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優勝作品專輯

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參展科別　生物化學

作品名稱　SeaUVeed Succeed

得獎獎項　二等獎

就讀學校　Heep Yunn School
指導教師　Chau Hay Lei
作者姓名　Maisie Cheung Cheuk Yu、Ashley Wong Sin Hang、Sophie Chan Fei

作者照片



Abstract

In the first part of this project, distilled water and 95% ethanol were used to extract ultra-violet(UV)-absorbing and anti-oxidizing compounds from different types of algae including kelp, wakame, sea grape and nori. Activated charcoal was used in attempt to purify the extracts and removed excessive pigments. It was found that the charcoal was more effective in adsorbing pigments from ethanol extract in which up to 80 to 100% pigments could be removed. The UV-absorbing and anti-oxidizing properties of the algae extracts were also studied. All algae extracts showed significant UV-absorbing and anti-oxidizing properties. In particular, extract formed by using 3 g kelp powder in distilled water could significantly reduce 50% UV intensity and react 96.5% DPPH solution which acts as a source of free radicals.

In the second part of the project, three applications of algae were explored in details. Firstly, it was found that kelp, wakame and nori extracts by using over 2 g of algae in 30 mL olive oil could absorb UVA and UVB by over 90%, which is comparable to the performance of zinc oxide, a common ingredient in commercial sunscreen products. Costs of preparing the sunscreens were also compared. Except for wakame extract, all other extracts were cheaper than using zinc oxide. Moreover, the kelp extract was found to maintain its UV-absorbing and anti-oxidizing abilities after at least 30 days of storage under room conditions. Lastly, sodium alginate was successfully extracted from kelp with a product yield up to 30%. The alginate solution was then used to form a calcium-alginate protective coating on plastic slides to reduce UV intensity by up to 50%. This aims to apply on nails or fingers during UV nail gel polish to protect against UVR.

1. INTRODUCTION

1.1 Hazards of ultra-violet (UV) lights

Sunlight is composed of a wide spectrum of electromagnetic waves, including ultra-violet radiation (UVR). There are three types of UVR, namely UVA, UVB and UVC [1].

UVR	Wavelength (nm)
UVA	315-400
UVB	280-315
UVC	100-280

Table 1. Wavelength of different UVR[2]

UVA has the longest wavelength while UVC has the shortest. The longer the wavelength, the higher the penetrating power it has. UVC, having the least penetrating power, is mainly absorbed by the Earth's atmosphere before reaching the surface. However, UVA and UVB can penetrate the atmosphere and reach our skin upon exposure (Fig. 1). UVA and UVB are responsible for about 95% and 5% of the UVR penetrated into our skin respectively [4].

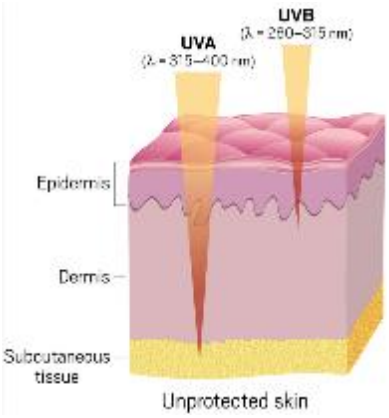


Fig.1. Penetration of UVA and UVB into skins [3]

Exposure to both UVA and UVB can induce the formation of free radicals [5,6], which is primarily reactive superoxide ions (O_2^-) with unpaired electron, in our skin that can degrade collagen and elastin. Photoaging and skin damage are resulted. In more serious case, long-term exposure to UVA and UVB can lead to DNA damage and even higher risks of developing skin cancer [7].

UV-induced diseases have become a worldwide health concern. According to the World Health Organization, 10% of the 15 million people who became blind due to cataracts are possibly caused by exposure to UVR[8]. And about 60% of a type of malignant skin cancer are suspected to be caused by UVR exposure [9]

1.2 Problems with commercial sunscreens

Sunscreens contain ingredients that can either absorb or reflect UV light from the skin. Organic aromatic ingredients in sunscreens like oxybenzone and octinoxate work by absorbing UV light and dissipating the energy as heat[10]. As for inorganic ingredients, like nano-zinc oxide and nano-titanium oxide, reflect and scatter the UVR off the skin[11].

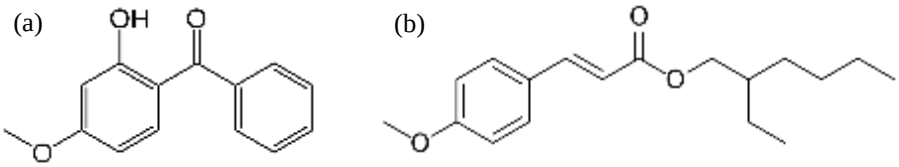


Fig.2. Chemical structures of (a) oxybenzone and (b) octinoxate

Oxybenzone
Octinoxate
Nano-zinc oxide
Nano-titanium oxide

Table 2. Common ingredients in commercial sunscreens

However, oxybenzone and octinoxate are derived from petroleum, which are non-renewable and causes much pollution along its production process. Besides, it is found that oxybenzone and other hydrocarbons in sunscreens are toxic to many marine organisms like algae and phytoplankton[12]. A study conducted by Stanford University found that corals take in oxybenzone and convert it into toxins after exposing to sunlight[13]. About 14,000 tonnes of sunscreens are washed to the coral reef areas each year[14]. In fact, Hawaii, Thailand and some other countries have banned the use of these hydrocarbon derivatives in sunscreen products in 2021.

In addition, nano-zinc oxide used in sunscreens has adverse effects on the growth of urchin[15] and it is toxic to algae[16] and other marine organisms.

1.3 Problems with the use of UV gel nail polish

In recent years, UV gel nail polish has become a popular trend in the cosmetic industry. The gel nail polish is made of methacrylate, which act as a monomer that will undergo polymerization to form a hard solid upon exposure to UV light. As the UV gels require the use of UV light of about 350 nm to set, most UV gel nail machines primarily emit a significant amount of UVA light.



Fig. 3. UV nail gel polish

During the process, a hand with nails coated with the UV gel polish is put under the UV lamp in the machine up to 5 minutes until the gel is hardened (Fig. 3). And this treatment is repeated about every two week[17].

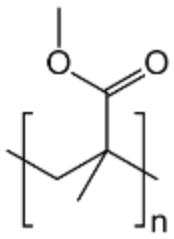


Fig. 4. Chemical structure of polymer formed from methacrylate

However, it is found that the chronic use of the UV gel nail lamp can damage DNA and mitochondrion in skin cells[18]. And nails might also be weakened and become more brittle. These adverse effects are especially prominent as the hand will be placed in close proximity to the UVR source during the polish treatment.

1.4 UV-absorbing and anti-oxidizing properties of algae

There are three main types of algae, which include brown algae (*Phaeophyceae)*, green algae (*Chlorophyta*) and red algae (*Rhodophyta*)[19].

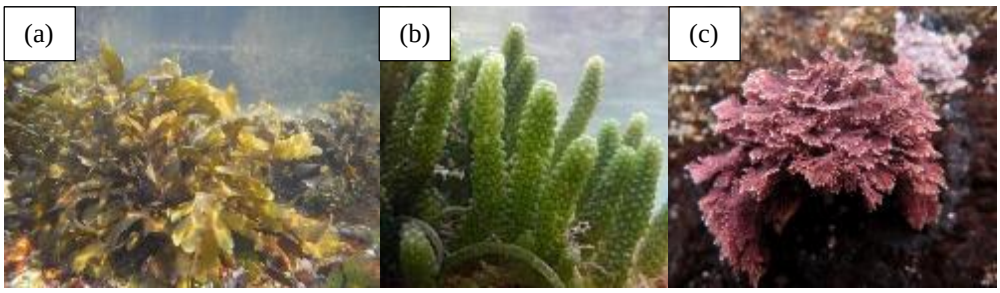


Fig. 5. Examples of (a) brown algae (b) green algae (c) red algae

They are known to contain a wide variety of bioactive compounds which attribute to various properties, such as UV-absorbing, anti-oxidizing and anti-bacterial properties. Two of the key compounds are mycosporine-like amino acids (MAAs) and fucoidan[20].

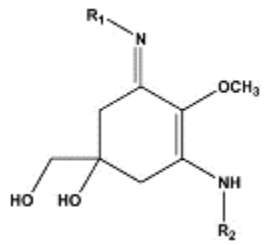


Fig. 6. Chemical structure of MAAs

MAAs are a group of UV-absorbing compounds that are usually produced by organisms living under exposure of high intensity of UV light[21]. The compounds are water-soluble with the major absorptions of UV light at 310 and 360 nm[22]. Therefore, they possess UV-absorbing property.

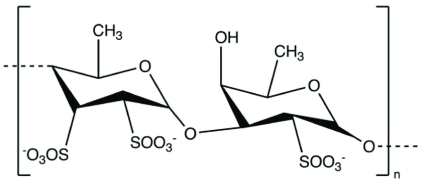


Fig. 7. Chemical structure of fucoidan

Besides, cell walls of many algae contain fucoidan, which is a type of polysaccharide that can remove free radicals formed and thus possesses strong anti-oxidizing property[23].

