Extraction of bioactive phenolic and antioxidant compounds from lignified Salicornia ramosissima

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Extraction of bioactive phenolic and antioxidant compounds from lignified *Salicornia ramosissima*

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Background

Desalinisation: Globally each year an agricultural area the size of Portugal gets lost due to salinisation [1]. Halophyte plants, meaning salt tolerant plants, have the ability to take up large amounts of salt from soils. This is called bioremediation.

Phenolics - The forgotten 'vitamins': Phenolic compounds, previously referred to as Vitamin P, are a group of healthy and high value biochemical compounds found in large quantities in deeply coloured fruits and berries [2]. Recent discovery of these compounds in salt tolerant plants, halophytes, has opened a new possibility for profitable biorefinery of biomass from marginal lands with high salinity.

Biorefinery of *Salicornia ramosissima*: By extracting the phenolic compounds from *S. ramosissima* grown in saline marginal soils, the salts can be removed, and the phenolic compounds can be used in feed/food/pharma, hence also creating circular economy in rural areas.

Solvent optimisation

- Four different solvents in a Soxhlet system.
  1. Ethyl acetate (EtOAc)
  2. Water
  3. Ethanol (EtOH)
  4. 40 % EtOH
- Solvents were based on:
  - Hansen Solubility Parameters (HSP)
  - Toxicity and environmental impact
  - Hydrolysis capabilities

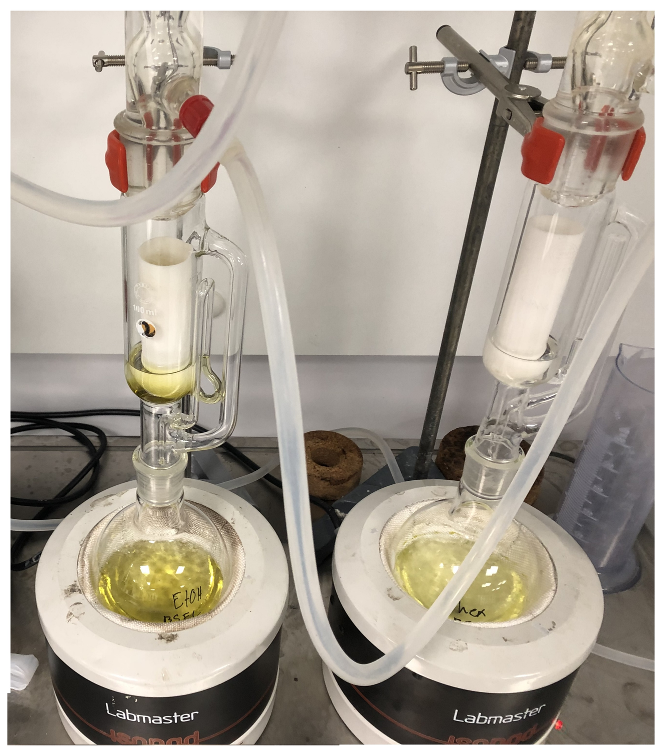

Fig. 1: Soxhlet extraction for reproducibility.

Solvent	HSP values	Hydrolysis capabilities	Toxicity
1.	Good	Bad	Medium
2.	Bad	Good	None
3.	Fair	Medium	Low
4.	Fair	Fair	Low

Table 1: Solvents compared.

Extraction of phenolics

To extract the bioactive phenolic compounds from the lignocellulosic matrix of *S. ramosissima*, different extraction methods were chosen. Water was used as solvent as it is the most sustainable solvent amongst the four, even though many of the phenolic compounds do not dissolve in water. Antioxidant capacity and total phenolics were analysed using the assays DPPH and Folin-Ciocalteu.

