Marine plant extracts offer superior dermal protection

Marine plants, or macroalgae, have long been recognised for their therapeutic ability on the skin. Whole seaweed was traditionally used in various cultures for relieving joint pain, inflammation and burns via direct application to skin, or the addition to bath water and topical pastes. With modern technology and the advancement of extraction methods, certain components of seaweed have been able to be isolated for more targeted use in cosmetic formulations. These components include algae carbohydrates such as alginites and fucoidans and pigments such as polyphloroglucinol and fucoxanthin. Carbohydrate components, while typically used for stability, viscosity and conditioning functions, have in more recent years gained recognition for their extensive array of bioactivities; particularly those of fucoidans.

Fucoidans refer to a class of fucose-rich polysaccharides found only in brown macroalgae. They are highly branched and heterodisperse compounds, which are both sulfated and acetylated. Fucoidans differ greatly in their molecular structure and bioactivity, depending on the type of macroalgae they are sourced from and the extraction method that is used. For example, fucoidan from Undaria pinnatifida seaweed (as seen in Figure 1) is highly sulfated and acetylated with a backbone containing both fucose and galactose, while fucoidan extracted from Fucus vesiculosus has a backbone made predominantly of fucose with little acetylation.

In nature, fucoidans protect algae by creating a barrier between plant cells and the ocean, to provide strength and flexibility and maintain correct ionic balance. They also work together with pigment molecules, such as polyphloroglucinol, to protect the plant from other harsh elements of the environment: UV rays, pathogens and pollutants.

Fucoidans have been shown to have a vast array of bioactivities once isolated from their natural source, including anti-viral activity, anti-inflammatory effects, UV protection, enzyme inhibition and immune modulating activity. Polyphloroglucinols have potent antioxidant effects and are known for their ability to reduce damage caused by UV, as well as inhibiting ageing enzymes.

Commercial-scale extraction of both of these compounds has been developed by Australian biotechnology company Marinova, for use in cosmetic formulations. Wild-grown Undaria pinnatifida and Fucus vesiculosus macroalgae were sourced to extract two well characterised, certified organic fucoidan compounds: Maritech® Reverse and Maritech® Bright. Maritech® Reverse is a highly sulfated, high purity fucoidan, while Maritech Bright is a high purity compound comprised of both fucoidan and marine polyphenols.

Extensive clinical and in vitro testing showed both extracts offer superior cosmeceutical benefits, particularly through anti-glycation, immune boosting and enzyme inhibitory mechanisms and UV protecting and soothing activity. Maritech Reverse was particularly effective at increasing the expression of wound-healing genes, while Maritech Bright was shown to clinically reduce age spot and wrinkle appearance. The demonstrated bioactivity of the extracts at low concentrations, in addition to their certified organic and environmentally sustainable status, position Maritech Bright and Maritech Reverse as two highly valuable ingredients for cosmetic formulations.

ABSTRACT

Two specialty macroalgae-derived extracts have been developed by leading Australian biotechnology company Marinova, for use in cosmetic formulations. Wild-grown Undaria pinnatifida and Fucus vesiculosus macroalgae were sourced to extract two well characterised, certified organic fucoidan compounds: Maritech® Reverse and Maritech® Bright. Maritech® Reverse is a highly sulfated, high purity fucoidan, while Maritech Bright is a high purity compound comprised of both fucoidan and marine polyphenols. Extensive clinical and in vitro testing showed both extracts offer superior cosmeceutical benefits, particularly through anti-glycation, immune boosting and enzyme inhibitory mechanisms and UV protecting and soothing activity. Maritech Reverse was particularly effective at increasing the expression of wound-healing genes, while Maritech Bright was shown to clinically reduce age spot and wrinkle appearance. The demonstrated bioactivity of the extracts at low concentrations, in addition to their certified organic and environmentally sustainable status, position Maritech Bright and Maritech Reverse as two highly valuable ingredients for cosmetic formulations.

Figure 1: Graphic of Undaria pinnatifida fucoidan molecule.

Skin protective properties

The human skin plays a similar role to fucoidan in marine plants. Being the largest organ of the human body, skin plays a fundamental role in our existence – not only providing definition, character and recognition, it is also the first line of defence against foreign bodies, UV radiation and other external hazards. Premature ageing, physical damage and diseases of the skin reduce its ability to maintain such functions. This is why application of effective anti-ageing, protective and healing cosmetic ingredients is so highly valued in today’s modern world of densely populated, polluted and stressful living conditions. The following studies were undertaken to define the likely cosmetic bioactivity of Undaria pinnatifida extract and Fucus vesiculosus extract.

Glycation inhibition

Glycation is a marker for ageing skin, defined as a non-enzymatic reaction
between amino acids and hydroxyl groups of naturally occurring sugars (i.e. Maillard’s reaction). When this reaction occurs in the dermis, it stiffens the linkages between certain elements within the extracellular matrix, causing a loss of flexibility and permanent damage. Glycation is a natural process induced by ageing, with signs appearing around the age of 30. External factors, including UV damage, pollution and smoking, can accelerate this process.

