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Potential medicinal value of some South African seaweeds

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Eleven macroalgae were collected from the KwaZulu-Natal coast and nineteen species from the cooler Western Cape coast in March and April 2000. Ethanolic and aqueous extracts were made and tested for biological activity in the Cox-1 anti-inflammatory assay, in a nematode mortality bioassay for anthelminthic activity, an IC_{50} anticancer assay and a MIC antimicrobial assay. The ethanolic extracts were very active in the Cox-1 anti-inflammatory assay for almost all of the species tested. The aqueous extracts were not active. No anthelminthic mortality was detected in extracts from any of the species tested. Many of the extracts had cytotoxic activity against three cancer cell lines tested, with those from representative species of the Chlorophyta and Rhodophyta being the most effective. The extracts had much lower cytotoxic activity when tested on normal mouse fibroblasts (NIH3T3). Extracts from only a few species had antimicrobial activity with those of the Chlorophyta tested being the most effective against both the Gram-positive and Gram-negative bacteria.

Introduction

Many drugs used to treat ailments and diseases have severe side effects especially if used in long term treatment. Pathogens also develop resistance to drugs. It is thus necessary to constantly improve and develop new drugs. There is a trend in developed countries towards utilising natural drugs rather than synthetic drugs. This is not a new concept with many cultures having a history of traditional medicine. The World Health Organization (WHO) estimates that almost 65% of the world's population has incorporated herbal remedies into its primary health care (Fabricant and Farnsworth 2001).

The use of seaweed in traditional medicine is not nearly as extensive as the use of land plants. There are records in ancient Chinese medicinal literature of seaweed being used both as food and medicine prior to 2000BC (Abbott 1996). Seaweeds are eaten extensively in Asia while algal products such as agar, alginate and carageenans are widely used in food products in Western countries (Abbott 1996). Examples of medicinal use of seaweeds include *Sargassum* tea to treat diseases such as goiter and excess phlegm (Yan *et al.* 1998) and *Caulerpa taxifolia* used in herbal medicines in the Philippines as an antifungal and hypertensive agent (Ninomiya *et al.* 1998).

Algae are a diverse group of organisms which occupy a wide variety of ecological niches not occupied by land plants. Algae are also, evolutionarily-speaking, far older than land plants which are already known as a rich source of biologically active compounds. Natural products from land plants have already provided many important leads in pharmaceutical research (see Fabricant and Farnsworth (2001) for an extensive list of drugs derived from plants). In many ways, algae are similar to higher plants in their biochemical makeup. They also have a number of secondary metabolites that serve as chemical defence mechanisms against herbivory, fouling by epiphytes and larval settlement, and competitors for space (De Nys *et al.* 1998, De Lara-Isassi *et al.* 2000). It is thus highly probable that algae have the potential to provide an alternative source of leads to solving biomedical problems (Baker 1984, Bustos *et al.* 1992, Apt and Behrens 1999).

Seaweeds are biologically active in many assays and some bioactive compounds have been identified. For example, algae and Cyanobacteria show antiviral activity towards the mumps, influenza and the *Herpes simplex* viruses (Schaeffer and Krylov 2000, Huleihel *et al.* 2001). Some algae have anticoagulant activity (Matsubara *et al.* 2001), antifungal activity (Ballesteros *et al.* 1992) and anti-oxidant activity (Le Tutour *et al.* 1998, Yan *et al.* 1998, Ruberto *et al.* 2001). Water extracts of *Chlorella vulgaris* contain a prophylactic agent which controls allergies which are characterised by the production of allergen specific IgE (Hasegawa *et al.* 1999).

Antimicrobial activity has been the most widely investigated medicinal property in algae. Antibacterial activity in algae was first reported in the 1940s in the microalga *Chlorella vulgaris* where the fatty acid chlorellin was the active constituent (Robles Centeno and Ballentine 1999). There have since been numerous surveys highlighting algae as potential sources of antimicrobial substances (Sastry and Rao 1994). Results vary depending on the bacterial strains tested in conjunction with the solvents used for extraction. Ethanol is the most commonly used extracting solvent and is generally the most efffective at extracting the antibacterial compounds (Vlachos *et al.* 1996).