1.5 Objectives and scope of the project

In everyday life, it is almost inevitable for us to come in contact with UV lights. However, prolonged exposure to UV lights imposes irreversible threats to our health. Nonetheless, not only are the major ingredients in commercial sunscreens made from non-renewable petroleum, they also pollute the marine life and induce allergic reactions to some people.

Therefore, it is our aim to obtain extracts from various types of algae (kelp, wakame, sea grape and nori) and evaluate their UV-absorbing and anti-oxidizing properties. In particular, three potential applications (sunscreens, titanium white paint and protective coating for UV nail polish) of algae extracts were studied in details in hope of providing a new insight to make good use of this renewable materials to offer economic and environmentally friendly protection against UVR and free radicals.

2. PREPARATION OF ALGAE EXTRACTS

2.1 Choices and sources of algae

In this project, samples from each family of algae were chosen for investigation (Fig. 8). For brown algae, kelps (*Saccharina japonica*) and wakame (*Undaria pinnatifida*) were chosen. For green algae, sea grapes (*Caulerpa Racemose*) was chosen. And for red algae, nori (*Porphyra*) was chosen. They were chosen as they were commonly available in local markets.

The samples of kelps, wakame and sea grapes used in this project were originated from Hokkaido, Naruto and Okinawa in Japan respectively. They were purchased from local supermarkets and food retailers. Nori was originated from Fujian in China and they were purchased through local wet markets.

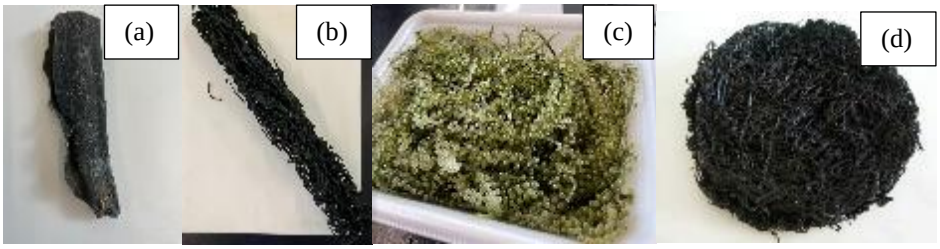


Fig. 8. (a) Kelp; (b) Wakame; (c) Sea grape; and (d) Nori

2.2 Pre-treatment of algae

All the algae samples (except for sea grapes) were washed gently with tap water and were air-dried. They were then ground into fine powder by using a domestic bean grinder (Fig. 9). The powder was then passed through a sieve of hole size of 0.5 mm to standardize the fineness of sample particles. For sea grapes, they were crushed into paste by using pestle and mortar (Fig. 10).

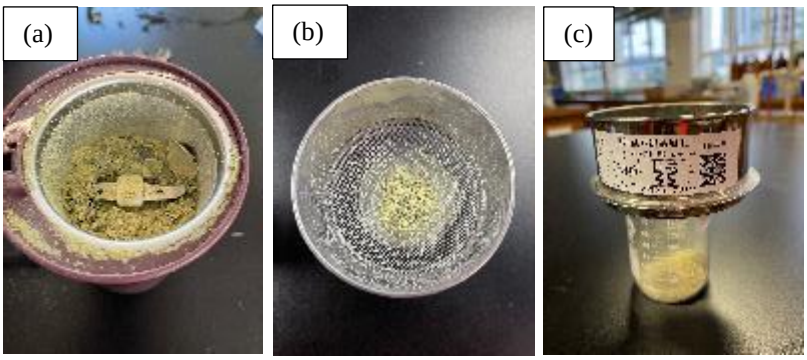


Fig. 9. (a) the bean grinder; (b) kelp powder on the sieve; (c) passing the powder through the sieve

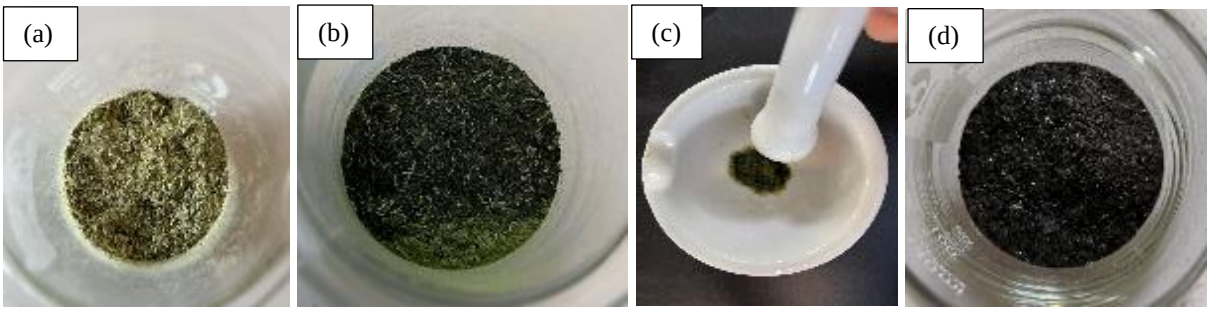


Fig. 10. (a) kelp powder; (b) wakame powder; (c) ground sea grape paste; and (d) nori powder

2.3 Solvent extraction

In order to extract the active compounds (for examples, MAAs and fucoidan) responsible for UV-absorbing and anti-oxidizing property from algae samples, solvent extraction was applied.

2.3.1 Choice of solvent

Distilled water and 95% ethanol were chosen to extract active compounds from the algae samples. The reason is that they are polar solvents, which can favour the extraction of polar molecules like MAAs and fucoidan [24, 25].

2.3.2 Method and experimental set-up

Method

1. Measure various masses (about 0.5 g, 1 g, 2 g and 3 g) of algae samples.
2. Immerse the weighed samples into 30 mL solvents respectively (distilled water and 95% ethanol).
3. Stir the mixture well to homogenize the content.
4. Allow the mixture to stand for 24 hours.
5. Filter the mixture to obtain the extracts.

Results

Appearance of extracts

(a) Extraction with **distilled water**

(i) **Kelp**



(ii) **Wakame**



(iii) **Sea grape**



(iv) **Nori**



Fig. 11. Algae extracts by using distilled water
(i) kelp;
(ii) wakame;
(iii) sea grape; and
(iv) nori

(b) Extraction with **ethanol**

(i) **Kelp**



(ii) **Wakame**



(iii) **Sea grape**



(iv) **Nori**



Fig. 12. Algae extracts by using 95% ethanol
(i) kelp;
(ii) wakame;
(iii) sea grape; and
(iv) nori

Discussion

According to the colours of the extracts in Fig. 11 and Fig.12, it can be concluded that a significant amount of pigments from the algae samples were extracted by both solvents. In particular, ethanol was able to extract much more pigments than distilled water which led to higher colour intensities in the extracts. The green colours can be explained by the fact that chlorophyll in algae is soluble in ethanol but not in water. Besides, purple colour was only observed in the nori extract by distilled water. This might be due to the presence of purple pigments phycoerythrin and phycocyanin[26].

In order to further study the effect of UV-absorbing property of other photosensitive compounds in the extracts, a purification method was proposed in the next section to remove the pigments to see if UV-absorbing property retains.

2.4 Removal of pigments from the crude extracts

It was observed in Fig. 11 and Fig. 12 in Section 2.3.2 of this project that the algae extracts have different intensities of yellowish green and purple colours. It is expected that the colour is due to the presence of chlorophyll, phycoerythrin and phycocyanin coming from the algae samples during the extraction. The effect of other photosensitive compounds in the extracts should also be studied. Therefore, the following experiment was carried out to remove the pigments and compare the UV-absorbing properties.

2.4.1 Use of colorimetry to estimate the relative pigment content

The major absorption wavelength of chlorophyll a is between 430 nm and 660-680 nm while that of chlorophyll b near 450 and 640 nm. As for phycoerythrin and phycocyanin, the peak absorption occurs between 530 and 570 nm; and 610 nm to 660 nm[28, 29, 30] (Fig. 13).

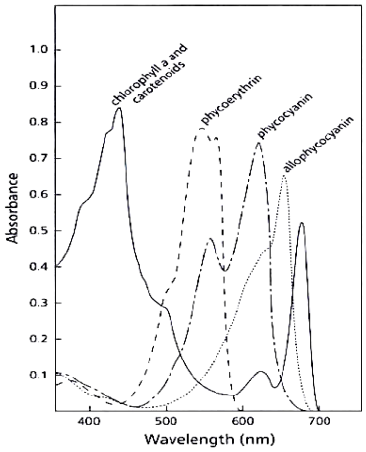


Fig. 13. Light absorbance of chlorophyll a, phycoerythrin and phycocyanin across various wavelengths [27]

To roughly compare the pigment content, a colorimeter was used (Fig.14.). Light filters of the complementary colours to the colours of the solutions were chosen. As chlorophyll absorbs blue light mostly, a blue filter was used in the colorimeter. As for phycoerythrin and phycocyanin, a green filter was used instead.

