Anticancer compounds from Seaweed

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Introduction

- Much evidence of the benefits of seaweed consumption
- Animal studies and human studies aimed at understanding the lower risk of certain cancers in countries with high seaweed intakes
- This makes seaweed an interesting target for the extraction of natural products
- Natural products have been studied as chemotherapeutic agents for many years
- Marine organisms good candidates for drug discovery
- Source of novel compounds not found in terrestrial plants

Marine Drugs

Table 1 Anti-cancer agents from marine sources which are currently in clinical development

Chemical name and synonyms	Class of molecule	Isolated from		Stage of study
		Species	Class	
Aplidine Dehydrodidemnin B Plitidepsin	Depsipeptide	*Aplidium albicans*	Ascidian	Phase II clinical trial
Bryostatin-1	Macrocyclic lactone	*Bugula neritina*	Bryozoa	Phase II clinical trials
Cryptophycins	Depsipeptide	*Nostoc* sp. *Dysidea arenaria*	Cyanobacteria sponge	Phase II clinical trials on cryptophycin 52 discontinued in 2002, phase II trials of analogues in progress
Didemnin B	Cyclic depsipeptide	Didemnidae	Ascidian	Phase II clinical trials
Dolastatins	Peptide	*Dolabella auricularia*	Mollusk	Phase II clinical trials
Trabectedin and Yondelis	Isoquinoline alkaloid	*Ecteinascidia turbinata*	Ascidian	Approved for sarcoma and ovarian cancer, other clinical trials ongoing (Phase II/III)
Halichondrin B	Polyether macrolide derivative	Found in a variety of marine sponges	Sponge	Phase II clinical trials
Kahalalide F	Depsipeptide	*Elysia rufescens*	Mollusk	Phase I/phase II clinical trials

Data summarised from Mayer and Gustafson (2008) and updated by searching PubMed and http://www.marinebiotech.org/dfsindex.html

Table 2 Drugs in current use originally derived from marine sources

Chemical name	Brand name	Chemical class	Use	Isolated from	Class
Cytarabine	Cytosar-U® Depocyt®	Nucleoside	Anti-cancer	*Cryptotheca crypta*	Sponge
Vidarabine	Vira-A®	Nucleoside	Anti-viral	*Tethya crypta*	Sponge
Trabectedin	Yondelis®	Isoquinoline	Anti-cancer	*Ecteinascidia turbinata*	Sea squirt
Ziconotide	Prialt®	Peptide	Pain reliever	*Conus magus*	Snail

Introduction

Problems

- Synergistic or antagonistic effects in a crude mixture
- Adequate supply / industrially feasible?
- Variation in seasonal / spatial distribution of the bioactive of interest

Some solutions

- Environmentally sound, sustainable harvesting
- Mariculture / Aquaculture
- Find (semi)synthetic route to drug

Anticancer vs cytoprotective

Anti-cancer drugs

- Cytotoxic - kills cancer cells
- Used as a chemotherapeutic agent

Cytoprotective

- Protects cells
- Reduce DNA damage

May be a matter of dose

- Low dose may prevent oxidative damage

Overview

Aim: to determine the cytotoxicity of several crude seaweed extracts rich in polyphenols

Seven species of seaweed

- Four Brown, Two red, One green

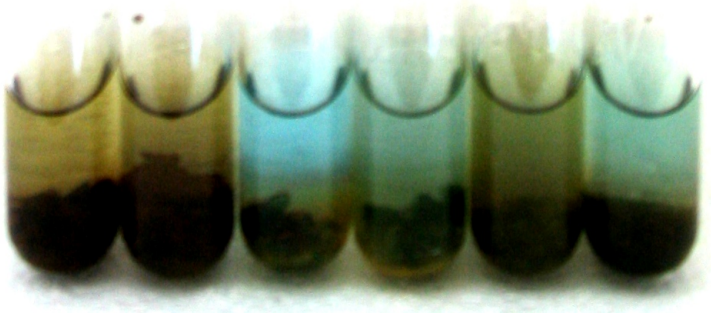
Several extraction methods

- Water, Enzyme assisted, Solvent

Methods

Polyphenol content was quantified by Folin-Ciocalteu method

- Standard method for total polyphenol determination
- Reacts with phenols and other reducing substances. Interferences have been shown to account for less that 5 % response
- Reacts to give a mixture of blue complexes which absorb between 725 and 750 nm
- The absorbance is proportional to the amount of polyphenols in the extract
- Phloroglucinol or gallic acid can be used as standards;
- Phloroglucinol for phlorotannins, gallic acid for red and green seaweed polyphenols



