



Determination of bioactive properties of extracts derived from Icelandic edible brown seaweed *Saccharina latissima*.

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Kelps

- **Kelps** are large seaweeds (algae) belonging to the brown algae (Phaeophyceae) in the order **Laminariales**. There are about 30 different genera
- Kelps contain high levels of iodine and therefore have been used to treat goiter since medieval times
- Kelps are rich in alginates that are especially used in food industry as thickeners in ice creams, jellies, dressings, toothpastes and dog food. Alginates found also an application in dentistry to make impressions of upper and lower arches
- Kelps contain large fractions of sulfated polysaccharides – **fucoidans** that show many health beneficial effects like anti-inflammatory, anti-diabetic or even anti-cancer



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Saccharina latissima (before: *Laminaria Saccharina* or *Fucus saccharinus*) – Sugar kelp, Royal Kombu, Sugar Wrack, Sea belt



http://en.wikipedia.org/wiki/File:Saccharina_latissima_NOAA.jpg



http://pendiva.com/seaweed/wp-content/uploads/2010/02/sugar_kelp.jpg



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Extractions

- **Cold water extraction:** 1:10 seaweed/ water 3h, room temperature on shaker
- **Hot water extraction:** 1:100 seaweed/ water, 30 min in 95 °C bath
- **Ethanollic extraction:** 1:25 seaweed / ethanol 70% for 24 h at room temperature with shaking
- All supernatants were spinned for 15 min at 3000 rpm and freeze dried
- Powders were prepared in dilutions for further experiments.
The stock concentration was 10 mg/ml



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THP-1 culture

- THP-1 cell line (monocytic, leukemia)
- Differentiated into macrophages by 200 ng/ml PMA, 72h incubation
- Extracts were prepared in dilutions in complete growth media and placed under UV light for 20 min.
- Tested extracts were added to medium at 5 concentrations: 1, 10, 100, 500 and 1000 µg/ml; incubation 24h at 37° C
- The control was with complete growth medium
- Collected medium was centrifuged at 3 500rpm for 10 min, aliquot and frozen at - 80° C for ELISA assay



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Methods

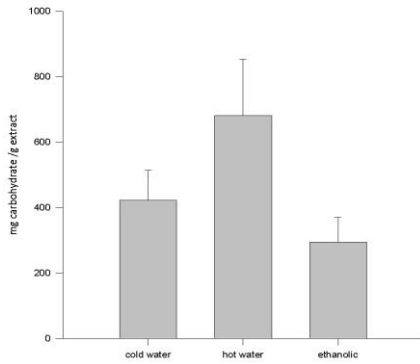
- **Total Carbohydrate Content** (phenol - sulphuric method) expressed in mg of fucose/ g dry extract
- **Total Polyphenol Content** (Folin Ciocalteu) expressed as Gallic Acid Equivalent (GAE)/ 100 g extract
- **ORAC** (Oxygen Radical Absorbance Capacity) for extracts expressed on µM of Trolox Equivalent/ g of extract
- **XTT** proliferation assay for 1, 10, 100, 500, 1000 µg/ml of saccharina extracts, 24h incubation, expressed as % of viability compare to the control
- **ELISA** assay for **IL-10, TNF-α and IL-6**
- **Statistics** by Sigma Stat and Sigma Plot, Multiple Comparisons versus Control Group (Holm-Sidak method):

Overall significance level = 0,05



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Total carbohydrate content

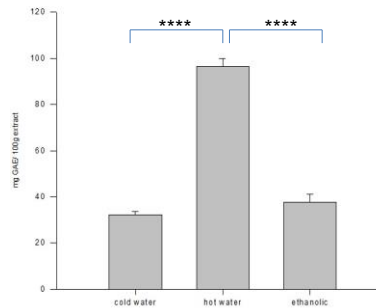


No statistically significant difference between groups $P=0,216$



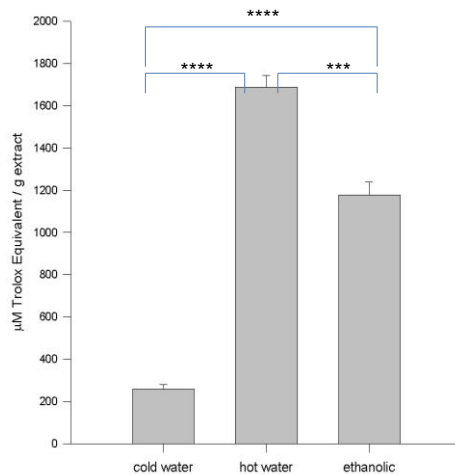
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Total Polyphenol Content (TPC)



	P	significant
hot water vs. cold water	0,00000414	yes
hot water vs. ethanolic	0,000007	yes
ethanolic vs. cold water	0,226	no

ORAC (Oxygen Radical Absorbance Capacity)



	µM T.E./g extract	SEM
cold water	258,987	21,623
hot water	1686,413	57,333
ethanolic	1176,467	63,018