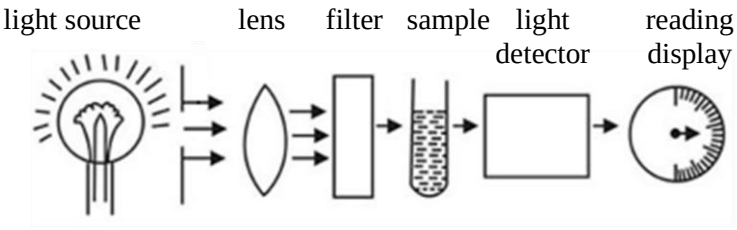


Fig. 14. Working pathway of a colorimeter [31]

The higher the absorbance of blue or green light recorded by the colorimeter, the higher the content of chlorophyll or phycoerythrin and phycocyanin. This method can give a brief idea of the change in pigment content in the algae extracts.

2.4.2 Use of activated charcoal to remove pigments

Activated charcoal is commonly used to remove and filter water in daily life. In the laboratory, it is used to remove acid-base indicator from the salt solution obtained by titration.

As a highly porous material, activated charcoal provides a large surface area of 300 m^2 to 2000 m^2 per gram to adsorb any impurities and pigments[32]. Therefore, it is expected that activated charcoal can remove a certain amount of pigments from the algae extract obtained from the previous section.

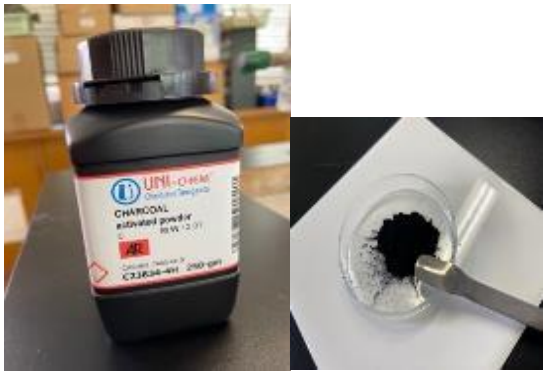


Fig. 15. Activated charcoal used in this experiment

2.4.3 Changes in relative pigment content after treatment

Method

1. Fill a test tube with 10 mL algae extract
2. Measure the light absorbance of the extract by using a colorimeter
3. Add 0.06 g activated charcoal into the extract
4. Mix the charcoal well in the extract
5. Allow the mixture to stand for 3 minutes
6. Filter the mixture to obtain the filtrate
7. Measure the light absorbance of the filtrate by using a colorimeter



Fig. 16. Colorimeter used in this experiment

Results

1. Appearance of purified extracts
   (a) Extraction with **distilled water**
   (i) Kelp (ii) Wakame





   (iii) Sea grape (iv) Nori





Fig. 17. Algae/water extracts treated with activated charcoal (i) kelp; (ii) wakame; (iii) sea grape; and (iv) nori

   (b) Extraction with **ethanol**
   (i) Kelp (ii) Wakame





   (iii) Sea grape (iv) Nori





Fig. 18. Algae/ethanol extracts treated with activated charcoal (i) kelp; (ii) wakame; (iii) sea grape; and (iv) nori

2. Percentage decrease in colorimeter readings

The colorimeter readings can be interpreted by using Beer-Lambert Law. In the Beer-Lambert equation (Equation 1), light absorbance of a coloured solution is directly proportional to the concentration of the coloured substance in the solution:

$$A = \varepsilon c l$$

Equation 1. Beer-Lambert equation

where A is the absorbance of light;
ε is the molar absorption coefficient;
c is the concentration of coloured substance; and
l is the optical path length in the colourimeter

From Equation 1, percentage decrease in pigment content can be approximated as follows:

$$\textit{Percentage decrease in pigment content}$$

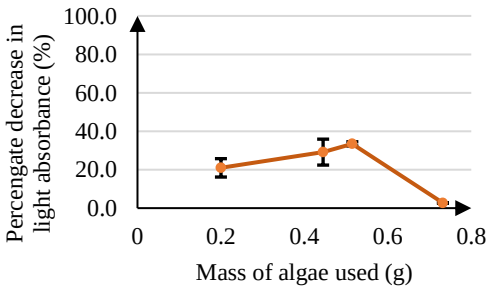
$$= \frac{\textit{Initial concentration} - \textit{Final concentration}}{\textit{Initial concentration}} \times 100\%$$

$$= \left(1 - \frac{\textit{Final absorbance}}{\textit{Initial absorbance}}\right) \times 100\%$$

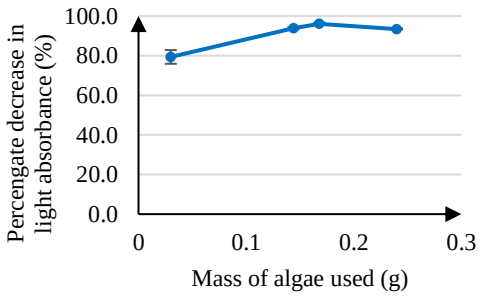
Equation 2. Percentage decrease in pigment content

(a) **distilled water extracts** *(for raw data, please refer to Supplementary File P.S-5)*

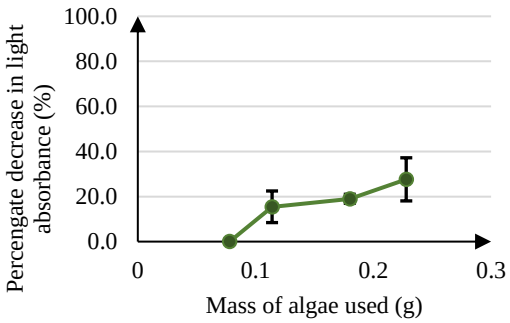
(i) Kelp



(ii) Wakame



(iii) Sea grape



(iv) Nori

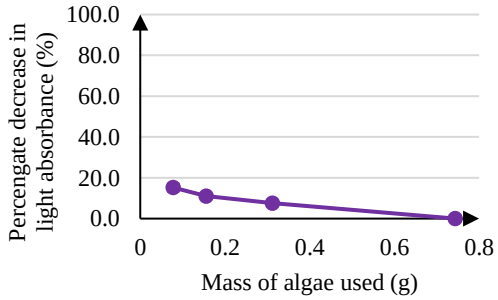
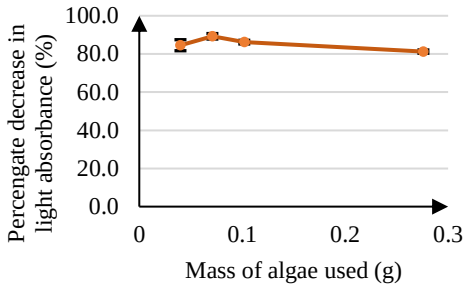


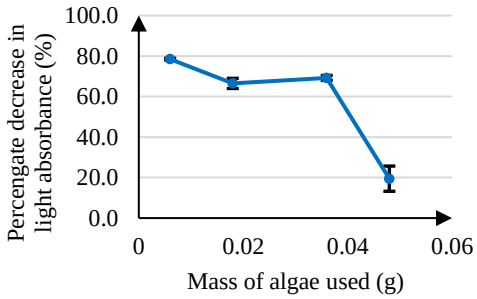
Fig. 19. Percentage decrease in pigment content in water extract after treated with activated charcoal (i) kelp; (ii) wakame; (iii) sea grape; and (iv) nori

(b) **ethanol extract** *(for raw data, please refer to Supplementary File P.S-5)*

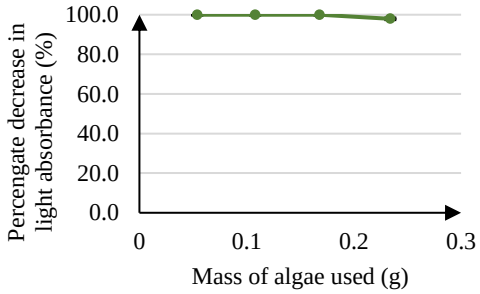
(i) Kelp



(ii) Wakame



(iii) Sea grape



(iv) Nori

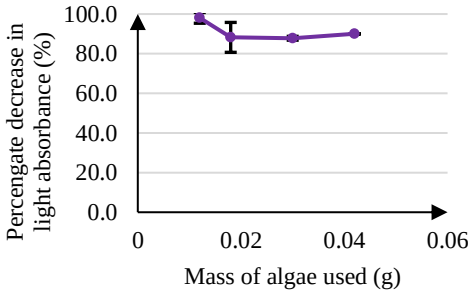


Fig. 20. Percentage decrease in pigment content in ethanol extract after treated with activated charcoal (i) kelp; (ii) wakame; (iii) sea grape; and (iv) nori

Discussion

By comparing the colours of extracts in Fig. 17 and Fig. 18, it can be concluded that pigments in ethanol extracts were more efficiently adsorbed by activated charcoal than in water extracts. This is supported by the results in Fig.19 and Fig. 20 that the percentage decrease in light absorbance of ethanol extracts after treatment was much higher than that of water extract (except for wakame). The differences were particularly significant in kelp extracts and nori extracts. There were about 40 to 60% difference in pigment contents between ethanol and water extracts in both cases.

In particular, the performance of activated charcoal was the poorest in the case of nori/water extract as shown in Fig. 19. It is suggested that purple pigments phycoerythrin and phycocyanin in the nori extracts were not subjected to adsorption by activated charcoal. The performances of activated charcoal were also relatively low for the case of kelp/water extract and sea grape/water extract as shown in Fig. 19(a)(i) and (ii). From the appearance of the extract in Fig. 17 and the light absorbance data before treatment (see *Supplementary File P.S-5*), it can be concluded that very low concentration of pigments were extracted by distilled water in both cases. Therefore, the percentage decreases in absorbance after treating with activated charcoal were low as there was not much pigment to be removed in the first place.