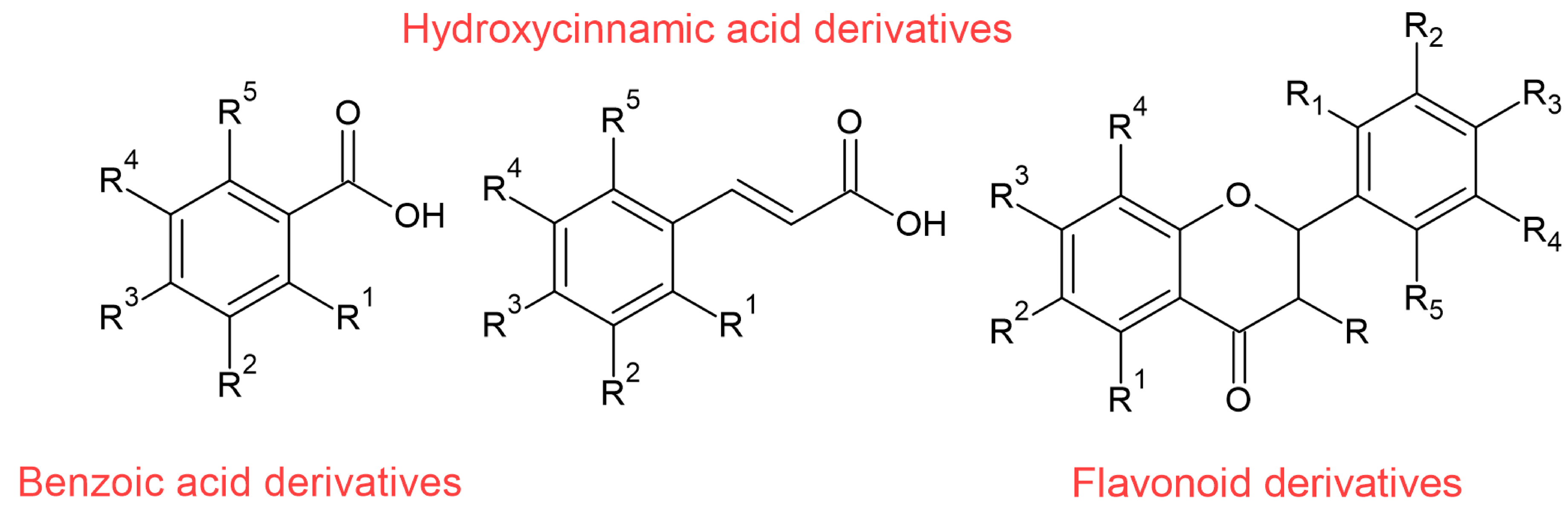

Fig. 2: Phenolic compounds comprises of different classes all including benzene rings, and at least one hydroxyl group. R^1-R_5 indicating carboxyl, hydroxyl, methyl, methoxy or glucosidic groups or hydrogen.

Fig. 3: *Salicornia* growing in the Danish Wadden Sea. Plants shown here are close to full lignification.

Phenolic compounds often contains hydroxyl and carboxyl groups, hence making them slightly acidic and often also possess antioxidant activities. Many of these compounds were bound to the lignocellulosic matrix, hence sometimes also glucosidic [3].

- **Extraction methods using water:**
  1. Maceration, 2 hours, 100 ° C
  2. Soxhlet, 8 hours, BP
  3. Ultrasound, 2 hours, 102 watt/L
  4. Sub-critical, 2 hours, 120 ° C

As the compounds stability was found highest when the compounds were protonated, the molecules in the extract were acidified below their pK_a values which also allowed for further separation of the compounds.

Results

Extraction method	Total phenolics [mg g_{DM}^{-1}]	Antioxidant capacity IC_{50} [µg g_{DM}^{-1}]
	Solvent optimisation	
1.	0	0
2.	3.31 ± 0.59	485.84 ± 96.44
3.	2.96 ± 0.26	302.73 ± 32.69
4.	**5.45 ± 1.00**	**916.69 ± 151.02**
	Extraction optimisation	
1.	2.29	259.43
2.	3.31 ± 0.59	485.84 ± 96.44
3.	2.41 ± 0.31	244.99 ± 20.81
4.	**4.00 ± 0.34**	**524.66 ± 51.07**

Table 2: Extraction methods compared.

Harvesting *S. ramosissima* for extraction of phenolic compounds can remove salt from salt affected fields, and provide healthy complex phenolic compounds for additional co-products in biorefining.

Conclusion

Investigating the extraction solvent and method, both total phenolics and antioxidant capacity have been evaluated. Water was chosen as the most sustainable and easiest to scale up, and shows good extraction efficiency at 120 °C subcritical extraction for 2 hours. This method can be used in rural areas to locally decrease salt concentrations in soil locally and create value for rural area farmers if followed by a down-stream process.

Fig. 4: Dried purified phenolic-rich extract.

References and acknowledgement

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[3] Chen, J. *et al.* (2020), "*Structure-antioxidant activity relationship of methoxy, phenolic hydroxyl, and carboxylic acid groups of phenolic acids*", Scientific Reports, Volume 10, Article number: 2611

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