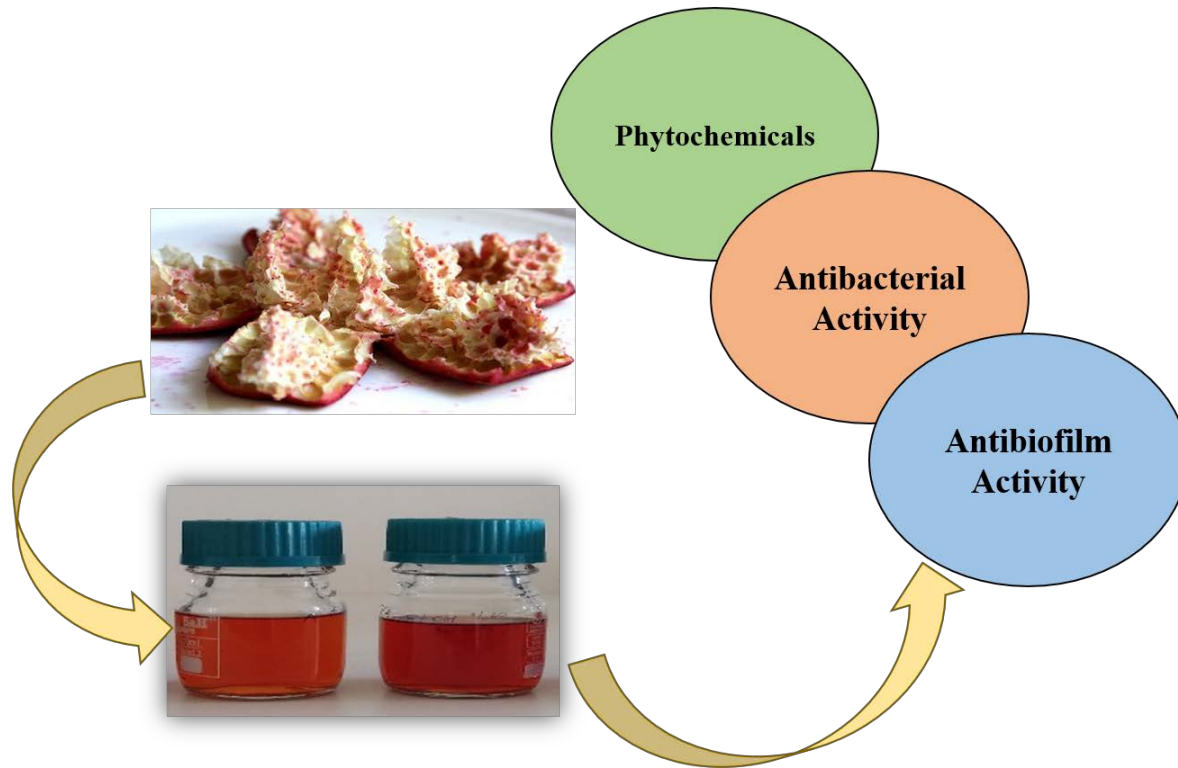


**Biofilm Removal**



Strain	OD <sub>570nm</sub>
<i>Bacillus cereus</i>	0.537 (0.003)
<i>Bacillus subtilis</i>	2.445 (0.090)
<i>Enterococcus faecalis</i>	0.889 (0.001)

- A. Methanol-acid;
- B. Methanol;
- C. Ethanol-acid;
- D. Ethanol
- E. Water
- Cl. Chlorine (200 ppm)



- Extracts from pomegranate peels represent a good source of **bioactive compounds**.
- **Methanol-acid** and **ethanol-acid** extracts exhibited the highest antimicrobial effects on all tested microorganisms, giving inhibition zones ranging in size from 17 mm to 36 mm.
- All extracts were generally more effective against **Gram-positive bacteria** than **Gram-negative ones**
- All PPL extracts diluted at different ratios inhibited and removed biofilms and the highest **antibiofilm** effects were up to **80%** by acid-treated extracts

## Screening for Bioactive Compound Rich Pomegranate Peel Extracts and Their Antimicrobial Activities

Extraction methods for increased antibacterial and antifungal properties







International Journal of Food Science and Technology 2021, 56, 4915–4924

4915

Original article

### **Antibiofilm effects of pomegranate peel extracts against *B. cereus*, *B. subtilis*, and *E. faecalis***

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HÖGSKOLAN  
I BORÅS

Thank you!

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