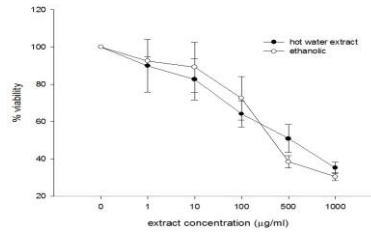
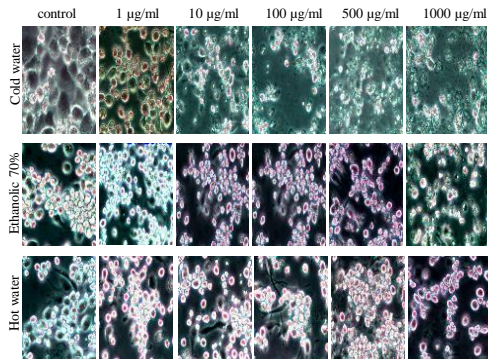
	P	significant
hot water vs. cold water	0,0000015	yes
ethanolic vs. cold water	0,0000141	yes
hot water vs. Ethanolic	0,00039	yes



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Cytotoxicity assessment



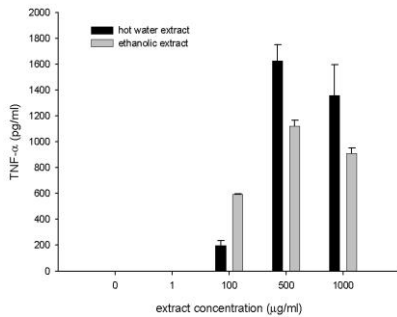
extract	concentration (µg/ml)	viability %	SEM
control	0	100	--
hot water	1	89.9	14.10
hot water	10	82.6	11.02
hot water	100	64.1	7.00
hot water	500	50.9	7.48
hot water	1000	35.1	3.08
ethanolic	1	92.5	2.15
ethanolic	10	89.2	13.36
ethanolic	100	72.5	11.67
ethanolic	500	38.5	3.28
ethanolic	1000	30.4	2.10



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Expression of TNF - α for hot water and ethanolic extracts



ethanolic extract (ug/ml)	TNF-α (pg/ml)	SEM
100	594,279	4,771
500	1120,39	44,361
1000	909,065	44,303

concentration	P	significant
100 vs. 500	0,0000504	yes
100 vs. 1000	0,00086	yes
500 vs. 1000	0,00624	yes

hot water extract (ug/ml)	TNF-α (pg/ml)	SEM
100	197,409	38,146
500	1625,442	126,754
1000	13562,2	241,048

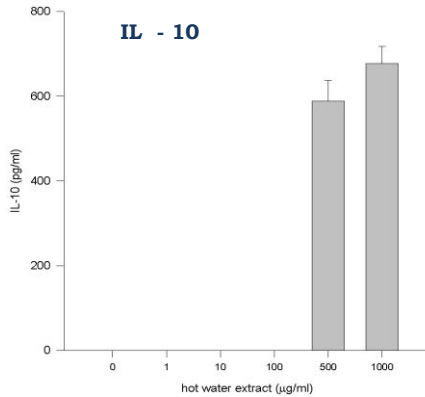
concentration	P	significant
100 vs. 500	0,000412	yes
100 vs. 1000	0,0018	yes
500 vs. 1000	0,22	no



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Cytokine expression



hot water extract (ug/ml)	IL-10 (pg/ml)	SEM
500	588,325	49,381
1000	677,529	39,659

IL-10 was expressed only for high concentrations of hot water extracts ($P=0,294$).



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Conclusions

- The antioxidant properties of extracts are dependent on extraction methods: hot water extraction provides extract with highest carbohydrate and polyphenol content that correspond well to estimated ORAC values
- Cold water extract was very cytotoxic to the cells even at low concentrations. After cytotoxicity assessed by microscopy further analysis was not performed
- Hot water and ethanolic extracts are relatively safe to the cells even at a quite high concentrations (100 ug/ml)
- Most likely hot water and ethanolic extracts are endotoxin free → IL -6 not expressed
- All extracts have pleasant flavor and therefore can be used as spice or food



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Sugar kelp salad (for brave ones)

Ingredients

- 500 g fresh (wet) sugar kelp or 30g dried seaweed
- 4 cloves of garlic, crushed
- 2 Tablespoons of apple cider vinegar
- 2 Tablespoons sesame oil (optional)
- 2 Tablespoons tamari soy sauce
- salt to taste
- 2 red chilies, finely chopped (optional)

Instructions

- Boil the kombu in water for 30 minutes (or until very tender - the dried kombu might need longer), then cool before cutting into thin strips (unless it's pre-cut into strips).
- Mix well with the crushed garlic, apple cider vinegar, sesame oil, and coconut aminos/tamari soy sauce.
- Add salt to taste and chopped red chilies for extra flavor and color.

<http://ancestralchef.com/simple-paleo-seaweed-kombu-salad/>



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