Possible novel UV-protectants from fish waste and by-catch

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Abstract. Nine fish tissue extracts which decrease or abrogate UVA and UVB damage in HaCaT keratinocytes were identified. Our results demonstrate that fish waste and by-catch may be a potential source of bioactive compounds in the development of new UV-protectors for skin care products.

Introduction

Solar UV radiation is a combination of UVB (280-320 nm) and UVA (320-340 nm) wavelengths. Both acute and chronic sun exposure induces different abnormalities in cutaneous and deep skin layers, such as erythema, photoaging, immunosupression, and skin cancer (Bernerd *et al*. 2003; Sarasin, 1999; Tzung *et al.* 1998).

There are a number of commercial sunscreens on the market, but some of them have undesirable side effects, such as allergies (Kiec-Swierczynska et al. 2005; Pustisek *et al*. 2005). To minimise complications and provide an efficient protection against UVA and UVB radiation a new generation of sunscreens are being developed. As most of the existing sunscreens acts as filters, new natural ingredients which penetrate into the skin and protect it at a biological level are being sought (Torita *et al*. 2004).

The aim of our project is to add value to fish waste and by-catch by identifying products for use in the cosmetic industry. UV skin protectants is one target.

Methods

Samples: Seven commercial fish species were supplied by Ngai Tahu Seafoods Ltd. By-catch species were collected aboard the NIWA research vessel, *Tangaroa* during trawling operations on the Chatham rise. All fish were frozen within 24 hr of capture and stored at -20° C.

Extraction: All extraction procedures were done using NIWA Standard Protocols.

UV-absorption assay: Ethyl-acetate tissue extracts were separated on a Thermo-Finnigan Surveyor HPLC system, followed by UV- absorption characterisation on a photodiode array (PDA) over the wavelength range of 200-600 nm. For each sample, 10 µl of tissue extract were separated on a C-18 reverse phase column (Waters Xterra C18 2.1 x 150 mm, 3.5 µm), using a gradient of water and acetonitrile (5-95%) over 45 min and a flow speed of 208 µl/min.

UV-stress assay method: HaCaT cells were seeded in 96-well plates and grown for 48 h. Cells were washed twice. Then 20 ul of extract was aliquoted into the wells. Ascorbic acid and Sunscreen (Nivea Children's Sun Lotion, SPF 35) were used as controls. Plates were kept at 37° C for 15 min and then irradiated.

UVA exposures were carried out under a Uvilite LF 215L illumination system (UVitec Ltd, UK). Cells were exposed to UVA irradiation for 65 min, resulting in a UVA dose of 129 kJ/m^2. UVB exposures were carried out under a Uvilite LF 106M illumination system (UVitec Ltd, UK). Cells were exposed to UVB irradiation for 2 min, resulting in a UVB dose of 0.8 kJ/m^2.

After irradiation cells were incubated in fresh medium for 24 h. The MTT assay was performed according to the standard procedure (Mossman , 1982).

Fractionation method: Fractionation method was performed using NIWA Standard Protocol.

Results and Discussion

Of ninety six solvent fish extracts analysed, 21 showed UV-absorptive properties. Most of the samples had several absorption peaks in the UVA and UVB wavelengths range. Data for four samples are presented in Table 1.

Twenty one positive extracts and 7 extracts showing scavenging activity in the TEAC antioxidant assay (data not shown) were further tested in the UV-stress assay. Data for HaCaT cells viability are shown on Figure 1. Nine fish tissue extracts enhanced survival HaCaT keratinocytes, 4 had protective effect against UVA irradiation (129 kJ/m2), and another 4 extracts enchanced survival of cells irradiated with UVB. Sample 8A conferred protection against both UVA and UVB radiation, and completely arrested cell death after UVA-irradiation experiments (Figure 1A, B) compared to no survival of cells in the UVA-irradiated control. Survival of HaCaT cells treated with 1E, 2E, 4E and 9E extracts was between 21-71 %, while ascorbic acid (positive control) protected cells to 75 % (Figure 1).

