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PROGRESS REPORT: 2011-2012 SUMMARY

Investigation of the natural product composition of the seaweed material *CEM-K*

1.1 Introduction

The approach towards optimizing recovery and identification of natural products initially required a comparative extraction processing of **minced** and **cell burst** samples. The percentage yield and chemical composition as revealed by phytochemical screening results were considered. The following outcomes were achieved.

1. Based on the preliminary results from pilot studies of both samples types, the partition extraction method was adopted. The decision was based on the high yield obtained from the methanol and water extracts, and so within a shorter period of time.
2. Comparison of the qualitative chemical profiles of compounds present from use of similar solvent systems on the two collections, showed a significant difference in the concentrations of the pigments between the **minced** and **cell burst** samples. This was determined by TLC. **Cell burst** sample contained higher concentrations of the targeted pigments.
3. Polysaccharide extractions and hydrolysis for preliminary analysis were performed in order to establish the presence or otherwise of fucoidans.
4. Phytochemical screening for the detection of natural plant product classes present in the seaweed extracts targeted phenolics, saponins, flavonoids and alkaloids. The collections were sampled from two separate times of the year. It was observed that there was no difference in phytochemical profile; however the winter collection displayed increased yields of secondary metabolites.

1.2 Results

	MeOH		H_2O	
	Minced	Cell burst	Minced	Cell burst
Amount	1.04	2.28	1.8676	2.1827

This table shows the relative amounts in mg of extraction yields using the indicated solvents.

Fractionation of the water extracts from both the summer and winter collections of the seaweed, gave a wide distribution of polysaccharides. Compositional analysis revealed the presence of a pentose (xylose), hexoses (glucose, mannose, galactose), and deoxy-hexoses (rhamnose, fucose). The relative monosaccharide distribution reflected the presence of fucoidans and xylomannans, as the major polysaccharide constituents, the latter having also been shown to exhibit antiviral activity[1] amongst other biological activities**.** Fucoidans, on the other hand, have been shown to possess antiviral[2], anti-inflammatory[3], as well as anti-neurological degeneration[4] properties. Further studies[5,6] have revealed that the anti-inflammatory properties of fucoidan are comparable to those of cortisone based products. Ion chromatographic analysis demonstrated a high level of sulfate groups, and these have been shown to be important for biological activities of associated polysaccharides particularly in brown algae such as *CEM-K*.

Brown algae generally contain phlorotannins as the only group of tannins. These are polymers of phloroglucinol (1, 3, 5-trihydroxybenzene) and may constitute up to 15% of the dry weight of brown algae [7,8]. Four phenolic compounds have been isolated following a bioassay-guided fractionation of the active *n*-hexane and ethyl acetate (EtOAc) soluble extracts, resulting in the isolation of the phlorotannins; phloroglucinol (1,3,5-trihydroxybenzene) (**1**), eckols; 4-(3,5-dihydroxyphenoxy)dibenzo [b,e][1,4]dioxine-1,3,6,8-tetraol (**2**), 8-(2,4,6-trihydroxyphenoxy)-4-(3,5dihydroxyphenoxy) dibenzo[b,e][1,4]dioxine-1,3,6-triol(**3**) a 7-phloroeckol, and 8-(2,4,6-trihydroxyphenoxy)-9-(3,5-dihydroxyphenoxy)dibenzo[1,4]dioxine-1,3,6-triol (**4**) a 2-phloroeckol, and a sterol; (8S,9S,10R,13R,14S,17R)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-17-(R,E)-5-isopropylhept-5-en-2-yl)-10,13-dimethyl-1H-cyclopenta [a]phenanthren-3-ol(**5**). These compounds are reported for this seaweed for the first time. The structures were characterized using NMR (1D and 2D) as well as mass spectroscopic techniques, and subsequent comparison with published data.[9,10,11]

1.2.1 Anti-oxidant assay

This was performed using the following methods[12,13,14]:

a) Oxygen radical absorption capacity (ORAC); acts on H^+ ion transfer.
b) Ferric reducing antioxidant power (FRAP); simple electron transfer.
c) Trolox equivalent antioxidant capacity (TEAC)

An estimation of the total phenolic contents of the seaweed extracts was also performed according to the Folin–Ciocalteu method with a slight modification; gallic acid was used as a standard and results are expressed as gallic acid equivalents (GAE). [15,16]

a) **Phenol content in mg GAE/g of some extracts and isolates present in *CEM-K.***

Extract/isolate	Hexane	DCM	EtOAc	MeOH	Cpd **1**	Cpd **2**	Cpd **3**	Cpd **4**	Cpd **5**
mg GAE/g	0.124	0.135	0.329	0.081	0.394	5.987	**26.570**	4.553	N/T

Compounds ***1*** *(Phloroglucinol),* ***2*** *(eckol),* ***3*** *(7-phloro-eckol) and* ***4*** *(2-phloro-eckol); 5 (Fucosterol)*

b) ORAC values of crude extracts and pure isolates present in CEM-K.