Anti-inflammatory activity is fairly well documented in seaweeds. Payá *et al.* (1990) and Bustos *et al.* (1992) tested seven Mediterranean seaweeds where both polar and non-polar extracts showed high anti-inflammatory activity in the Mouse Paw and Mouse Ear Edema assays. The red alga *Vidalia obtusaloba* has two bromophenols with anti-inflammatory activity, which are thought to be related to defence against marine herbivores (Wiemer *et al.* 1991). The brown alga *Caulocystis cephalornithos* has anti-inflammatory activity with the active principal identified as the trisubstituted aromatic ring 6-tridecylsalicylic acid (Kazlauskas *et al*. 1980). *Caulerpa taxifolia* is able to inhibit the lipooxygenase pathway and the active compound is a derivative of caulerpenyne (Ninomiya *et al.* 1998). Oxylipins are important in the treatment of many inflammatory diseases, heart diseases and ulcers and they have been found in many algal species from all the major taxonomic divisions. These show strong potential for development as new pharmaceutical agents or pharmacological probes (Gerwick *et al.* 1991). A few South African seaweeds have previously been screened using the Cox-1 inflammatory assay where almost all the ethanolic extracts were inhibitory and the aqueous extracts showed very little inhibitory activity (Stirk *et al.* 1996).

As with higher plants, the progress of developing marine natural products for pharmacological use proceeds in two ways. Firstly, there is the screening of crude extracts to identify sources of potentially important compounds. This screening must be for a diverse range of assays and should also determine seasonal and geographical variation. Secondly, one needs to isolate and identify the active principal and study its pharmacological properties (De Freitas 1990). Although a number of novel bioactive compounds have been identified in algae, none have been developed into commercial pharmaceutical products. The probability of developing a commercial product is directly correlated with the amount of screening and there has been considerably less attention focussed on the algae than on higher land plants (Apt and Behrens 1999).

The aim of this work was to screen some crude extracts of common South African macroalgae in a number of assays to determine their potential medicinal value and to indicate areas where more intensive screening should be conducted. The biological activities tested were the Cox-1 anti-inflammatory, anthelminthic, antifungal, anticancer and antimicrobial activity.

Material and Methods

The seaweeds were collected from Rocky Bay on the KwaZulu-Natal coast (30°23'S, 30°43'E) in March 2000 and from Kommetjie on the Western Cape coast (34°08'S, 18°43'E), South Africa in April 2000. The KwaZulu-Natal coast is subtropical where temperatures seldom drop below 20°C while the Western Cape coast is a cool temperate region affected by upwelling where the temperatures vary between 8–16°C and the waters are nutrient rich (Branch and Branch 1985). Seaweeds were transported to the laboratory in a cooler box where they were immediately identified, washed and epiphytes removed before being air-dried in front of a fan at room temperature. The dried seaweed was stored for up to one year at 10°C until needed.

The dried material was ground to a fine powder using liquid nitrogen. Ethanolic (100% ethanol) and aqueous (distilled water) extracts were prepared by adding 20ml of the solvent to 2g of the dried seaweed powder. The extracts were sonicated in an ultrasound bath for 30min and left to stand overnight at 10°C after which they were sonicated for a further 15min. The extracts were filtered under vacuum through Whatman No. 1 filter paper and then dried down in a stream of air. The resulting extract weights were recorded and the extracts stored at 10°C until needed. These extracts were used in the anti-inflammatory and anthelminthic assays.

For the antimicrobial and anticancer assays, 0.5–1g of the original dried material was extracted with 30ml 100% ethanol for 2h. After centrifugation at 15 000rpm at 4°C for 15min, the pellet was re-extracted using the same method. Both extracts were combined and purified using a C18 column to remove the chlorophyll and then dried at 35°C under vacuum. The dried extracts were dissolved in 200µl 50% methanol and 800µl 0.1M Tris.HCl (pH 7.5), filter sterilised using a 0.2µm filter and used in the antimicrobial and anticancer assays.

Cyclooxygenase-1 (Cox-1) assay for anti-inflammatory activity

The Cox-1 assay was performed according to the method of White and Glassman (1974) with modifications by Jäger *et al.* (1996) to test for anti-inflammatory activity where inhibition refers to the reduction of prostaglandin formation in comparison to an untreated control. Indomethacin was used as a positive control. Ethanolic and aqueous extracts (as described above) of the Cape algae were screened. The residue of the ethanolic extracts were resuspended in ethanol at 10mg ml^{-1} and the residue of the aqueous extracts were resuspended in distilled water at 2.5mg ml^{-1} to give the final test concentration of the extract as 250µg ml^{-1} and 500µg ml^{-1} for the ethanolic and aqueous extracts respectively. All samples were tested in duplicate in the assay to ensure accurate assay results. The means were determined and presented as percentage inhibition. This was calculated using the ^{14}C conversion for solvent blanks and untreated controls where no inhibitory compounds were added which gave the maximum conversion of arachidonic acid to prostaglandins in the assay conditions used.