Methods

Cytotoxicity against MCF-7 (breast cancer) cells was tested

- Viable cell counts measured by trypan blue staining
- Excluded by live cells, but permeates dead cells
- Viable cells appear clear under a microscope and dead cells are dyed blue
- Docetaxel used as a control - an extract from the bark of the Pacific yew tree *Taxus brevifolia*.

Water extracts

- Brown fucoids highest in polyphenol content
- 4.0-6.0 % for brown
- 0.3-1.5 % for red and green
- Moderate polyphenol content compared to commercial extracts

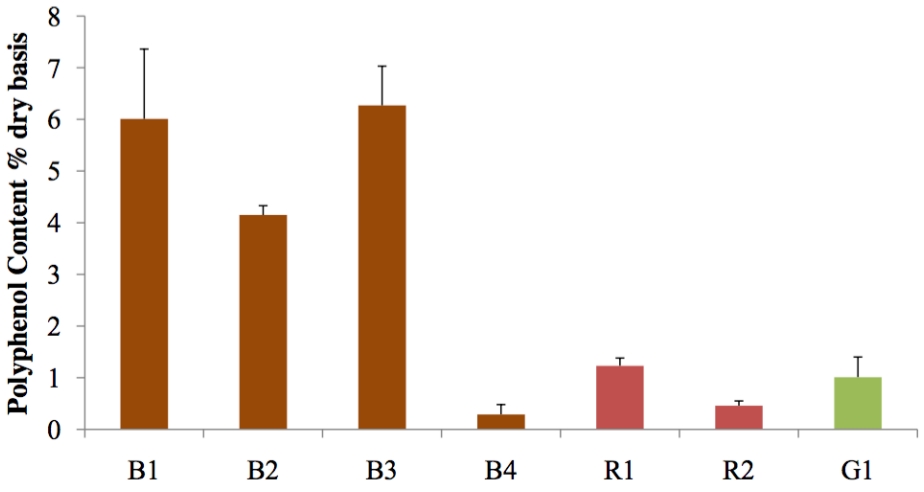


Figure 1: Polyphenol content of water based extracts

Water extracts

- All extracts had some cytotoxic effect
- In general, browns were better (50 % or more of cancerous cells killed compared to 75 % or less for red and green)