In order to study whether the treatment with activated charcoal removed the UV-sensitive compounds in the extracts, the UV-absorbing property of purified extracts were measured together with the untreated crude extracts in the following section.

3 PRELIMINARY STUDY OF UV-ABSORBING PROPERTY OF CRUDE EXTRACTS

The following experiments were conducted to investigate the UV-absorbing property of the algae extracts obtained from the previous section.

3.1 Design of an experimental set-up to measure UV intensity

A simple experimental set-up was designed by using common daily materials (paper box, plastic foam block etc.) to measure the intensity of UV light given off from an UV lamp after passing through each extract. (Fig. 21).

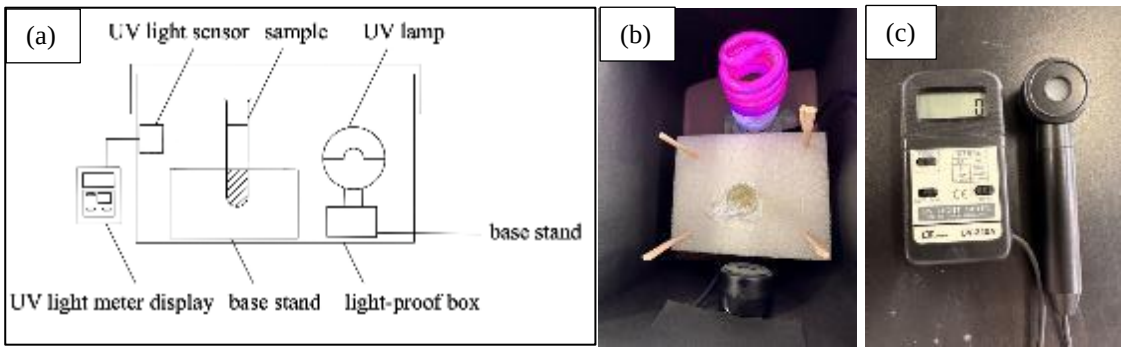


Fig. 21. (a) A design of set-up to measure the change in UV light intensity after passing through samples; (b) the internal structure of the set- up; and (c) the UV light meter and its sensor used in this project

Each tube of algae extract was placed in the base holder of the above apparatus between the UV lamp and the UV light meter at a fixed position. The UV light meter measures intensity of UV lights (in $\mu W/cm^2$) ranging from 290 nm to 390 nm, which include both UVA and UVB lights. The box was covered with black light-proof materials to prevent any leakage of UV lights or light interference from the surrounding.

3.2 Percentage decrease in UV intensity (UV-absorbing ability)

Method

1. Turn on the UV light sensor
2. Turn on the UV lamp to warm up until the reading on the sensor is steady
3. Insert the test tube of sample extract to the base holder and cover the apparatus
4. Record the reading until it is steady

Results

To estimate the UV-absorbing abilities of different algae extracts, intensities of UV light passing through the pure solvent (distilled water or ethanol) and the extracts were measured. The UV-absorbing ability was then estimated by the percentage decrease in UV intensity as compared with the value using pure solvent :

$$\textit{Percentage decrease in UV intensity}$$

$$= \frac{UV_{pure\ solvent} - UV_{sample}}{UV_{pure\ solvent}} \times 100\%$$

$$= \left(1 - \frac{UV_{sample}}{UV_{pure\ solvent}}\right) \times 100\%$$

Equation 3. Percentage decrease in UV intensity

where $UV_{pure\ solvent}$ and UV_{sample} are the intensities of UV lights detected after passing through the solvents (distilled water or ethanol) and the algae extracts respectively

1. Percentage decrease in UV intensity (arranged according to types of solvents used)

(a) **Before treated with activated charcoal**

(for raw data, please refer to Supplementary File P.S-6)

(i) using **distilled water** (ii) using **ethanol**

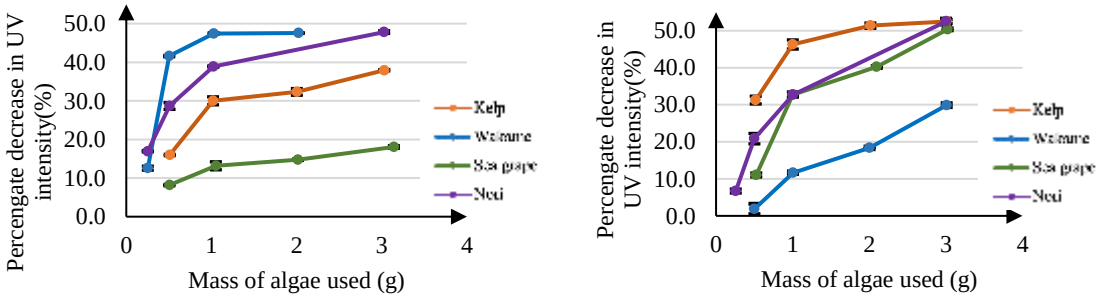


Fig. 22. Comparison of percentage decrease in UV intensity before treatment with activated charcoal (i) using distilled water (ii) using ethanol as solvent

(b) **After treated with activated charcoal**

(for raw data, please refer to Supplementary File P.S-6)

(i) using **distilled water** (ii) using **ethanol**

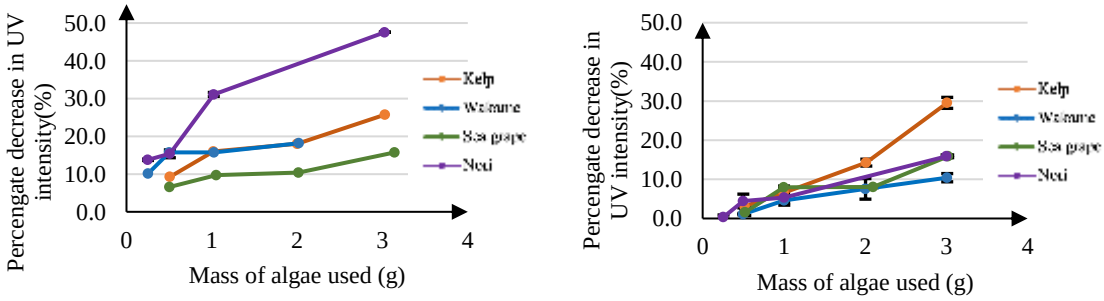
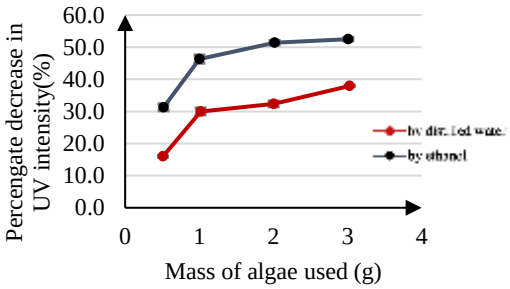


Fig. 23. Comparison of percentage decrease in UV intensity after treatment with activated charcoal (i) using distilled water (ii) using ethanol as solvent

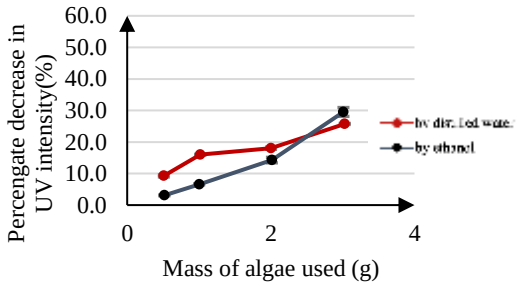
2. Percentage decrease in UV intensity (arranged according to the types of algae used)
(for raw data, please refer to Supplementary File P.S-5)