Nematode mortality assay for anthelminthic activity

Caenorhabditis elegans var. Bristol (N2) nematodes were cultured on nematode growth agar seeded with *E. coli* following the method of Brenner (1974). A simple nematode mortality bioassay described by Rasoanaivo and Ratsimamanga-Urveg (1993) and modified by McGaw *et al.* (2000) was used to screen the aqueous extracts of the Cape algae. Algal extracts were resuspended at 50mg ml^{-1} in distilled water and added to the sterile repli-dishes to give a final test concentration of 1mg ml^{-1}. These nematode cultures were incubated for 2h at 25°C in the dark. Nematode mortality was measured by comparing the movement, using a dissecting microscope, of the nematodes in the test extracts to those in the control and expressed as a percentage. The drug levamisole was used as an internal standard at the concentration 5µg ml^{-1}. The experiment was repeated twice.

Anticancer IC_{50} assay

Both Cape and KwaZulu-Natal macroalgae were screened for activity. This bioassay used cell lines of different histogenetic and species origin: the panel of cell lines used was the *MCF7* (human breast adenocarcinoma), *CEM* (human lymphoblastoid leukaemia) and *G361* (human malignant melanoma) cancer cell lines, with the *NIH3T3* (mouse fibroblasts) as a normal cell line. The cell lines were grown in DMEM medium (Gibco BRL) supplemented with 10% (v/v) foetal bovine serum and L-glutamine and maintained at 37°C in a humidified atmosphere with 5% CO_2. Each well of a 96 well plate was seeded with 10^4 cells, allowed to stabilise for at least 4h**,** after which 20µl of the algal test extracts was added at six concentrations ranging from 0.5–0.005g ml^{-1}. These assays were repeated on three occasions. The plates were incubated at 37°C and 5% CO_2 for three days after which Calcein AM solution (Molecular Probes) was added. After 1h, the fluorescence of the viable cells was quantified using Fluoroscan Ascent (Microsystems). The IC_{50} value, the extract concentration lethal to 50% of the tumour cells, was calculated from the dose response curve.

Antimicrobial MIC assay

Ethanolic extracts of Cape and KwaZulu-Natal algae were screened in this bioassay using the Gram-postive bacteria strains *Enterococcus faecalis* 4224, *Staphylococcus aureus* 3953 and 4223 and the Gram-negative bacteria *Pseudomonas aeruginosa* 3955 and *Escherichia coli* 3954, 3988 and 4225 (Czech Collection of Microorganisms, Brno). Bacteria were precultured for 2h at 37°C in 5ml Mueller-Hinton Broth and then diluted with distilled water in a 1:10 ratio and inoculated into the sterile wells containing the algal extract in the cultivation medium. The dried ethanolic extracts of the tested algae were resuspended in distilled water (1g ml^{-1}). The cultivating medium for Gram-positive bacteria contained 37g Brian Heart Infusion in 1l distilled water. The cultivating medium for Gram-negative bacteria consisted of 5g Protose-BE, 17.5g Casein Acid Hydrolysate and 0.06g Na_2CO_3 in 1l distilled water. Wells of microtiter plates containing serial two-fold dilutions of the extracts were inoculated with the bacteria inoculum and incubated for 18h at 37°C. The wells were then examined for the presence of bacterial growth. The lowest concentration of each extract dilution series that prevented bacterial growth was considered to be the minimum inhibitory concentration (MIC) of the extract. There were three replicates per assay and each assay was repeated twice.

Results

Cox-1 assay for anti-inflammatory activity

The ethanolic extracts from most of the species tested were highly inhibitory in the Cox-1 assay with eleven of the twelve species having similar or higher inhibition than that of the indomethacin standard (72% inhibition, Table 1). All of the water extracts showed less inhibition than that of the indomethacin standard (71% inhibition, Table 1). It was not possible to make water extracts for all the seaweeds as many formed a polysaccharide gel, such as alginate, when exposed to water.

