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OceanDerMX™ Research Study 2020

Introduction

OceanDerMX™ - produced by our patented 'clean beauty' TPT Xtraction™ technology is a result of five fruitful years of research, comprehensive testing and trials. **This 4 in 1** multitasking ingredient blend synchronises functional, structural, bioactive and sensorial properties of our biopolymers - abundantly present in biodiverse New Zealand *Rhodophyta* (red seaweed) and *Cyathea medullaris* (black fern Mamaku).

The unique milieu of sulphated glycosaminoglycans (sGAGs) within our red seaweed, acts as a;

- supreme bioactive delivery system for its own and added bioactives
- unrivalled immediate and long - lasting hydrator
- bio-identical structural scaffold to ensure the skin gains or maintains its suppleness, firmness, vitality and supreme hydration.

The sGAGs in our **OceanDerMX™** contain significantly high proportions of essential antioxidants and possess film forming - antipollution activity against environmental aggressors. This is further surmounted by the world's most intriguingly unique biopolymers, derived from the meristem of Mamaku - New Zealand native black fern.

Results summary

The sensorial profile of a face cream can be determined by evaluating parameters such as spreadability, greasiness, softness and film-forming properties. **OceanDerMX™'**s face serum was found to have a greater sensorial profile than a well-known competitor's face cream that also contains a seaweed extract.

Fucoidan is a sGAG derived from brown seaweed and is the current industry standard. However, **OceanDerMX™** was found to contain significantly more sGAGs than brown seaweed. Numerous scientific studies have also shown that the sGAGs from red seaweed have several bioactivies, compared to green and brown seaweeds.

The water-binding capacity of a compound is critical to how well it can perform as a skin moisturiser. The current industry standard is hyaluronic acid. **OceanDerMX™** is a better natural alternative, which significantly outperformed hyaluronic acid after only 1-minute administration of distilled water.

One of the main parameters the cosmetic industry focuses on when it comes to ingredient formulations is the total antioxidant capacity. Since brown seaweed derived fucoidan is commonly used in the industry, we used it as a standard and found that **OceanDerMX™** has comparably higher antioxidant capacity. **OceanDerMX™** acts as a carrier blend, meaning we can increase its antioxidant capacity further by introducing other naturally potent bioactives. E.g. Kawakawa

Another capability of **OceanDerMX™** is its ability to chelate heavy metals, indicating its antipollution properties, which are relevant to protecting the skin from environmental aggressors, especially in urban environments. The sGAGs from red seaweed and the biopolymers from Mamaku are responsible for these properties in **OceanDerMX™**. Mamaku especially, was found to be almost as strong as the industry standard synthetic EDTA.

Apart from protection and hydration, the skin also requires a good balance of nutrients. The bioactivities of **OceanDerMX™** are also attributed to its composition of chlorophylls, vitamin A and retinoid precursors. Based on the presence of sGAGs in **OceanDerMX™**, there is a high potential for these nutrients to get delivered into the skin and not remain on the surface of the skin. This can be demonstrated on the skin by applying oil-based bioactives / vitamins topped with **OceanDerMX™**.

1.0.0 Sample preparations

We optimised our water extraction method by combining it with ultrasonic assisted extraction (UAE) to create our patented 'clean beauty' **TPT Xtraction™** technology. This is because UAE has been well documented to enhance the bioactivity seen with conventional water or solvent extraction, as well to extract sulphated glycosaminoglycans from seaweeds [1]. We therefore used **TPT Xtraction™** to prepare all our **OceanDerMX™** samples for testing.

2.0.0 Content quantification methods

2.1.0 Sulphated glycosaminoglycans (sGAGs) quantification

A 0.02% Alcian Blue (AB) stock solution (pH 1) was prepared by dissolving 0.1 g of AB in 500 ml of 0.06% acetic acid with appropriate addition of 1 M HCl for pH adjustment. All extracts were mixed with AB in a 5:1 ratio in Eppendorf tubes.

Using a plate reader:

After 10 mins, the tubes were centrifuged at 10,000 rpm for 5 mins and the resulting supernatant were measured at 610 nm in a plate reader [SPECTROstar nano (BMG Tech)]. Tests were carried out as triplicates [2]. Fucoidan was used as a standard [3].