Both Undaria pinnatifida extract and Fucus vesiculosus extract were found to be effective inhibitors of glycation. In vitro testing based on the methods of Kiho5 and Choi6 demonstrated the two extracts were effective at inhibiting glycation as amino-guanidine, at 0.1 mg/mL and 0.2 mg/mL.

In a placebo-controlled study, 25 Caucasian subjects tested the ability of a 0.3% w/v active gel formulation to protect and soothe damage caused by UV radiation. UV radiation was administered to subjects from a solar simulator at 1.25x the minimum erythemal dose. Erythema (reddening of the skin) and trans-epithelial water loss (TEWL) were evaluated at different time points following UV exposure. The evaluation of erythema was performed by a Mexameter MX 18 (Courage+Khazaka electronic GmbH) and TEWL was assessed by a Tewameter 300R (Courage+Khazaka electronic GmbH). A quantity of 2 mg/cm² of active or placebo gel was applied to the skin, before and/or after UV exposure. Figures 3 and 4 display the per cent reduction in erythema and TEWL (respectively) in the study group that applied gel 30 minutes before and 20±4 hours after UV exposure. Both extracts provided significantly reduced erythema and TEWL compared to the placebo. While both extracts proved to be effective protective and soothing agents, Fucus vesiculosus extract showed marginally higher activity than Undaria pinnatifida extract.

Enhanced wound-healing gene expression

Undaria pinnatifida extract strongly activated the gene expression of wound healing genes in UVB and UVA irradiated reconstructed human epidermis cells.

### Table 1: Description of Fucus vesiculosus and Undaria pinnatifida extract for cosmetic use.

<table>
<thead>
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<th>INCI name</th>
<th>Fucus vesiculosus extract</th>
<th>Undaria pinnatifida extract</th>
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<td>Trade Name</td>
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<td>Maritech Reverse</td>
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Figure 2: Per cent of glycation inhibition of Undaria pinnatifida extract and Fucus vesiculosus extract compared to standard glycation inhibitor, amino-guanidine, at 0.1 mg/mL and 0.2 mg/mL.
In *vitro*, confirming previous research observations for *Undaria pinnatifida* extracts.\textsuperscript{7} Toll-like receptors (TLR) are critical to innate immune system function. They are expressed by keratinocytes (the most populated cells in the epidermis) in response to invading pathogens, aiding in the body’s first line of defence.\textsuperscript{8} Matrix metalloproteases (MMP) are a group of enzymes that are involved in a wide range of proteolytic circumstances, including inflammation, wound healing and angiogenesis.\textsuperscript{9}

In the study, *Undaria pinnatifida* extract was used to topically treat reconstructed human keratinocytes at 30 and 100 μg/mL concentrations and incubated for 24 hours *in vitro*, to replicate histologically very similar cultures to those that are observed *in vivo*. Results were compared to placebo treated cells. After incubation, cells were topically treated again (with *Undaria pinnatifida* extract or placebo) and irradiated with UVB and UVA, using a SOL500 sun simulator equipped with an H2 filter. Cells were then topically retreated (with active or placebo) and incubated for a further 4 or 24 hours and frozen at −80°C. mRNA was extracted and analysed on a dedicated PCR array containing 64 target genes.

Genes for TLR2 and TLR3 were strongly activated in the 100 μg/mL *Undaria pinnatifida* extract treated assay, at 387% and 229% respectively. In addition, there was a substantial increase in expression of certain extra cellular MMP genes. Gene expression of MMP1, MMP3 and MMP9 more than doubled in the *Undaria pinnatifida* extract treated group compared to the control at 4 hours. These results, as seen in Figure 5, indicate an enhanced immune system response and ‘wound-healing’ signal in UV-irradiated human keratinocytes.

Enzyme inhibition

Enzyme stimulation is a fundamental component of ageing.\textsuperscript{10} Elastase, collagenase and tyrosinase are three extracellular matrix enzymes, which cause degradation of the skin when stimulated. It is believed that degraded collagen fibrils actually induce further collagenase activity and prevent new tissue formation. Inhibiting collagenase activity is seen as an effective way to slow the skin ageing process and promote new tissue formation. Previous studies have noted the inhibitory effects of fucoidan fractions on matrix degrading enzymes.\textsuperscript{11,12}

Both *Fucus vesiculosus* extract and *Undaria pinnatifida* extract were effective elastase and collagenase inhibitors *in vitro*, with 99% inhibition at concentrations of 0.1 mg/mL. In addition, *Fucus vesiculosus* extract was a highly effective inhibitor of tyrosinase, with 99% inhibition at a concentration of 0.2 mg/mL *in vitro*. While *in vitro* findings cannot be directly inferred to *in vivo* activity, this provides positive indicative data in combination with earlier clinical findings.