Under UVB-irradiation (0.8 kJ/m2), survival of irradiated cell control was 13 %, whereas 7A and 6A preserved cell survival of HaCaT keratinocytes to 97 and 93 %, respectively (Figure 1B). Sunscreen in 1:10 dilution protected cells to 82 %.

All positive samples were re-extracted, fractionated with a size exclusion column, and re-tested in the UV-stress assay. Data on the protective activity of three aqueous samples fractions in UVB irradiation assay are shown in Table 2. For samples 5A, 6A and 7A, twelve fractions were collected, and fractions 2, 4, 6-9, and 11 all displayed protective effects on HaCaT skin cells. Presumably, several different groups of compounds are involved in UV-protection, in all five extracts. One group of early-eluting compounds (Fraction 2), with high molecular weight of 25-76 kDa, is probably proteins.

Other active fractions (Table 2) contain small molecules with MW between 1–6 kDa. The finding that UV-protective activity was observed in the same size fractions for 3 extracts, taken from two different fish species and one by-catch specimen, suggests that the

active compounds might belong to closely related classes of chemicals.

Our results demonstrate that fish waste and by-catch may be a potential source of bioactive compounds in the development of new UV-protectors for skin care products. Further studies will aim to confirm the stability of fish extracts, and to investigate possible protective mechanisms at the cellular level. Further fractionation of the most active extracts as well as structure elucidation of compounds will also be performed.

Acknowlegements

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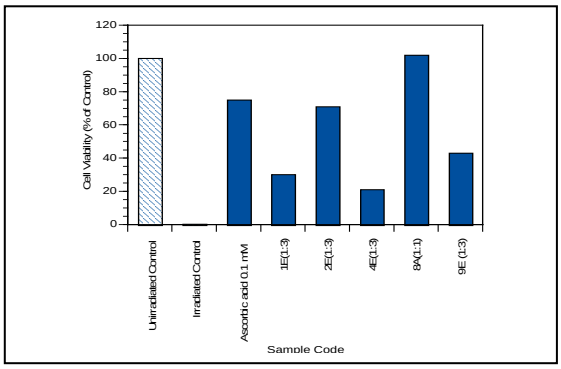
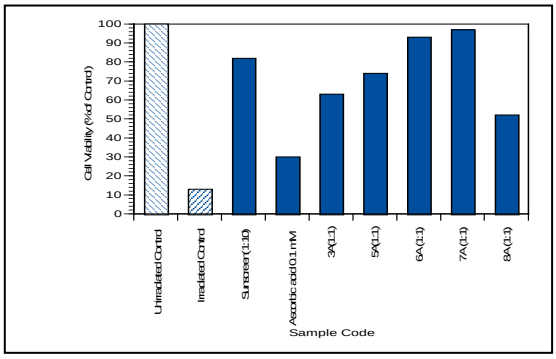
Table 1. UV absorption properties of four fish tissue solvent extracts

Sample Code	Wavelength	Absorbance (µabs)	UV-Stress Assay
1E	356 308 326	5000-10000 5000-10000 5000-10000	UVA positive UVB negative
2E	326	>10000	UVA positive UVB negative
4E	326	>10000	UVA positive UVB negative
9E	321 336 417	<5000 5000-10000 >10000	UVA positive UVB negative



A



B

Figure 1. Survival of HaCaT keratinocytes after UVA (A) and UVB (B) irradiation. Cells ($5x10^4$ per well) were preincubated with tested fish extracts for 15 min, and irradiated with the doses of 129 kJ/m^2 (UVA) and of 0.8 kJ/m^2 (UVB)

Table 2. UVB-protective properties of three fractionated aqueous fish tissue extracts. HaCaT cell survival in percent.

Sample	Raw Extract(%)	Fraction (%)											
		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
MW (kDa)	no size fractions	270000	76300	21500	6100	1700							
5A	101	8	18	9	20	14	41	71	95	101	103	102	93
6A	99	11	32	9	31	13	99	54	67	35	6	32	4
7A	73	4	32	5	31	8	53	29	50	31	5	35	16