(a) **Kelp**

(i) before treatment

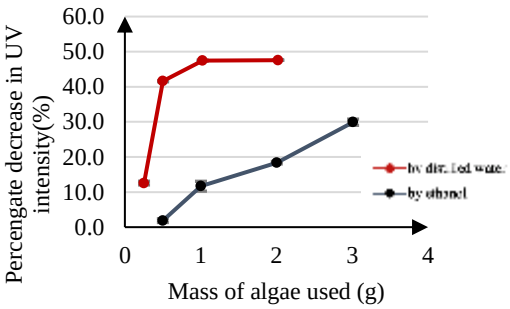


(ii) after treatment

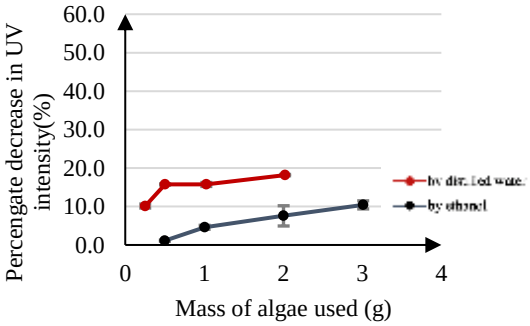


(b) **Wakame**

(i) before treatment

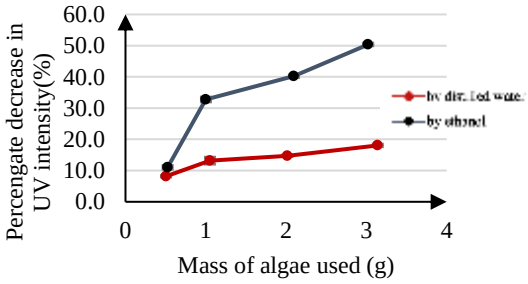


(ii) after treatment

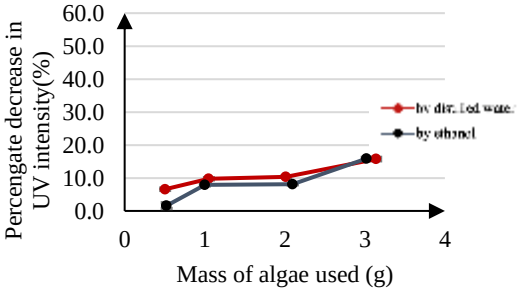


(c) **Sea grape**

(i) before treatment

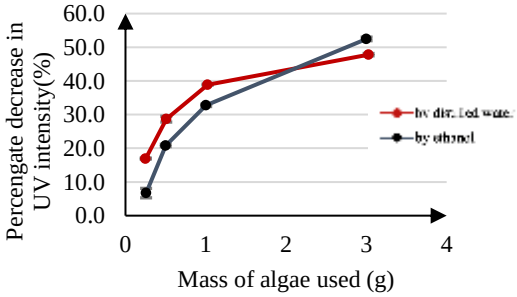


(ii) after treatment



(d) **Nori**

(i) before treatment



(ii) after treatment

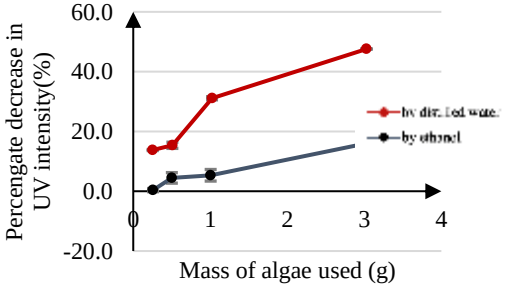


Fig. 24. Comparison of percentage decrease in UV light intensity before and after treatment with activated charcoal for various types of extracts

Discussion

Based on the results in Fig.22 to Fig.24, extracts by using both ethanol and distilled water showed certain degrees of UV-absorbing abilities. In particular, 3 g of kelp in 30 mL ethanol gave an extract with the highest UV-absorbing ability before treating with activated charcoal. It reduced the UV intensity by 52.5% as shown in Fig. 24(a)(i).

As from Fig. 24, it is worth noting that all UV-absorbing abilities of extracts dropped after treating with activated charcoal. In particular, the extent of decline in performance was much more significant in extracts prepared by using ethanol. The largest difference went with 39.7 % reduction in UV-absorbing ability for kelp/ethanol extract prepared from 1 g kelp. This suggests that the active compounds in the extract were most subjected to the action of activated charcoal. Among all, nori/water extract prepared from 3 g nori retained most of its UV-absorbing ability with only 0.2% decline after treatment with activated charcoal.

The results show that the pigments were responsible for part of the UV-absorbing abilities. The extracts maintained certain levels of UV-absorbing ability even after removal of the pigments. Therefore, algae extract could be a potential effective UV-absorbing ingredient even after purification.

4 ANTI-OXIDIZING PROPERTY OF ALGAE EXTRACTS

As mentioned in the introduction, exposure to UV light would lead to generation of free radicals which cause oxidative damage to the skin cells. In order to study the free-radical-removing property (anti-oxidizing property) of algae extracts and explore the possibility of using them as sunblock materials, the following experiments were carried out.

4.1 Use of DPPH solution

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable organic compound with a nitrogen radical[33]. It exists as dark purple crystalline form at room temperature and pressure (Fig. 25). This can be used to mimic the free radicals generated in skin when exposed to UV light.

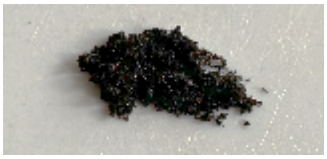


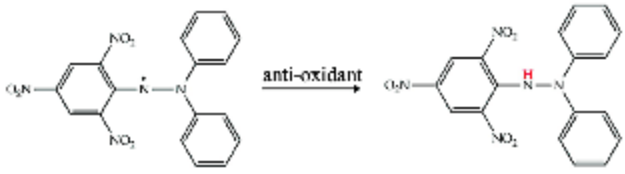
Fig. 25. Crystalline form of DPPH used in this project

To measure anti-oxidizing abilities of the algae extracts, DPPH solution should be used. DPPH solution can be prepared by dissolving the crystals into absolute ethanol. A purple solution with light absorbance at around 517 nm will be resulted[33] (Fig. 26).



Fig. 26. DPPH solution in absolute ethanol

When DPPH solution is mixed with anti-oxidant solution, a chemical reaction will occur and the free radical of the nitrogen atom in a DPPH molecule will receive an electron from the anti-oxidant. Thus, the odd electron is paired off (Equation 4). As a result, the solution will turn from deep purple to yellow. The extent of colour change depends on the anti-oxidizing power of the anti-oxidant used[33].



Equation 4. Equation of free-radical cleavage: pair up odd electron on free radical atom

Therefore, anti-oxidizing power of an algae sample can be estimated by the degree of scavenging the free radicals from DPPH and turning the solution yellow. The lower the intensity of purple colour of the final mixture, the higher the anti-oxidizing power of the algae sample.



Fig. 27. Discoloration of DPPH under the influence of ascorbic acid at different concentrations (25-125 µg/ml)[34]

In this study, 0.1 mM DPPH solution was prepared by directly dissolve the solid in absolute ethanol. The solution was wrapped with aluminium foil and stored in a fridge at around 4°C to prevent unwanted side reactions of the free radicals. The solution was freshly prepared only right before each of the experiments to reduce the effect of self-degradation of DPPH solution upon storage.

4.2 Anti-oxidizing property of different algae extracts

To estimate the anti-oxidizing power of different algae extracts, three solutions were prepared for each sample, including an algae sample solution, a control solution and a blank solution. Then, the light intensity of purple colour of these solutions were measured by a colorimeter.

Method

1. Prepare control solution by mixing 3 mL DPPH solution with 3 mL solvent (distilled water or 95% ethanol) and shake well.
2. Prepare a blank solution by mixing 3 mL algae extract solution with 3 mL absolute ethanol and shake well.
3. Add 3 mL DPPH solution into 3 mL algae extract solution and shake well.
4. Place the solutions in the dark for 30 minutes to allow the reaction to take place.
5. Measure the light absorbance of each solution at around 517 nm by using a colorimeter with a green light filter.
6. Repeat the steps with all other algae extract solutions.

Results

1. Colours of the **control solutions**
   A mixture of 3 mL DPPH solution with **distilled water (the left)** and **95% ethanol (the right)**



Fig. 28. Colours of DPPH solution mixed with distilled water and 95% ethanol

2. Colours of the DPPH/algae extract mixtures after 30 minutes
   (a) distilled water extracts

(i) Kelp



(ii) Wakame



(iii) Sea grape



(iv) Nori



Fig. 29. Final colour of the mixture of DPPH solution and different water extracts after 30 minutes

(b) ethanol extracts

(i) Kelp



(ii) Wakame



(iii) Sea grape



(iv) Nori

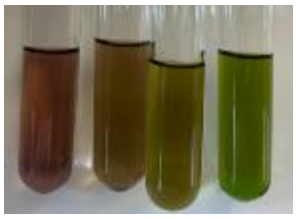


Fig. 30. Final colour of the mixture of DPPH solution and different ethanol extracts after 30 minutes

3. Anti-oxidizing power of algae extracts

The anti-oxidizing power of the algae extracts, in terms of free radical scavenging power, was estimated by calculating the percentage decrease of light absorbance at 517 nm by using the following formula[35,36]:

Anti-oxidizing power of algae extract
(percentage decrease in light absorbance at 517 nm)

$$= (1 - \frac{A_{sample} - A_{blank}}{A_{control}}) \times 100\%$$

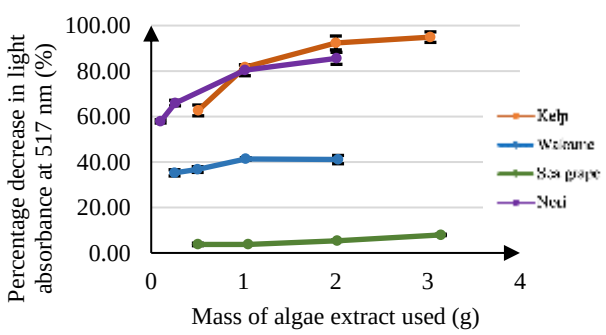
Equation 5. Percentage decrease in light absorbance at 517 nm

where A_{sample}, A_{blank} and $A_{control}$ refer to the light absorbance of sample solution, blank solution and control solution at 517 nm

Percentage decrease in light absorbance at 517 nm

(a) Data arranged according to **types of solvents** used
(for raw data, please refer to Supplementary File P.S-7)