Nematode mortality assay for anthelminthic activity

The levamisole internal standard showed 100% mortality and the water control showed 100% nematode survival after 2h incubation. In all the algal test extracts, there was 100% nematode survival indicating no anthelminthic activity in the aqueous extracts of the Cape algae.

Table 1: Cox-1 inhibitory activity of ethanol and water extracts of the Cape macroalgae. The final test concentration of the extracts was 250µg ml^{-1} for the ethanolic extract and 500µg ml^{-1} for the aqueous extract. nt = not tested

Species	Inhibition (%)	
	Ethanolic extract	Aqueous extract
Indomethacin (20µM)	72	71
Chlorophyta		
Cladophora capensis	91	56
Ulva sp.	76	nt
Phaeophyta		
Bifurcaria brassicaeformis	78	55
Ecklonia maxima	97	68
Laminaria pallida	nt	49
Macrocystis angustifolia	78	48
Sarcothalia stiriata (gametophyte)	83	nt
Sarcothalia stiriata (sporophyte)	79	nt
Rhodophyta		
Hypnea spicifera	5	50
Mazzaella capensis	71	nt
Carradoriella virgata	90	nt
Porphyra capensis	95	nt
Suhria vittata	80	nt
Aeodes orbitosa	nt	43
Hymenena venosa	nt	53

Anticancer IC_{50} assay

The algal extracts were active against all of the three cancer cell lines tested. Higher effectiveness of the algal extracts was found when tested on the cell lines bearing various mutations or deletions in the cell-cycle-associated proteins e.g. CEM cell line. This indicates that these extracts should be effective in tumours with various alterations of tumour suppressor genes such as p53 and pRb. Growth of the normal mouse fibroblasts was also affected but only when subjected to concentrations at least 10 times higher than those experienced by the cancer lines. Generally the Phaeophyta were the least effective with high concentrations of extract required to obtain IC_{50}. The Chlorophyta tested showed the most biological activity against the CEM cell line, with very low concentrations of *Caulerpa filiformis* and *Halimeda cuneata* extracts needed to obtain IC_{50}. These chlorophyte extracts also effectively inhibited growth in the other three cancer cell lines. The Rhodophyta tested were generally able to inhibit all the cancer cell lines at low extract concentrations with the KwaZulu-Natal algae *Amphiroa ephedraea*, *Arthrocardia* sp. and *Corallina* sp. (all Corallinaceae) and the Cape *Gigartina clathrata*, *G. polycarpa* and *Sarcothalia scutellata* (all Gigartinaceae) showing the most effective biological activity (Table 2).

Antimicrobial MIC assay

Extracts from the chlorophytes tested had the most biological activity, with those from *Caulerpa filiformis* and *Halimeda cuneata* inhibiting both Gram-positive and Gram-negative bacteria. None of those from the Phaeophyta tested inhibited bacterial growth and only three extracts of the Rhodophyta tested, *Arthrocardia* sp., *Hypnea spicifera* and *Suhria vittata*, were able to inhibit Gram-positive bacteria (Table 3).

Discussion

As in previous Cox-1 anti-inflammatory screenings of South African seaweeds (Stirk *et al.* 1996), the aqueous extracts had low inhibitory activity and the ethanolic extracts were highly active for most of the seaweed extracts. The ethanolic extracts were tested at a lower concentration than that of the previous screening study (250µg ml^{-1} as opposed to the earlier 500µg ml^{-1}), suggesting that the active compound(s) is/are very effective. Seasonal variation in biological activity is known to occur in some seaweed species. For example, of the five South African seaweed species tested for seasonal variation in antimicrobial activity, only one species,

Table 2: IC_{50} inhibitory concentrations (mg $well^{-1}$) of the Cape (C) and KwaZulu-Natal (KZN) macroalgae tested against cancer cell lines. n = 3