2.1.1 Results

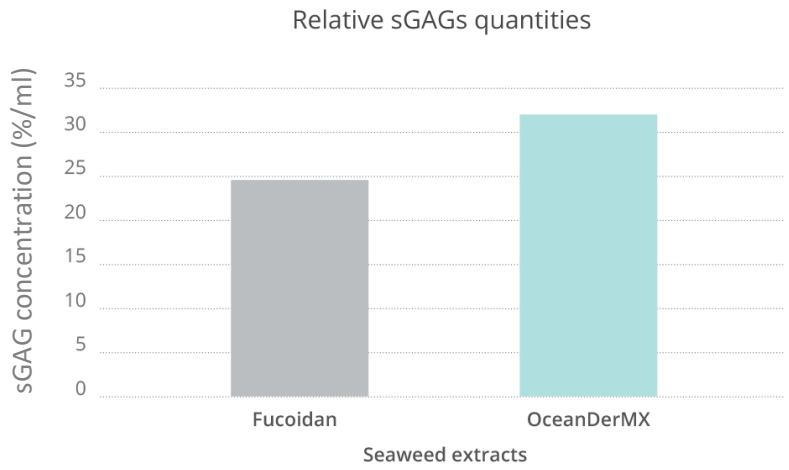


Fig 1. The total amount of sulphated GAGs [glycosaminoglycans] per milliliter present in **OceanDerMX™** compared to **Fucoidan**. (n=3)

Fucoidan is a sGAG derived from brown seaweed and is the current industry standard. However, **OceanDerMX™** was found to contain significantly more sGAGs than brown seaweed (Fig 1). Numerous scientific studies have also shown that the sGAGs from red seaweed have several bioactivies, compared to green and brown seaweeds [4].

2.2.0 Carotenoid content estimation

Approximately 500 mg of seaweed was kept in a pestle and mortar with 10 ml of 80% acetone and it was ground well, and the homogenate was centrifuged at 3000 rpm for 15 minutes and the supernatant was stored. The pellet was re-extracted by repeated washing with 5 ml of 80 % acetone till it became colourless. All the extracts were pooled and utilized for pigment quantification. The process was followed for all the samples [5].

The amount of chlorophyll present in the algae was estimated by the method of Arnon (1949). Absorbance was measured at 645 nm and 663 nm in a spectrophotometer.

The chlorophyll content was determined by using the following formula Arnon's (1949) equations (1-4):

1) Chlorophyll ai = 12.7 (A663) - 2.69 (A645)

2) Chlorophyll bi = 22.9 (A645) - 4.68 (A663)

3) Chlorophyll a = $\frac{\text{[Chlorophyll ai] x 10ml}}{\text{W}}$

4) Chlorophyll bi = $\frac{\text{[Chlorophyll bi] x 10ml}}{\text{W}}$

5) C = $\frac{\text{A x Fd}}{\text{ƐΛ}}$

Where, **A** = Absorbance is measured absorbance, **ƐΛ =** molar absorption coefficient (L/mol/cm)**, C =** molar concentration (mol/L)**, d =** 1 cm, Volume of extract (ml), **W** = weight of the sample (g).

To determine carotenoid composition, we used Table 1 to substitute parameters mentioned in the protocol above for chlorophyll. We used equation 5 above to calculate results [6].

Table 1. Carotenoid standards for equation 5 [6].

Compound	Solvent	Λ max	Molar coeff	m[g/mol]
Beta-carotene	Acetone	452	140663	537
Lycopene	Acetone	448	120600	537
Zeaxanthin	Acetone	452	133118	568.88
Lutein	Ethanol	445	144900	569

2.2.1 Results

The bioactivities of **OceanDerMX™** are also attributed to its composition of chlorophylls, vitamin A and retinoid precursors (Fig 2 & 3). Since **OceanDerMX™** acts as a bioactive delivery system, it helps deliver these nutrients into the skin and not remain on the surface like other competitor products.