(i) **distilled water**



(ii) **ethanol**

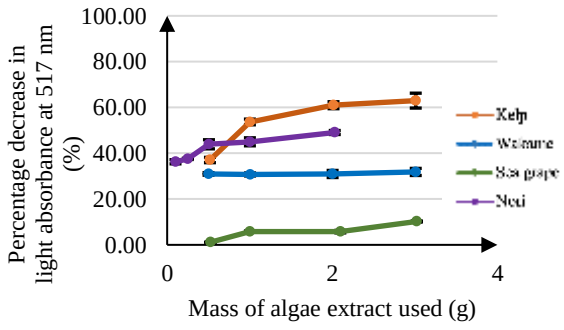
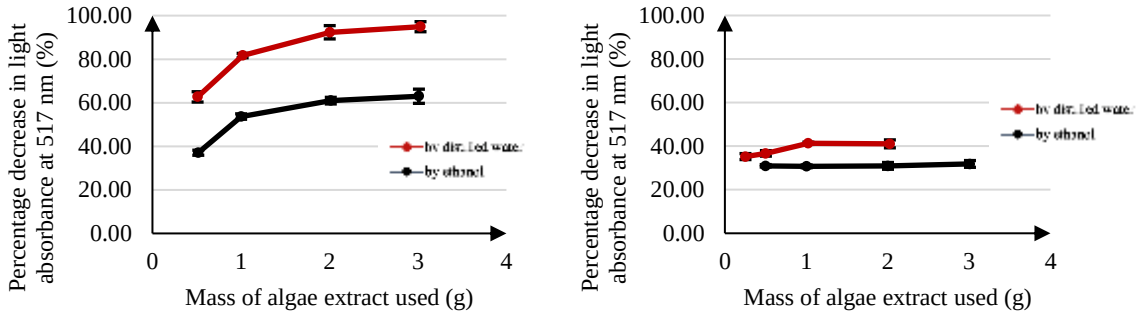


Fig. 31. Comparison of percentage decrease in light absorbance at 517 nm after mixing DPPH solution with different algae extracts

(b) Data arranged according to **types of algae**
(for raw data, please refer to Supplementary File P.S-7)

(i) Kelp (ii) Wakame



(iii) Sea grape (iv) Nori

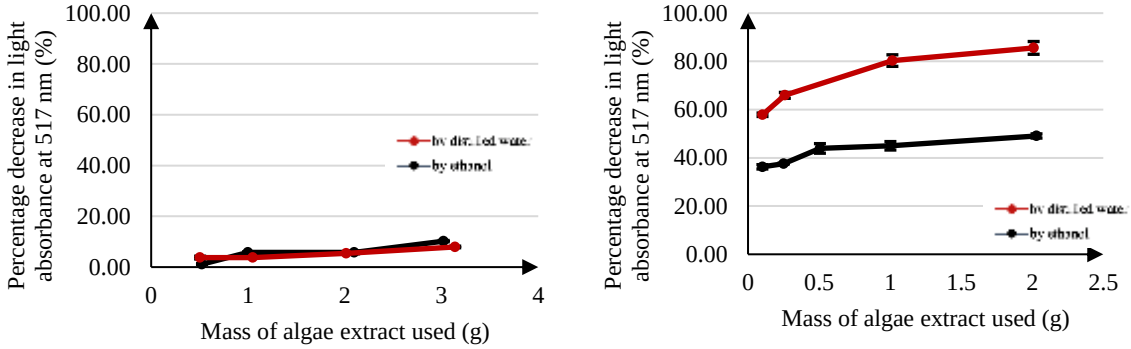


Fig. 32. Comparison of percentage decrease in light absorbance at 517 nm after mixing DPPH solution with extracts prepared by different solvents

Discussion

According to Fig. 31, kelp extracts exhibited the highest anti-oxidizing power to scavenge the free radicals in DPPH among all other extracts. In particular, kelp extract prepared from 3 g kelp in 30 mL water exhibited the highest anti-oxidizing power by reacting with 96.5% DPPH as shown in Fig. 31(a)(i).

However, both sea grape extracts performed the worst among all other extracts. The largest percentage decrease in light absorbance at 517 nm was 10.4% for sea grape/ethanol extract, which was still 20% lower than the lowest percentage decrease in wakame/ethanol extract prepared from 0.501 g wakame.

Besides, from Fig. 32(b)(i), the percentage decreases in absorbance for kelp/water extract were much larger than that by ethanol extract. It is suggested that water could extract the anti-oxidants from kelp more effectively.

5 APPLICATION I: AS RAW MATERIALS FOR SUNSCREENS

5.1 Preparation of nano-zinc oxide suspension as a standard

As mentioned in section 1.2, nano-zinc oxide is one of the most commonly used ingredients in commercial sunscreen to block UVR.

In common commercial sunscreens, nano-zinc oxide was homogenized with some plant oils to make a lotion or a cream. Therefore, nano-zinc oxide was mixed with olive oil in this study to prepare a series of mixture to explore the UV-absorbing ability of zinc oxide and to compare with the performances of the algae extracts.



Fig. 33. Nano-zinc oxide powder used in this project

Methods

1. Measure about 0.1 g, 0.2 g, 0.5 g, 1 g, 2 g and 3 g nano-zinc oxide powder
2. Add 30 mL olive oil to the powder and stir well
3. Measure the intensities of UVA and UVB lights after passing through the controls prepared

Results

1. **Appearance** of nano-zinc oxide suspension
   Creamy yellowish white suspension were obtained with high viscosity.



Fig. 34. Suspension of nano zinc oxide in olive oil

2. Percentage decrease in intensities of UVA and UVB
   (for raw data, please refer to Supplementary File P.S-8)

Mass concentration (g/dm^3)	Average percentage decrease in intensities of	
	UVA (%)	UVB (%)
3	97.9	69.6
7	98.3	76.8
17	98.7	83.9
34	98.7	87.5
68	99.1	87.5
100	99.4	94.6

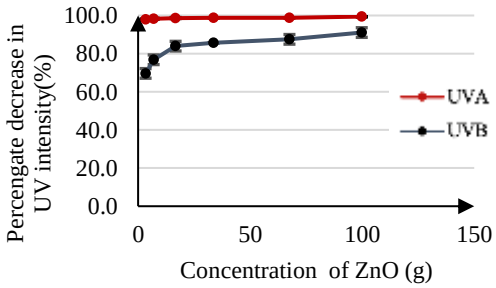


Table 3. and **Fig.35** Average percentage decrease in UVR intensities after passing through standard zinc oxide suspension

Discussion

The nano-zinc oxide suspensions prepared exhibited excellent UV-absorbing abilities. In particular, it was more effective in absorbing UVA than UVB with the largest difference of 28.3% in the case of 3 g/dm^3 concentration.

5.2 Choice of oil for extraction from algae

In order to explore the possibility of the algae extracts to replace zinc oxide in sunscreens, olive oil PEG-7 esters (CAS：103819-46-1) was used to extract photo-sensitive compounds from the algae samples. Olive oil PEG-7 esters is commonly used to make sunscreen lotions and perfume in cosmetic and skincare industry. It is water soluble mixture of olive oil and esters, which is non-irritant and non-toxic to skin and eyes.

5.3 Preparation of algae/olive oil extracts

The algae/olive oil extracts were prepared through the following procedure:

Method

1. Measure various masses (about 0.5 g, 1 g, 2 g and 3 g) of algae samples.
2. Immerse the weighed samples into 30 mL olive oil
3. Stir the mixture well to homogenize the content
4. Allow the mixture to stand for 24 hours.
5. Centrifuge the mixture to obtain the supernatant as the extract

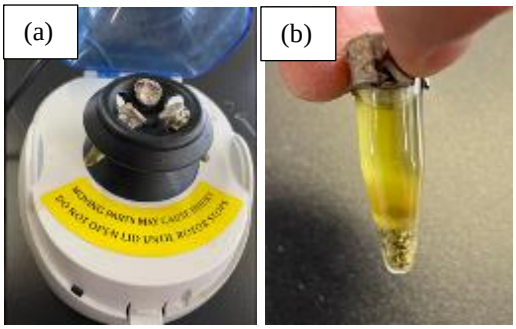


Fig. 36.(a) Centrifuge machine and (b) kelp/olive oil extract after centrifugation

5.4 Properties of algae/olive oil extracts

To explore the possibility of using algae/olive oil extracts in sunscreens, UVA- and UVB-absorbing abilities of the extracts were studied. The UV light measurement box prepared in section 3.1 was modified by replacing the UV lamp with an UVA light torch of wavelength 340 nm and UVB light torch of wavelength 295 nm (Fig. 37).

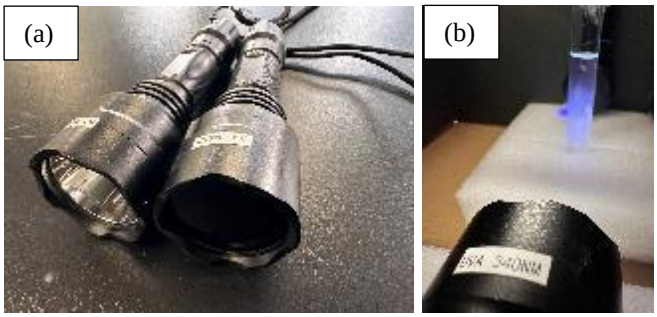


Fig. 37.(a) UVA and UVB light torches; and (b) internal structure of the UV light measurement box

Besides, DPPH test was also carried out to find out the anti-oxidizing properties of the oil extracts.