Species	Province of origin	Cancer cell lines		Normal cell lines	
		MCF7	*CEM*	*G361*	*NIH3T3*
Chlorophyta					
Caulerpa filiformis	KZN	0.50 ± 0.10	0.004 ± 0.0005	0.47 ± 0.12	1.02 ± 0.21
Halimeda cuneata	KZN	>0.20	0.007 ± 0.0003	0.13 ± 0.02	0.43 ± 0.10
Ulva sp.	KZN	0.40 ± 0.08	0.13 ± 0.02	1.38 ± 0.14	1.55 ± 0.21
Cladophora capensis	C	1.80 ± 0.25	1.00 ± 0.18	>2.0	>2.0
Ulva sp.	C	2.10 ± 0.35	0.94 ± 0.11	>2.0	>2.0
Phaeophyta					
Sargassum heterophyllum	KZN	1.40 ± 0.26	0.11 ± 0.02	1.62 ± 0.19	1.85 ± 0.24
Bifurcaria brassicaeformis	C	>2.0	>2.0	>2.0	>2.0
Splachnidium rugosum	C	3.40 ± 0.80	1.00 ± 0.24	>2.0	>2.0
Laminaria pallida	C	>5.0	1.40 ± 0.26	>2.0	>2.0
Macrocystis angustifolia	C	1.00 ± 0.21	0.33 ± 0.07	1.75 ± 0.32	>2.0
Rhodophyta					
Amphiroa bowerbankii	KZN	8.20 ± 2.70	0.06 ± 0.015	1.03 ± 0.18	1.28 ± 0.23
Amphiroa ephedraea	KZN	0.06 ± 0.009	0.04 ± 0.006	0.95 ± 0.18	1.06 ± 0.25
Arthrocardia sp.	KZN	0.03 ± 0.005	0.03 ± 0.007	0.78 ± 0.16	0.95 ± 0.21
Cheilosporum sp.	KZN	1.10 ± 0.29	<0.02	0.12 ± 0.02	0.45 ± 0.09
Corallina sp.	KZN	0.03 ± 0.004	0.03 ± 0.005	0.39 ± 0.06	0.67 ± 0.12
Jania sp.	KZN	1.30 ± 0.21	0.01 ± 0.002	0.12 ± 0.02	0.35 ± 0.06
Aeodes orbitosa	C	0.90 ± 0.21	0.08 ± 0.005	1.12 ± 0.21	1.15 ± 0.23
Gigartina clathrata	C	0.07 ± 0.012	0.06 ± 0.009	0.68 ± 0.14	0.65 ± 0.10
Gigartina polycarpa	C	0.09 ± 0.01	0.05 ± 0.008	0.53 ± 0.12	0.74 ± 0.22
Sarcothalia scutellata	C	0.10 ± 0.02	0.07 ± 0.014	0.14 ± 0.09	0.32 ± 0.10
Hymenena venosa	C	0.28 ± 0.09	0.09 ± 0.02	0.29 ± 0.08	0.54 ± 0.12
Hypnea spicifera	C	0.46 ± 0.11	0.16 ± 0.08	0.92 ± 0.32	1.23 ± 0.28
Mazzaella capensis	C	2.20 ± 0.34	0.06 ± 0.02	0.18 ± 0.05	0.45 ± 0.11
Nothogenia erinacea	C	0.12 ± 0.02	0.05 ± 0.008	0.34 ± 0.08	0.52 ± 0.13
Plocamium corallorhiza	C	1.14 ± 0.25	0.26 ± 0.09	0.91 ± 0.18	1.25 ± 0.24
Carradoriella virgata	C	1.56 ± 0.35	0.49 ± 0.12	1.32 ± 0.26	1.54 ± 0.32
Porphyra capensis	C	0.38 ± 0.11	0.18 ± 0.05	0.63 ± 0.12	0.85 ± 0.14
Sarcothalia stiriata	C	0.24 ± 0.06	0.04 ± 0.002	0.31 ± 0.07	0.56 ± 0.12
Suhria vittata	C	0.46 ± 0.15	0.16 ± 0.05	0.85 ± 0.21	1.18 ± 0.35

Table 3: Antimicrobial activity (MIC) of extracts of Cape (C) and KwaZulu-Natal (KZN) macroalgae. *Sa* = *Staphylococcus aurens*, *Ef* = *Enterococcus faecalis*, *Ec* = *Escherichia coli*, *Pa* = *Pseudomonas aeruginosa*. Bold figures highlight the most active extracts