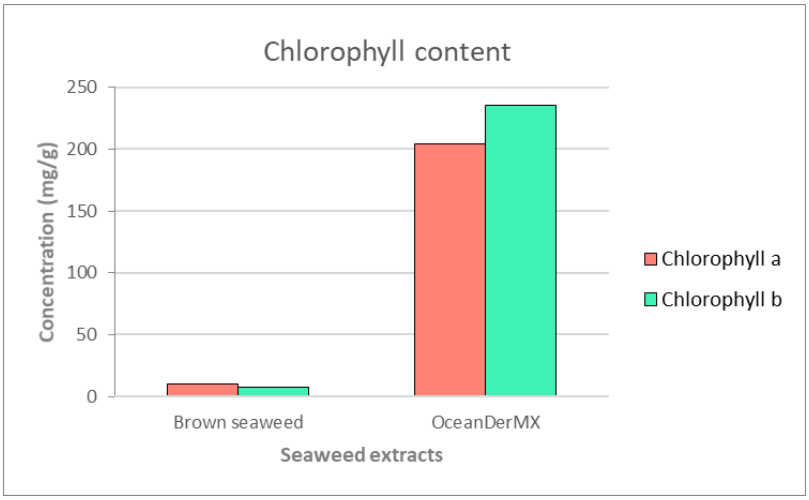


Fig 2. The total amount of Chlorophylls present in **OceanDerMX™** compared to **Brown seaweed** extract. (n=3)

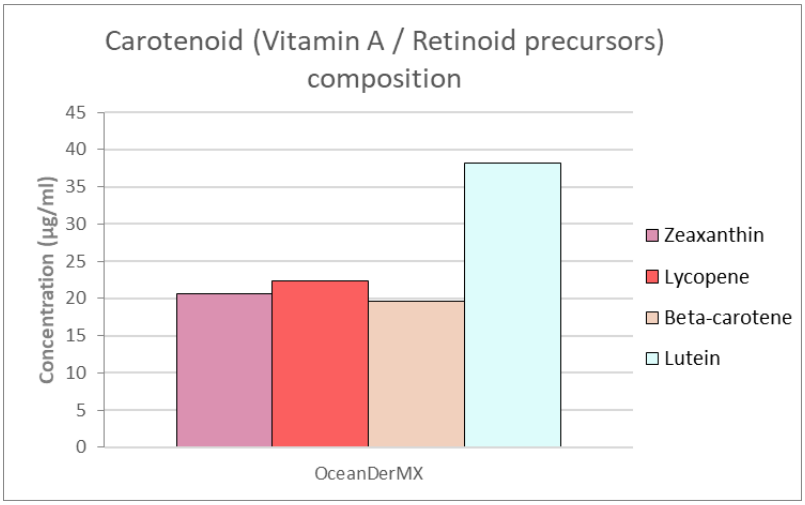


Fig 3. The carotenoid (Vitamin A / Retinoids precursors) composition present in **OceanDerMX™.** (n=3)

3.0.0 Antioxidant assays

3.1.0 Reducing activity

To test the reducing activity of potential hydrophilic and lipophilic antioxidants within our extracts, the CUPRAC method was used. The absorbance of Cu(I)-neocuproine (Nc) chelate can be measured at 450nm in a plate reader [SPECTROstar nano (BMG Tech)].

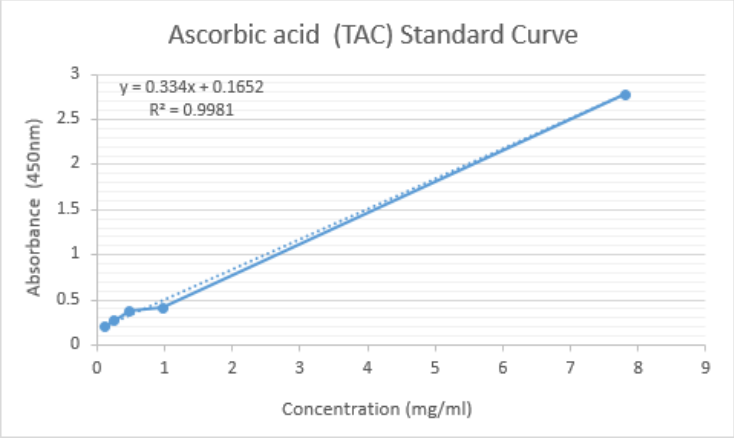
A 10mM copper(II)chloride ($CuCl_2$) solution was prepared from distilled water. A 1M sodium acetate (NaAc) buffer at pH 7.0 was prepared by dissolving NaAc (81.57 g) to distilled water (800 mL) and acetic acid (0.3198 mL), and then diluting to 1L volume with distilled water. A 96% ethanol solution was used to make a 7.5 mM solution of Neocuproine, the end volume was dependent on how much was needed on the day. **Note:** Neocuproine solution should not be used after 2 days due to its stability.