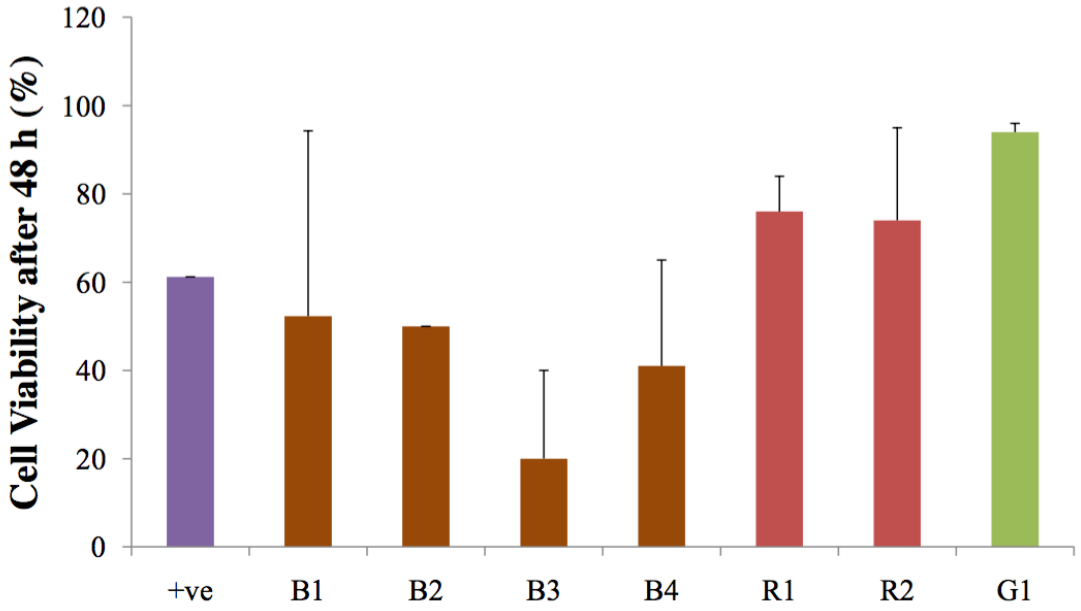


Figure 2: Cell viability after treatment with water based extracts

Enzyme assisted extracts

- Reports in the literature of using enzymes to enhance the extraction of bioactives*
- Cell wall can be degraded to make the intracellular contents more accessible for extraction
- Flavourzyme (protease) and Celluclast (carbohydrase) were used

*Wang et al., LWT - Food Science and Technology 43 (2010) 1387-1393 Li et al., Separation and Purification Technology 48 (2006) 189–196

Enzyme assisted extracts

- Celluclast extracts worked best (cell viability was reduced to 50-90 %)
- Not as good as water extracts
- Polyphenol content (not shown here) was not as high as for water extracts with exception of *Palmaria palmata*

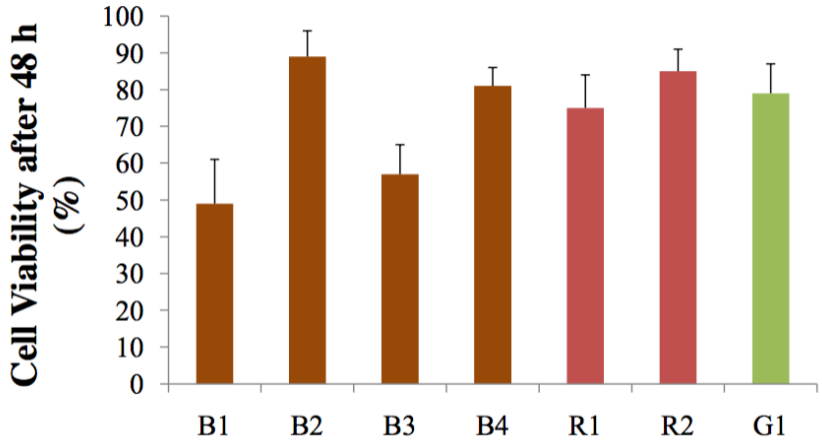


Figure 3: Viability of MCF-7 cells after 48 h treatment with celluclast based extracts

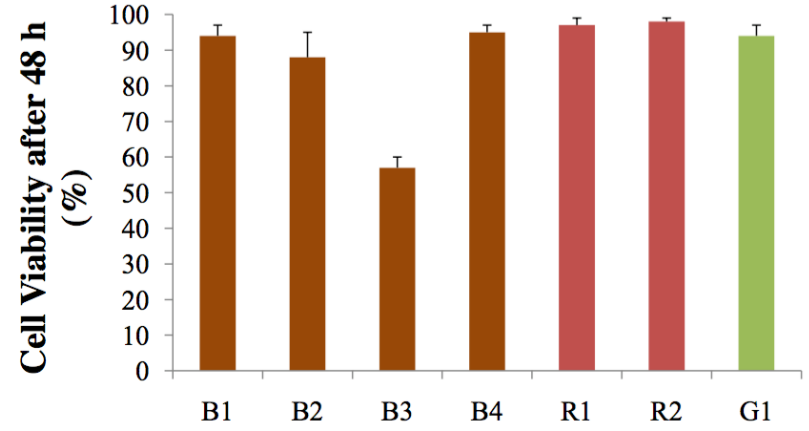


Figure 4: Viability of MCF-7 cells after 48 h treatment with flavourzyme based extracts