Species	Province of origin	Minimal Inhibitory Concentration (mg ml^{-1})						
		Gram-positive bacteria			Gram-negative bacteria			
		*Sa*3953	*Sa*4223	*Ef*4224	*Ec*4225	*Ec*3988	*Ec*3954	*Pa*3955
Chlorophyta								
Caulerpa filiformis	KZN	**0.0065**	**0.0065**	**0.0325**	**0.0325**	**0.065**	>0.0325	**0.0065**
Halimeda cuneata	KZN	**0.025**	**0.025**	**0.025**	**0.025**	**0.013**	**0.025**	**0.025**
Ulva sp.	KZN	>0.15	>0.15	>0.15	0.15	**0.075**	0.15	**0.075**
Cladophora capensis	C	**0.0625**	**0.0625**	0.125	>0.25	0.125	>0.25	>0.25
Ulva sp.	C	>0.25	>0.25	>0.25	0.25	0.125	0.25	0.125
Phaeophyta								
Sargassum heterophyllum	KZN	>0.175	>0.175	>0.175	>0.175	>0.175	>0.175	>0.175
Bifurcaria brassicaeformis	C	>0.25	>0.25	>0.25	>0.25	>0.25	>0.25	>0.25
Splachnidium rugosum	C	>0.25	>0.25	>0.25	>0.25	>0.25	>0.25	>0.25
Laminaria pallida	C	>0.25	>0.25	>0.25	>0.25	>0.25	>0.25	>0.25
Macrocystis angustifolia	C	0.25	0.25	>0.25	>0.25	0.25	0.25	>0.25
Rhodophyta								
Amphiroa bowerbankii	KZN	0.25	0.25	>0.25	>0.25	>0.25	>0.25	>0.25
Amphiroa ephedraea	KZN	>0.22	>0.22	>0.22	>0.22	>0.22	>0.22	>0.22
Arthrocardia sp.	KZN	0.13	**0.065**	>0.13	>0.13	>0.13	>0.13	>0.13
Cheilosporum sp.	KZN	0.12	0.12	>0.12	>0.12	>0.12	>0.12	>0.12
Corallina sp.	KZN	0.1375	>0.1375	>0.1375	>0.1375	>0.1375	>0.1375	>0.1375
Jania sp.	KZN	0.1425	>0.1425	>0.1425	>0.1425	>0.1425	>0.1425	>0.1425
Aeodes orbitosa	C	>0.25	>0.25	>0.25	>0.25	>0.25	>0.25	>0.25
Gigartina clathrata	C	>0.25	>0.25	>0.25	>0.25	>0.25	>0.25	>0.25
Gigartina polycarpa	C	0.25	>0.25	>0.25	>0.25	>0.25	>0.25	>0.25
Sarcothalia scutellata	C	>0.22	>0.22	>0.22	>0.22	>0.22	>0.22	>0.22
Hymenena venosa	C	>0.25	>0.25	>0.25	0.125	0.125	0.25	0.25
Hypnea spicifera	C	**0.0425**	**0.0425**	**0.0425**	>0.17	0.17	>0.17	0.17
Mazzaella capensis	C	>0.25	0.25	>0.25	>0.25	>0.25	>0.25	>0.25
Nothogenia erinacea	C	>0.25	>0.25	>0.25	>0.25	>0.25	>0.25	>0.25
Plocamium corallorhiza	C	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Carradoriella virgata	C	0.125	0.125	>0.25	>0.25	>0.25	>0.25	>0.25
Porphyra capensis	C	0.1475	0.1475	>0.1475	>0.1475	0.1475	>0.1475	>0.1475
Sarcothalia stiriata	C	>0.25	>0.25	>0.25	>0.25	>0.25	>0.25	>0.25
Suhria vittata	C	**0.0625**	**0.0625**	**0.0625**	>0.25	>0.25	>0.25	0.25

Osmundaria serrata (Rhodophyta), showed increased activity in material collected during winter (Vlachos *et al.* 2001). Fourteen species collected from the coast of India all had increased antibacterial activity after the monsoon season (Vidyavathi and Sridhar 1991). A comparison of the Cox-1 anti-inflammatory activity from summer (1994; Stirk *et al.* 1996) and autumn (2000; current study) extracts suggests that these active principals are not greatly influenced by season.

There is ethnopharmacological evidence that seaweeds contain active anthelminthic compounds. In China, children infected with intestinal worms are treated with the seaweeds *Digenea simplex* and *Caloglossa leprieurii,* both of which contain the anthelminthic kainic acid, and *Chondria* species, where the active principal is domoic acid (Tseng 2001). Only the water extracts of the Cape algae were tested in the current screening study as McGaw *et al.* (2000) showed that, in higher land plants, the water extracts show more inhibitory activity than ethanol extracts. None of the macroalgae tested were able to kill the nematodes. However, other side effects, such those on the reproductive potential, were not considered.