Appropriate concentration ranges were constructed for each test sample via serial dilution with distilled water. Each of the made-up concentrations should be individually mixed with $CuCl_2$, neocuproine and sodium acetate buffer at a 1:1:1:1 ratio. Reaction mixtures were dispensed into 96-well plates, in triplicates and results were calculated using a standard curve with Ascorbic acid acting as the positive control. The standard curve's equation was used to calculate ascorbic acid equivalents. You will need a range of concentrations to test ascorbic acid [7,8,9].

We used the following equation to determine Total Antioxidant Capacity (TAC):

1) **TAC = (Af/ Ɛ) (Vf/Vs) dR(Vcup/m)**

Where Af = reaction absorbance, **Ɛ = 0.334,** Vf = final reaction volume, Vs = Sample analysis volume, dR = dilution ratio, Vcup = orginal extraction volume, m = original dry weight of test sample.



3.1.1 Results

One of the main parameters the cosmetic industry focuses on when it comes to ingredient formulations is the total antioxidant capacity. Since brown seaweed derived fucoidan is commonly used in the industry, we used it as a standard and found that **OceanDerMX™** has comparably higher antioxidant capacity (Fig 4). **OceanDerMX™** acts as a carrier blend, meaning we can increase its antioxidant capacity further by introducing other naturally potent bioactives. E.g. Kawakawa (Fig 5).

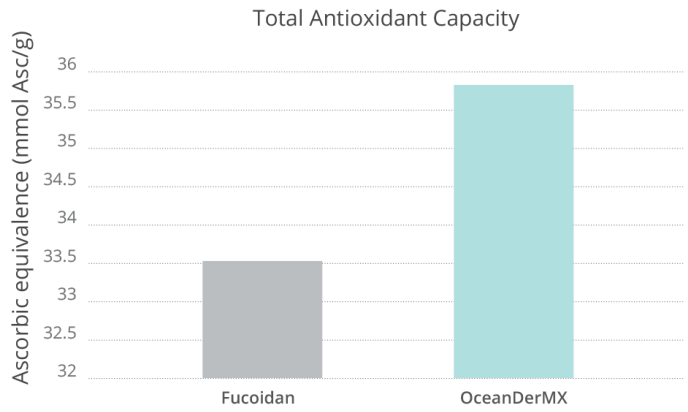


Fig 4. The Total Antioxidant Capacity (TAC) of **OceanDerMX™** compared to **Fucoidan** at 1% concentration. (n=3)

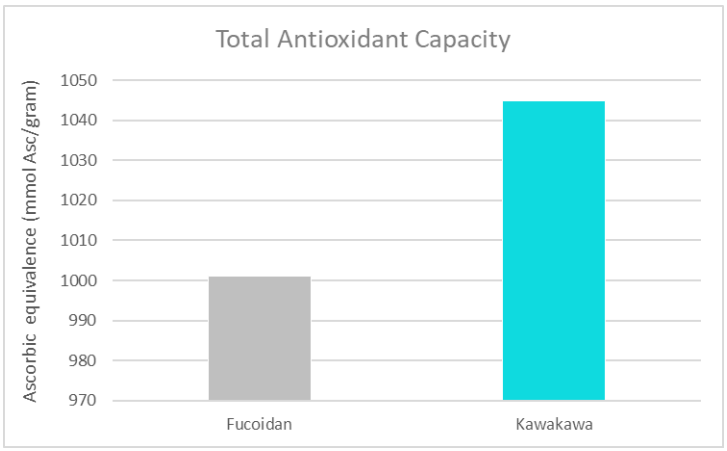


Fig 5. The Total Antioxidant Capacity (TAC) of native **Kawakawa leaves** compared to **Fucoidan** at 5% concentration. (n=3)