Solvent extracts

- Polyphenol contents were higher than water extracts (7-9 %)
- Also good cytotoxicity

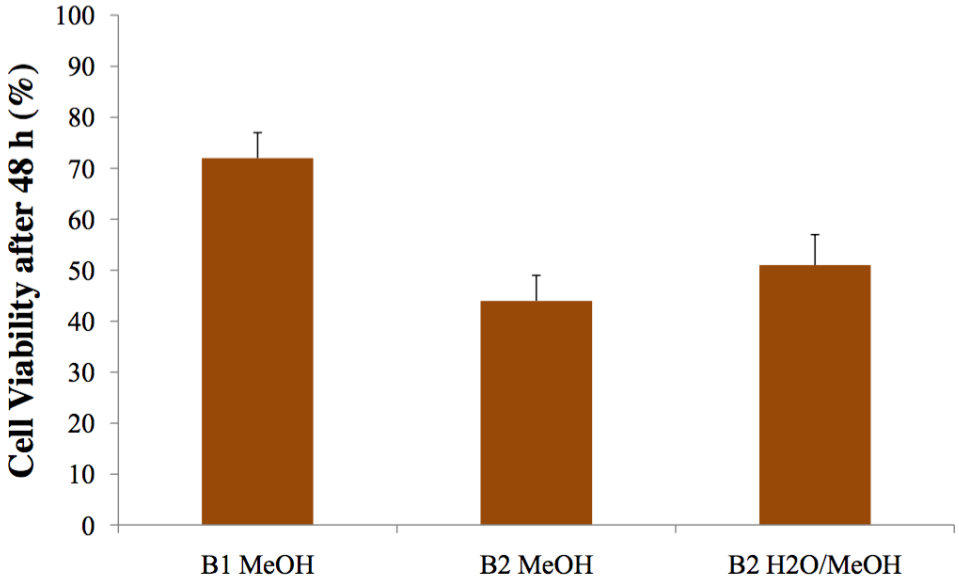
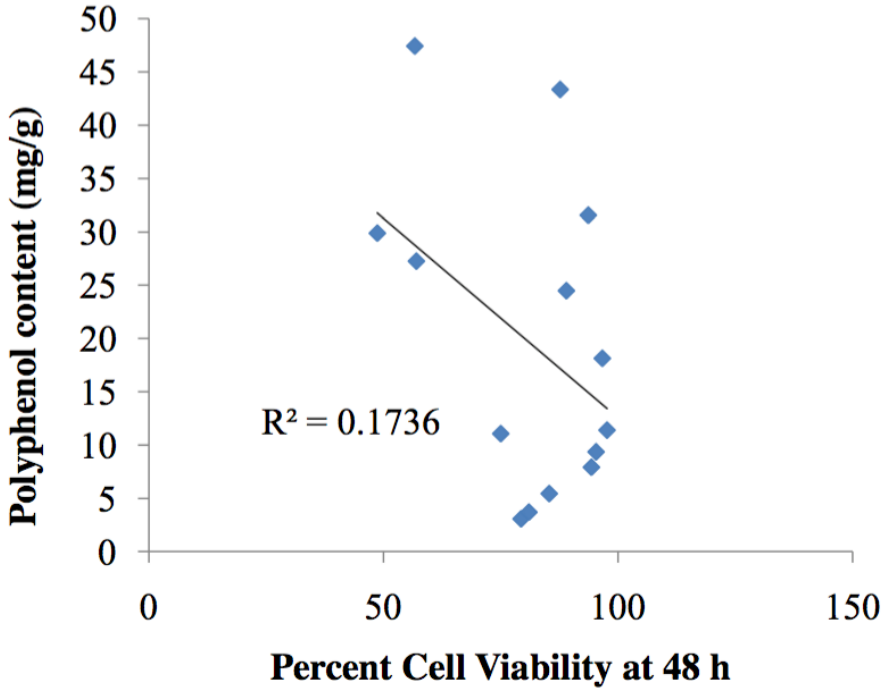