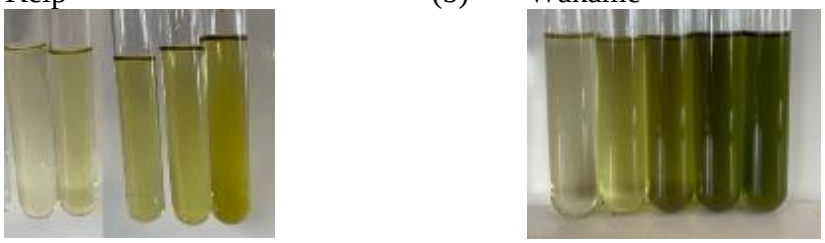
Methods

1. Measure the change in intensity of UVA and UVB lights passing through the extracts and zinc oxide suspensions by using the modified set-up as in Fig. 36(b)
2. Carry out the DPPH test with the sample according to procedure stated in section 4.2.

Results

1. **Appearance** of the olive oil extract

(a) Kelp (b) Wakame



(c) Sea grape (d) Nori

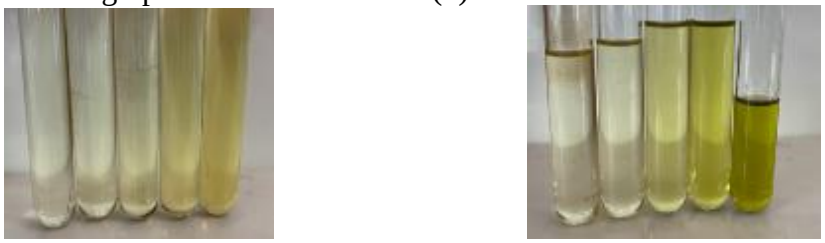


Fig. 38. Algae/olive oil extracts with different masses of algae used

2. **Percentage decrease in intensities of UVA and UVB**
(for raw data, please refer to Supplementary File P.S-9 to S-11)

(a) Data arranged according to **types of UVR**

(i) **UVA** (ii) **UVB**

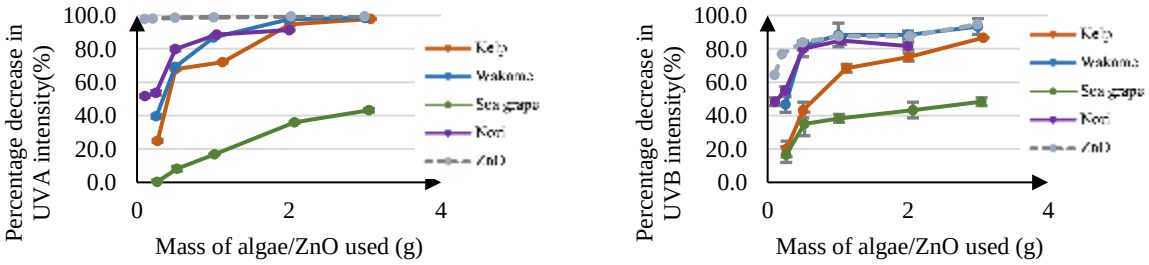


Fig. 39. Comparison of percentage decrease in UV light intensity among different algae extracts

(b) Data arranged according to **types of algae**

(i) Kelp (ii) Wakame

(ii) Sea grape (iv) Nori

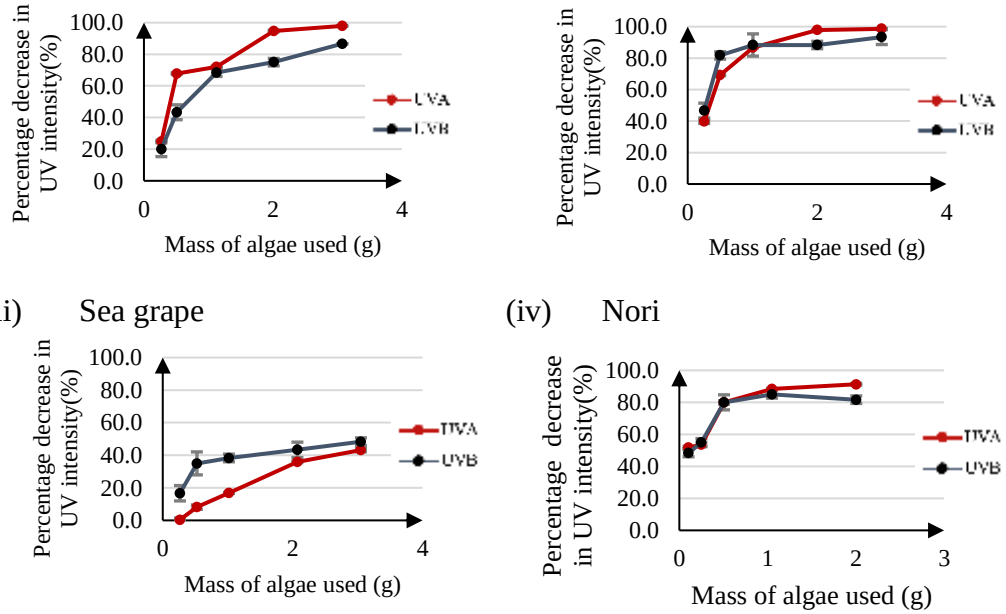


Fig. 40. Comparison of percentage decrease in UVA and UVB lights for each algae extract

3. DPPH test

Only kelp/olive oil extract could lead to reduction in purple intensity in the DPPH solution:

Mass of kelp used (g)	Average percentage decrease in light absorbance at 517 nm (%)
0.270	13.9
0.508	20.2
1.126	21.4
2.012	26.4
3.076	27.3

Table 4. Average percentage decrease in light absorbance at 517 nm among olive oil extract by using different masses of kelp

Discussion

All algae/olive oil extracts showed significant abilities to absorb UVA and UVB as shown in Fig. 39. In particular, wakame and kelp extract could reduce UVA intensity by 98.8% and 97.9% respectively. However, the UVA-absorbing ability of sea grape were found to be weaker than others.

When there were over 2 g of algae used, UVA- and UVB-absorbing ability of the kelp, wakame and nori extracts were comparable to that of nano-zinc oxide suspension (Fig.39(a)), which is a major component in commercial sunscreen. For the case of 3 g of wakame used, the difference in UVA and UVB absorbance was only 0.8% and 1.3% respectively compared with zinc oxide. It shows that these extracts are potential replacement of zinc oxide in sunscreen products,

It is worth noting that the increasing trends in UV-absorbance began to slow down when more than 1 g of algae used in 30 mL solvent (as shown in Fig. 34 and Fig.40). A possible explanation for this is that the oil solvent became saturated with the extracted components at those concentrations after 24 hours.

With the good anti-oxidizing ability (Table 4) and UVR-absorbing ability, kelp, in particular, is expected to be a promising material which can provide an extra protection against free radicals and UVR.

5.5 Sustainability of performance of algae/olive extracts

To investigate whether the kelp/olive oil extract can sustain its UV-absorbing ability and anti-oxidizing ability after a prolonged period of storage, the following experiments were carried out.

5.5.1 Performances after storing for different periods of time

A series of kelp extracts of mass concentration about 133 g/dm^3 were prepared. They were then stored under room conditions ($25\pm3\,^{o}$C, 1 atm) in the dark for different periods of time. The UV-absorbing ability and anti-oxidizing ability were then measured after the designated storage time. The procedures of measurements were identical as those in sections 5.4 and 4.2 respectively.

Results

(a) Percentage decrease in UV intensities (**UV-absorbing ability**)
(for raw data, please refer to Supplementary File P.S-12)

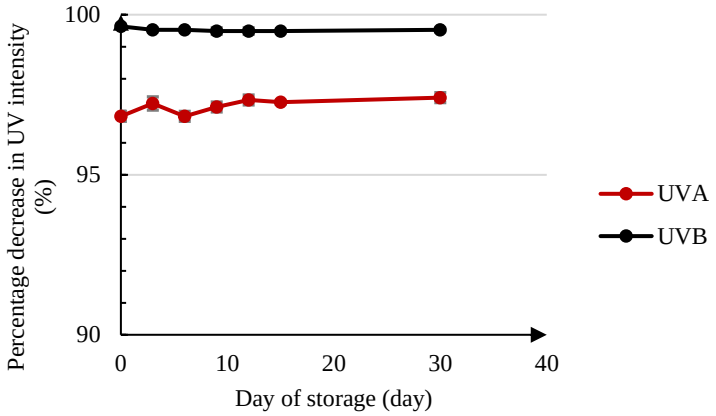


Fig. 41. Percentage decrease in UVA and UVB intensities after storage

(b) Percentage decrease in light absorbance at 517 nm (**anti-oxidizing ability**)
(for raw data, please refer to Supplementary File P.S-12)

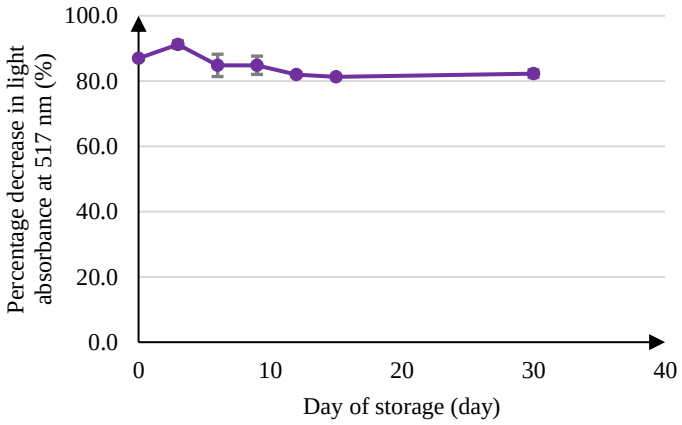


Fig. 42. Percentage decrease in light absorbance at 517 nm after storage

5.5.2 Performances after exposing to UVR for different periods of time

A series of kelp extracts of mass concentration about 133 g/dm^3 were prepared. The extracts were then exposed to UVR from a UV lamp for different periods of time. The UV-absorbing ability and anti-oxidizing ability were then measured right after. The procedures of measurements were identical as those in sections 5.4 and 4.2 respectively.