3.2.0 Metal chelation activity

Metal chelation activity of all extracts will be calorimetrically quantified at an absorbance of 562nm via a plate reader [SPECTROstar nano (BMG Tech)]. This is done to assess the Fe^{2+} scavenging capabilities of the extracts and EDTA (control), in the presence of the synthetic Fe^{2+} scavenger (Ferrozine). In slightly acidic medium (pH 6), phenolic compounds bind a certain amount of Fe^{2+}, but the remaining Fe^{2+} reacts with ferrozine, forming a blue-colored complex that can be monitored spectrophotometrically.

Using a plate reader:

A concentration range between 2500 to 50 µg/ml, was constructed for each extract via serial dilutions with distilled water. Solutions of 2.0 mM ($FeCl_2$ x 4H2O) and 5.0 mM Ferrozine were made up in distilled water.

Using 1.5 ml Eppendorf tubes, 100 µl of each extract were incubated with 50 µl of $FeCl_2$ x 4H2O and 100 µl of 90% methanol for 1 min. The reaction was initiated by addition of Ferrozine at 200 µl and tubes were centrifuged at 10,000 rpm for 5 mins, after the colour of the reaction mix had stabilized. Samples were measured in triplicates on 96-well plates.

The equation below was used to describe chelation activity. Where 'C' is the absorbance of ferrozine mixed with methanol and $FeCl_2$ without a metal chelator, while 'S' is the absorbance of the test sample reaction with ferrozine and methanol. Methanol was used as a blank [10].

$$Iron\ chelation\ (\%) = \left(C - \frac{S}{C}\right) x\ 100$$

3.2.1 Results

Another capability of **OceanDerMX™** is its ability to chelate heavy metals, indicating its antipollution properties, which are relevant to protecting the skin from environmental aggressors, especially in urban environments. The sGAGs from red seaweed and the biopolymers from Mamaku are responsible for these properties in **OceanDerMX™**. Mamaku especially was found to be almost of strong as the industry standard synthetic EDTA (Fig 6).

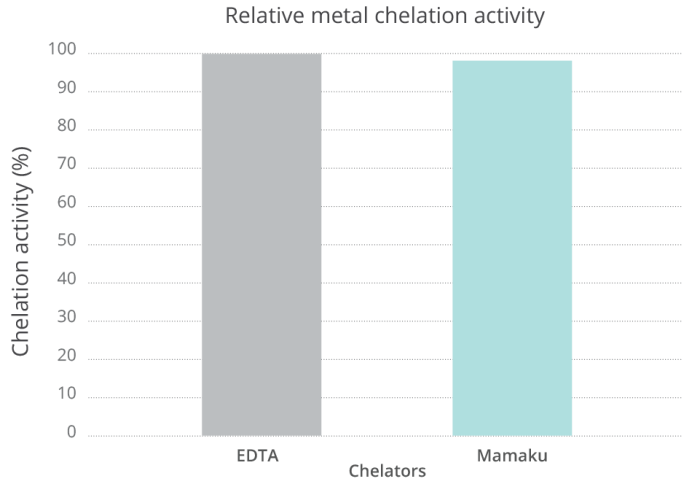


Fig 6. The total metal chelation activity (%) of **Mamaku**, compared to **EDTA**. **Note:** Chelation activity is related to anti-pollution activity. (n=3)

4.0.0 Physical properties

4.1.0 Spreadability

The spreadability of our test samples were tested by using two glass plates of equal length and width. One plate was taped and a marker was used to draw on to the surface a square grid with boxes of 5x5 mm. The center box on the plate is where our test samples were dispensed at 0.5ml. The glass plate with no tape was then dropped down carefully to sit on top of the bottom glass plate with our test sample. After 1 min, the top glass plate was lifted and spread diameter of test samples were measured and subbed into equations 1 and 2 [11].