Figure 5 Viability of MCF-7 cells after 48 h treatment with solvent based extracts

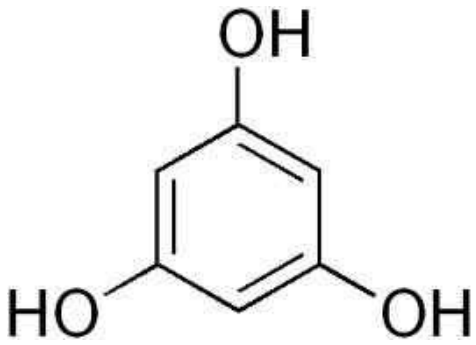
Correlation between polyphenol content and cytotoxicity?

- No correlation seen (R^2=0.17)
- Polyphenols not responsible for cytotoxic effect?



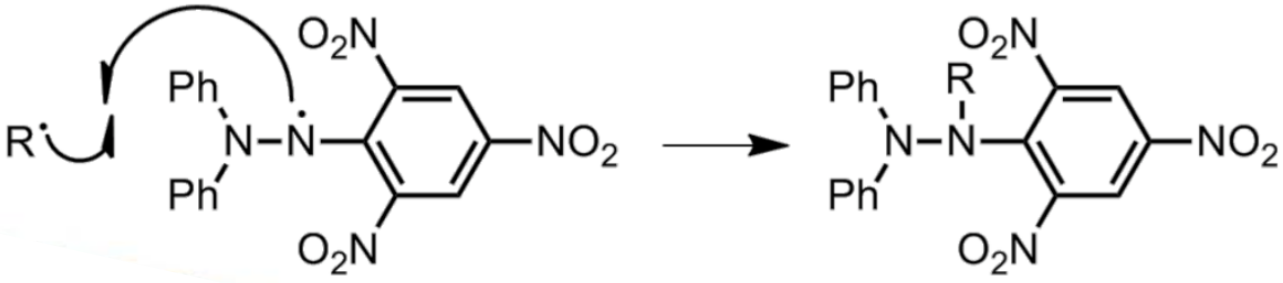
Cytoprotective effect

- Antioxidants may slow or prevent the development of cancer
- Protect cells from the action of free radicals which may damage DNA
- In vitro evidence for this, evidence from human clinical trials is not so clear
- Vitamin C, E, lycopene, **polyphenols**



Methods

- DPPH method for radical scavenging potential
- Commonly used
- DPPH (1,1-diphenyl-2-picrylhydrazyl) is a stable free radical
- Reduction of DPPH. Due to the presence of an odd electron it has a strong absorbance at 517 nm.
- Once this electron is paired (e.g., by the action of a free radical scavenging antioxidant/DPPH is reduced), the absorbance falls.



Results

- Extracts had good anti- radical effects
- A correlation between polyphenol content and anti-radical power was found (R^2=0.86)
- May be good cancer protective agents