Extract/isolate	Hexane	DCM	EtOAc	MeOH	Cpd **1**	Cpd **2**	Cpd **3**	Cpd **4**	Cpd **5**
µmol TE/g	1.44	1.58	4.01	0.70	7.166	59.87	**255.27**	59.21	N/T

c) FRAP values (Ferric Reducing Antioxidant Power) of crude extracts and isolates present in CEM-K.

Extract/isolate	Hexane	DCM	EtOAc	MeOH	Cpd **1**	Cpd **2**	Cpd **3**	Cpd **4**	Cpd **5**
µmol AAE/g	0.248	0.256	0.995	0.055	0.411	14.130	**93.249**	10.849	N/T

d) ABTS (TEAC) values (Trolox equivalent antioxidant capacity) of some extracts and isolates present in CEM-K.

Extract/isolate	Hexane	DCM	EtOAc	MeOH	Cpd **1**	Cpd **2**	Cpd **3**	Cpd **4**	Cpd **5**
µmolTrolox®E/g	0.750	0.880	1.412	0.342	1.693	22.196	**83.153**	19.745	N/T

The foregoing data is in support of the high anti-oxidant properties of the polyphenolic compounds, with compound 3 being the strongest in this regard. Such properties may also be linked to the well-known anti-inflammatory properties of these compounds[17,18], in addition to bactericidal[19] and anti-allergy properties[20] and protection against UV radiation.[21] On the other hand compound 5, fucosterol, has been shown to possess anti-cancer properties.[10] Finally, given that both fucoidan and the phenolic compounds described above have been shown to possess anti-inflammatory properties, it may well be that the potency of CEM-K based products is due to a synergistic effect between these two sets of constituents.

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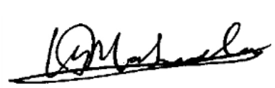

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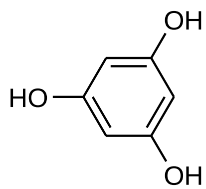

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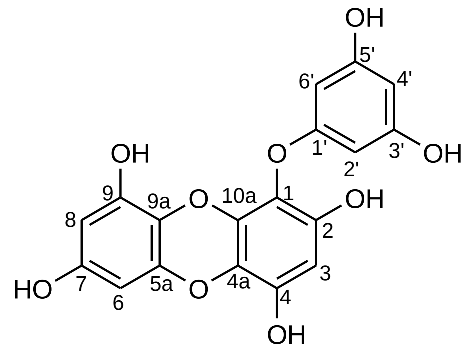

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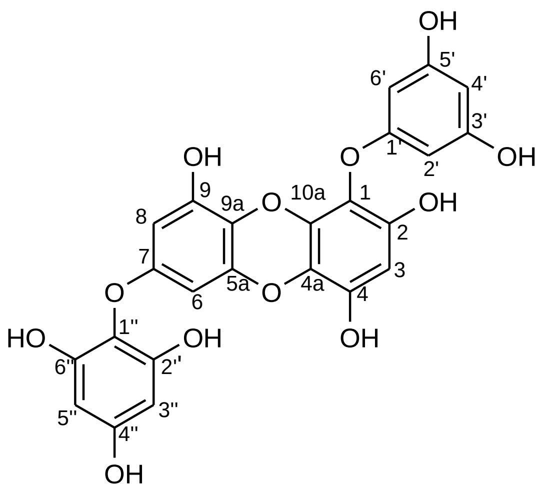

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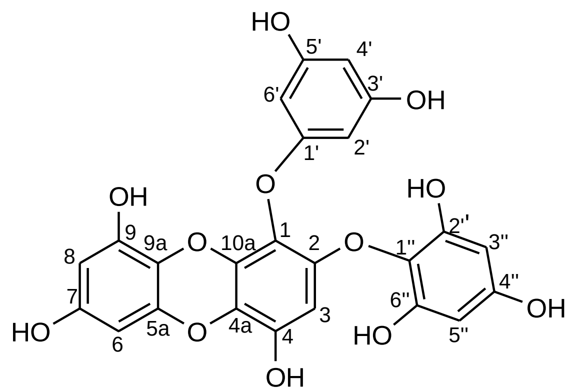

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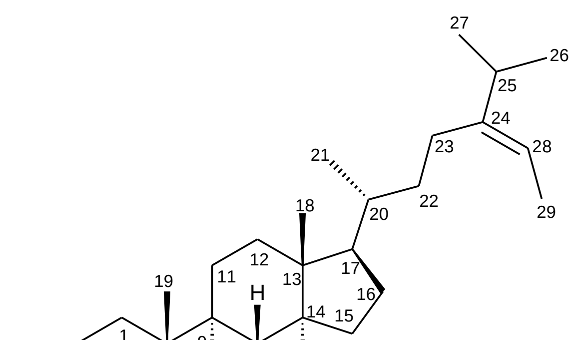

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Figure 1: *Structures of phloroglucinol (**1**) and phlorotannins **2**, **3**, **4**, and a sterol **5** isolated from CEM-K.*