1) **$Ei = d^2 \pi/4$** ; Where Ei (mm^2) = spreadability of the sample weight for a given sample, d = diameter (mm).

2) **Sf = A/W**; Where Sf = spreading factor (mm^2/g), A = total area (mm^2), W = total weight (g)

4.1.1 Results

The sensorial profile of a face cream can be determined by evaluating parameters such as spreadability, greasiness, softness and film-forming properties. **OceanDerMX™'s** face serum was found to have a greater sensorial profile than a well-known competitor's face cream that also contains a seaweed extract (Fig 7).

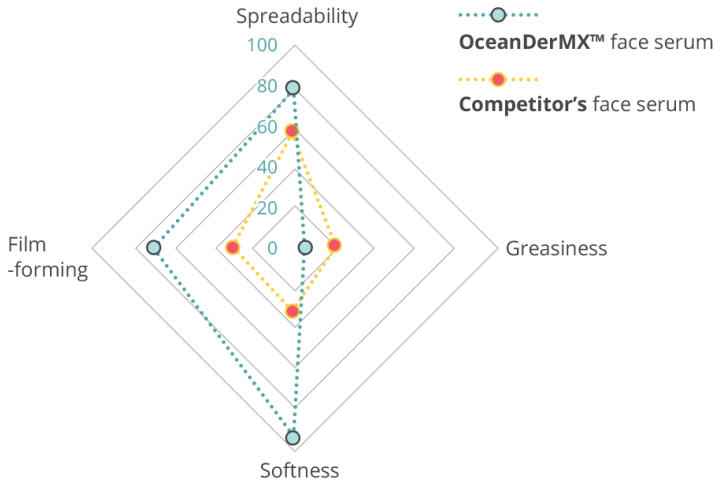


Fig 7. Sensorial profile of **OceanDerMX™'s** face serum, against **competitor's** face serum. (n=3)

4.2.0 Water-binding capacity

To determine the water-binding capacity of our samples, we firstly used hyaluronic acid as the standard. We ensured the initial dry weight of the standard and our samples were the sample, before administering 1ml of distilled water. After 1 min, the excess water was removed and the final weighted was noted. The results were then substituted in equation 1 below [12]:

1) **Water-binding capacity (%) = mt- mo / mo**

Where mo = original dry weight, mt = final wet weight without excess water

4.2.1 Results

The water-binding capacity of a compound is critical to how well it can perform as a skin moisturiser. The current industry standard is hyaluronic acid. **OceanDerMX™** is a better natural alternative, which significantly outperformed hyaluronic acid after only 1-minute administration of distilled water (Fig 8).

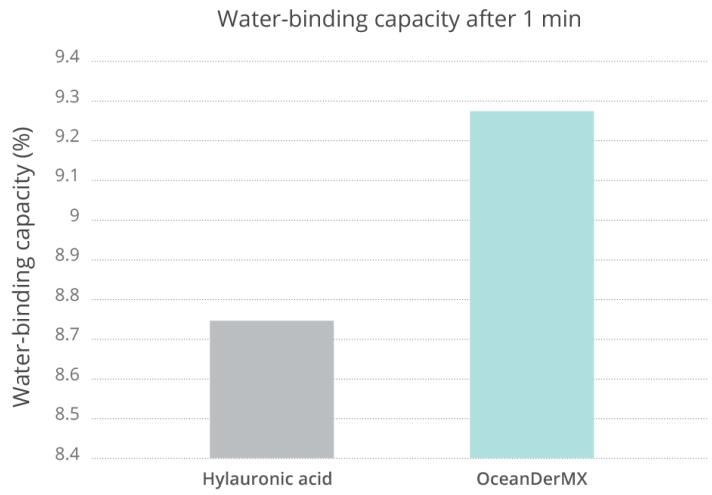


Fig 8. Water-binding capacity of **OceanDerMX™** compared to **Hyaluronic acid**, at 1 ml for 1 min. (n=3)

Note: Higher water-binding capacity is indicative of higher water retention capacity.

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