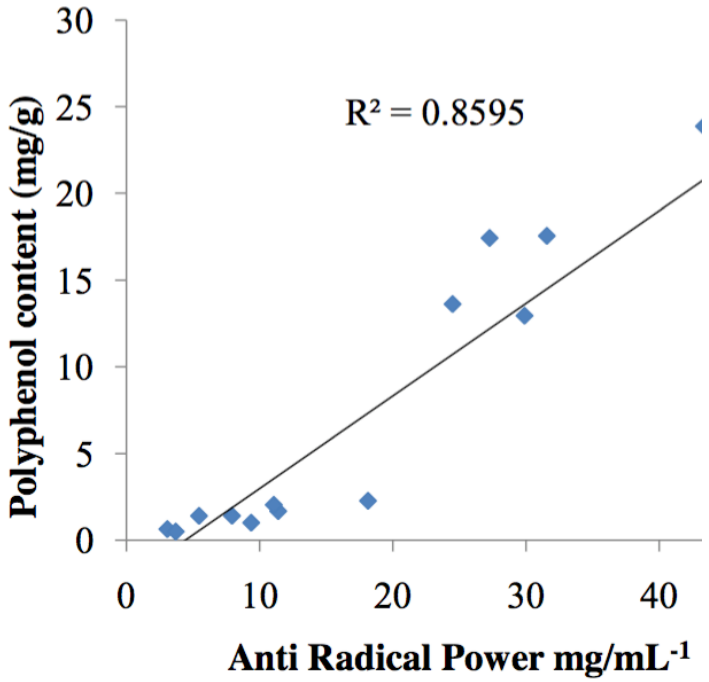


Figure 7 Correlation between polyphen content and anti-radical activity

Conclusions

- Good cytotoxic effect
- Cytotoxicity not related to polyphenol content
- Other compounds may be responsible: sulphated polysaccharides, carotenoids, other small molecules?
- Good antioxidant activity
- Maybe there is an application for high polyphenol extracts as cancer-protective agents
- Seaweed a good target for extraction of natural products

Future work

- Fractionate most promising extracts by semi- preparative HPLC and re-assay for cytotoxic effect
- Use tandem mass spectrometry as an aid towards the structural elucidation of compounds of interest

Acknowledgements

Komal Kaluskar and Alwynne Cleary who analysed samples for cytoxicity

Fore more information

See review article:

Murphy, C., Hotchkiss, S., Worthington, J. & McKeown, S. (2014). The potential of seaweed as a source of drugs for use in cancer chemotherapy. Journal of Applied Phycology. 26: 2211-2264.

J Appl Phycol
DOI 10.1007/s10811-014-0245-2

The potential of seaweed as a source of drugs for use in cancer chemotherapy

Catherine Murphy · Sarah Hotchkiss · Jenny Worthington · Stephanie R. McKeown

Received: 19 September 2013 / Revised and accepted: 21 January 2014

Abstract This review discusses studies on marine macroalgae that have been investigated for their potential as sources of novel anti-cancer drugs. The review highlights the very large number of studies of crude, partially purified and purified seaweed extracts, collected from many locations, which have shown potential as sources of potent anti-cancer drugs when tested in vitro and/or in vivo. The activity of polysaccharides, polyphenols, proteinaceous molecules, carotenoids, alkaloids, terpenes and others is described here. In some reports, mechanistic studies have identified specific inhibitory activity on a number of key cellular processes including apoptosis pathways, telomerase and tumour angiogenesis. However, despite the potential shown by these studies, translation to clinically useful preparations is almost non-existent. It is hoped this review will serve as a source document and guide for those carrying out research into the potential use of macroalgae as a source of novel anti-cancer agents.

Keywords Macroalgae · Tumour · Sulphated polysaccharide · Carbohydrate · Polyphenol · Carotenoid

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Published online: 20 February 2014

Introduction

This review provides an overview of the evidence that macroalgae-derived natural products provide a potential source for novel anti-cancer drugs. To contextualize their use, a short initial discussion of the issues relating to cancer treatment is provided.

Approaches to cancer treatment

The initial presentation of a tumour is the result of genetic changes which occur in critical genes within a cell that control its normal growth and development. This leads to unregulated proliferation of the cell and its progeny which results, except in the case of most haematological malignancies, in the development of a solid tumour. The increase in tumour size depends on a large number of interacting factors including a reduction in cell death (particularly through the apoptosis pathway), an increase in proliferation and the generation of new blood vessels to provide oxygen and nutrients to support the growing mass (King and Robins 2006). Tumours are classified in relation to their tissue of origin to include squamous cell carcinomas, adenocarcinomas, sarcomas, lymphomas and neuroectodermal cancers; however, even within each group, there is much heterogeneity (Weinberg 2007). Six main characteristics of tumours have been proposed: the ability to proliferate without reliance on external growth signals, insensitivity to anti-growth signals, resistance to apoptosis, limitless replicative potential, the ability to encourage angiogenesis and the ability to metastasise (Hanahan and Weinberg 2011). In many instances, this final characteristic is the one that results in death of the individual since there are very few effective treatments of metastatic cancer. As tumours grow, the genome becomes increasingly unstable with the acquisition of further deleterious mutations that promote malignant progression.

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