Powerful antioxidant ability

*Fucus vesiculosus* extract was found to have superior antioxidant capability, due to its high content of the marine polyphenol, polyphloroglucinol. *Fucus vesiculosus* extract was tested using the ORAC 5.0 assay, which involves measuring the antioxidant activity of the extract in reference to five common free radicals: peroxyl, hydroxyl, superoxide, singlet oxygen and peroxynitrite. Superoxide is of particular interest in a cosmetic setting, as it is a precursor of other free radicals and is highly toxic to cells, contributing to DNA damage. ORAC 5.0 assay results showed the extract to have a total antioxidant power of 27,187 μmol TE/g, with particularly high activity towards the superoxide free radical (85% of total antioxidant activity), as seen in Figure 3. The exceptional antioxidant power of *Fucus vesiculosus* extract towards the superoxide anion measures at 23,025 μmol TE/g, which, to put into perspective, is considerably higher than that of other well-known antioxidant compounds such as green tea extract (14,000 μmol TE/g) and cocoa extract (406 μmol TE/g).

Reduction in wrinkles and age spots

*Fucus vesiculosus* extract was used in a double-blind, placebo-controlled hemi-face clinical trial to assess its effect on wrinkle depth and the appearance of age spots. The study was carried out over 60 days with 20 Caucasian participants, who applied either a 0.3% w/v formulation containing *Fucus vesiculosus* extract or a placebo, morning and night. Subjects were assessed on days 0, 15, 30 and 60 for wrinkle depth (by Primos 3D) and the melanin index of age spots (by Mexameter MX 18 probe). Results of the study found the extract to be an effective active ingredient, reducing wrinkles and age spot appearance, as seen in Figures 7 and 8.

**Organic and sustainable extracts**

The demand for organic, sustainable, plant-based cosmetic ingredients is increasing throughout the globe, with organics being the fastest growing segment of the personal care industry. Increased consumer awareness of personal health and hygiene and rising concerns towards environmental pollutants is driving growth in this area. The Asia Pacific region is the fastest-growing region in the world in regards to demand for organic cosmetics, with the sector expected to grow at a CAGR of 9.5% by 2020.\textsuperscript{13}
In order to receive organic certification, extracts must derive from organic agriculture that respect biodiversity, use natural resources responsibly and utilise manufacturing processes that respect human health and the environment. They must also integrate ‘Green Chemistry’ – methods that involve minimal waste and exclude harsh chemical use. This rigorous set of rules is stringently monitored through comprehensive inspections and auditing processes by the certifying body.

Both Fucus vesiculosus extract and Undaria pinnatifida extract are produced in the world that are certified organic with Australian Certified Organic (ACO), Cosmetic Organic and Natural Standard (Cosmos) and the United States National Organic Program (NOP).

Wild growing Fucus vesiculosus and Undaria pinnatifida macroalgae are sourced to produce these cosmetic ingredients, from the purest ocean waters of Nova Scotia and Patagonia respectively. All seaweed is hand harvested at their peak growth cycle to ensure minimal environmental impact and is then immediately sun-dried. Dried seaweed then undergoes a unique cold-water extraction process to produce the isolated ingredients, in cGMP and ISO 9001 accredited facilities. All by-products of this process are utilised in nutrient-rich horticultural products, thus making this unique process zero-waste and environmentally sustainable.

Safe and non-sensitising
All clinical tests have shown Undaria pinnatifida extract and Fucus vesiculosus extract to be non-sensitising and non-allergenic. Both extracts are safe to apply topically at high concentrations, although clinical testing demonstrates their bioactivity at the very low concentration of 0.3 % w/v.

Conclusion
Maritech Reverse, an extract of Undaria pinnatifida macroalgae and Maritech Bright, an extract of Fucus vesiculosus macroalgae, are two certified organic and effective anti-ageing cosmetic ingredients.

Sourced from marine plants grown in the purest ocean waters, Maritech Bright and Maritech Reverse have demonstrated anti-glycation activity and were highly effective collagenase and elastase inhibitors in vitro. Maritech Reverse increased the expression of wound-healing genes in vitro and demonstrated clinical UV protecting and soothing effects at 0.3% w/v. Maritech Bright demonstrated marginally higher UV protecting and soothing effects than Maritech Reverse, as well as reducing the appearance of age spots and wrinkles in clinical testing. This is most likely due to its high polyphenol content and demonstrated antioxidant ability.

Clinically active at the very low concentration rate of 0.3% w/v with organic certification and a ‘pure source’ guarantee, Maritech Reverse and Maritech Bright combine the best elements of science, sustainability and marketability. These two extracts are novel bioactive ingredients that exceed the ever-increasing global demand for natural, sustainable, safe and effective cosmeceutical formulations.