The results obtained from the anticancer assay were particularly promising with many of the species tested being cytotoxic to the different histogenetic cancer cell lines. On the other hand, cytotoxicity of the algal ethanolic extracts for normal mouse fibroblasts was more than 10x lower. Further studies are needed in this respect. There are also some other studies confirming that algal extracts are cytotoxic. Ballesteros *et al.* (1992) found that 35% of the 71 seaweeds tested using kidney cells of monkey were cytotoxic, with over half of the chlorophytes tested being cytotoxic. *Chlorella vulgaris* extracts administered orally to tumour-bearing mice significantly prolonged their survival by enhancing phagocyte production and quality (Justo *et al.* 2001). Caulerpenyne from *Caulerpa taxifolia* is also cytotoxic and inhibits growth in human cancer cell lines by modifying the microtubule network (Barbier *et al.* 2001).

Ballesteros *et al.* (1992) found only 6% of the 71 Mediterranean seaweeds tested had antibacterial activity. Similarly, only a few of the macroalgae tested in this study had antimicrobial activity with 23% of the 30 species tested being active. The Chlorophyta predominated, with four of the five species tested being active against both Gram-positive and Gram-negative bacteria. The Rhodophyta tested showed limited antibacterial activity while none of the

Phaeophyta tested showed antibacterial activity. Particularly promising is that the chlorophyte species tested were effective against the Gram-negative bacterial strains. Many antibiotics are less active against Gram-negative than Gram-positive bacteria, probably as a result of the more complex cell wall structure with additional lipopolysaccharides on the outer surface of the Gram-negative bacteria which makes penetration of the extracts more problematic (Rang and Dale 1987).

Previously, Vlachos *et al.* (1997) screened 56 South African seaweeds for antimicrobial activity using an agar-overlay diffusion method. Unlike the present study, the authors found that the Phaeophyta they tested were the most effective antimicrobial agents, mainly against Gram-positive and a few Gram-negative bacterial strains, while the Rhodophyta and Chlorophyta investigated showed limited inhibitory activity against the Gram-positive bacterial strains tested (Vlachos *et al.* 1997, 1999). However, they used different extraction methods, boiling the extracts which could have denatured heat sensitive compounds (Vlachos *et al.* 1997). This extraction method was established in a previous comparative study (Vlachos *et al.* 1996) designed to optimise the protocol for testing antimicrobial activity in seaweeds and testing different bacterial strains. Vlachos *et al.* (1997) also did not mention the concentration of the seaweed extracts tested so comparisons between bioassays or species cannot be made. The agar diffusion method used by Vlachos *et al.* (1997) relies on the size, diffusibility and chemical properties (eg. hydrophobicity) of the inhibitory compound/s which could influence the results.

Before specific algae can be chosen for the isolation and identification of the active compounds, seasonal and geographic variation also needs to be considered. The comparison of the results from the Cox-1 anti-inflammatory assay in this study and from Stirk *et al.* (1996) indicates that the active anti-inflammatory compounds are not sensitive to seasonal changes. However, this is not necessarily true for all biologically active compounds. Robles Centeno and Ballentine (1999) grew male and female gametophytes and tetrasporophytes of the red alga *Spyridia filamentosa* over a range of irradiance conditions and then tested the extracts against five microorganisms for antibiotic activity. Even small changes in irradiance conditions resulted in different activities and the degree of activity for the various extracts. The various gametophyte and tetrasporophyte cultures also showed different biological activity. This shows that there are optimal conditions for the synthesis of secondary compounds. Thus, screening studies based on a single collection probably underestimate potential biological activity. This emphasises the need for many such screening studies to be undertaken using different bioassays so that seasonal and geographical assessment of biological activity can be made.

This study has shown that seaweeds are biologically active in many assays and that they do contain many secondary metabolites which can potentially be utilised for pharmaceutical research. Future work needs to use a wider variety of assays before the full potential of seaweeds as pharmaceutical agents can be realised. The areas highlighted in this study with the most potential are anti-inflammatory, cytotoxic and antimicrobial biological activity. Of the organisms currently tested, those belonging to the Chlorophyta generally produced the most effective inhibitors and those belonging to the Phaeophyta had the least biological activity.

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