Results

(a) Percentage decrease in UV intensities (UV-absorbing ability)
(for raw data, please refer to Supplementary File P.S-13)

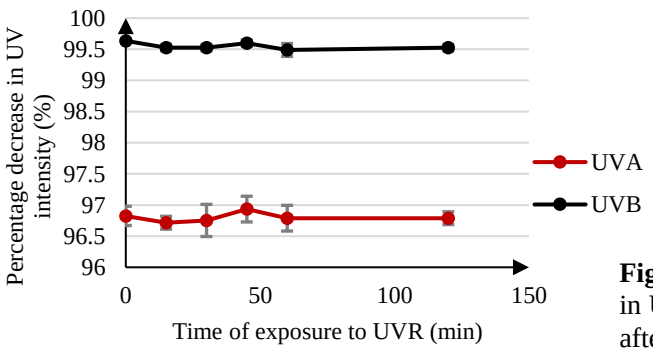


Fig. 43. Percentage decrease in UVA and UVB intensities after UV exposure

(b) Percentage decrease in light absorbance at 517 nm (anti-oxidizing ability)
(for raw data, please refer to Supplementary File P.S-13)

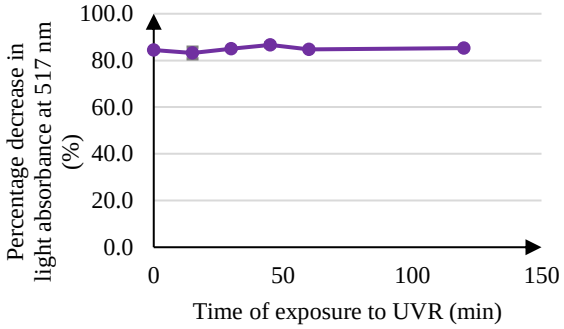


Fig. 44. Percentage decrease in light absorbance at 517 nm after UV exposure

Discussion

From both Fig.41 and Fig. 42, it can be concluded that kelp/olive oil extract can be stored for a prolonged period of time (at least 30 days which was limited by the duration of this project) without a significant drop in UV-absorbing and anti-oxidizing abilities. It is believed that its storage time can be much longer if more time is given for investigation.

The results in Fig. 43 and Fig. 44. suggested that the UV-absorbing and anti-oxidizing abilities of kelp extract retain after exposing to UV light for at least 4 hours. It shows that the performance of kelp extract as sunscreen retained during use even with continuous exposure of UV light.

5.6 Comparison of Cost

5.6.1 Original cost of raw materials purchased

	Supplier	Mass / volume	Total cost (HKD)
Nano-zinc oxide	Naturalland	30 g	40
Olive oil	Naturalland	100 cm^3	70
Kelp	Aeon	45 g	39.8
Wakame	Tak Wah Trading	20 g	78
Sea grape	Tak Wah Trading	100 g	85
Nori	Local market	12 g	6

Table 5. Suppliers and cost of each raw material in making sunscreens

5.6.2 **Comparison of cost of sunscreens** using nano-zinc oxide and algae extracts

	Nano-zinc oxide suspension (commercial)	Kelp extract	Wakame extract	Sea grape extract	Nori extract
Cost of 30 cm^3 olive oil (HKD)	$\frac{70}{100}\times30 = \$21$				
Cost of 3 g active ingredient (HKD)	$\frac{40}{30}\times3$ = \$4	$\frac{39.8}{45}\times3$ = \$2.65	$\frac{78}{20}\times3$ = \$11.7	$\frac{85}{100}\times3$ = \$2.55	$\frac{6}{12}\times3$ = \$1.5
Total cost of a 30-mL sunscreen with 0.1 g/cm^3 (HKD)	21+4 = \$25	21+2.65 = \$23.65	21+11.7 = \$32.7	21+2.55 = \$23.55	21+1.5 = \$22.5
Total cost per cm^3 of sunscreen (HKD/cm^3)	$\frac{25}{30}$ **= \$0.833/cm^3**	$\frac{23.65}{30}$ **= \$0.788/cm^3**	$\frac{32.7}{30}$ **=\$1.09/cm^3**	$\frac{23.55}{30}$ **\$0.785/cm^3**	$\frac{22.5}{30}$ **=\$0.75/cm^3**

Table 6. Cost of preparing each of the sunscreens

Discussion

According to the calculation in Table 6, the cost of sunscreen prepared by using nano-zinc oxide, which is the active ingredient in commercial sunscreens, was higher than those made by kelp extract, sea grape extract and nori extract. At the same time, algae extracts like wakame and nori could absorb a level of UVA and UVB comparable to nano-zinc oxide. In comparison, the cost/performance ratio of sunscreen made by kelp extract was higher than commercial sunscreen made by nano-zinc oxide. Besides, the preparation process (as in section 5.3) was simple and did not require any heating. Thus, it can be concluded that sunscreens made from algae extracts are cost- and energy-effective.

6 APPLICATION II: ACT AS A PROTECTIVE COATING AGAINST UV LIGHT IN GEL NAIL POLISH

To investigate the application of algae extracts on protecting nails or skins during UV gel nail polish, the possibility of forming coatings of algae extracts was explored in the following experiments.

6.1 Comparison of viscosity of various algae extracts

During the earlier preparation of algae extracts in section 2.3.2, it was noticed that kelp/water extract was much more viscous than the other extracts. In fact, a muslin cloth was required to filter the mixture in order to obtain the extract solution (Fig. 45) instead of using a filter paper.



Fig. 45. Gelatinous mixture of kelp and water during filtration

A simple experiment was conducted to compare the viscosity of the extracts. A bottle lid was drilled to form a hole at the bottom. The bottle lid was immersed into each of the extracts. When the lid was taken out of the liquid, the time taken for the liquid stream to fall out of the hole continuously was measured until it started to drip (Fig. 46). The longer the time taken, the more viscous the liquid is

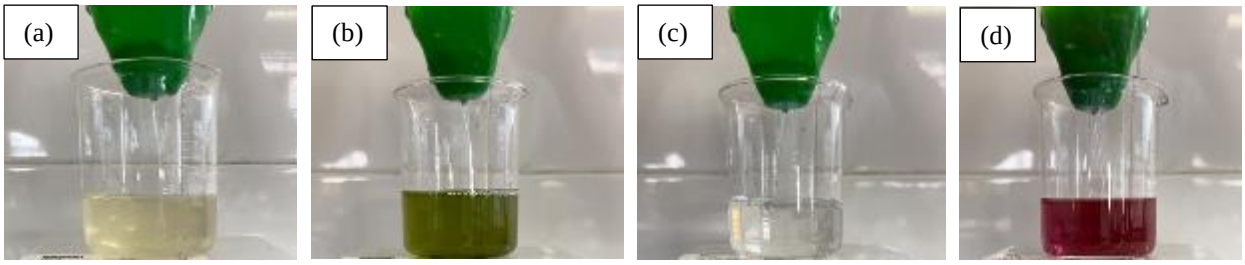


Fig. 46. Liquid stream flow out of the cup during viscosity test: (a) kelp/water extract; (b) wakame/water extract; (c) sea grape/water extract; and (d) nori/water extract

Method

1. Immerse 3 g of powdered algae samples into 30 mL distilled water
2. Allow the mixture to stand for 24 hours
3. Filter the mixture to obtain the extract
4. Immerse the entire viscosity cup into each of the extract
5. Take out the cup and hold at a fixed distance
6. Measure the time taken for the liquid stream to fall out of the hole continuously until the liquid starts to drip

Results

Time taken for the liquid started to drip:
(for raw data, please refer to Supplementary File P.S-14)

	Distilled water	Kelp/water extract	Wakame/water extract	Sea grape/water extract	Nori/water extract
Average time (s)	41	45	42	40	37

Table 10. Time taken for different algae/water extract to start dripping from the cup

Discussion

As kelp/water extract took the longest time to complete the above experiment as shown in Table 10, it can be concluded that it had the highest viscosity among other extracts.

As alginate is well-known to form a gel-like solution in food industry, it was suspected that some alginate was obtained from kelp during extraction with distilled water. In order to explore the potential of using the alginate from kelp to create coatings against UVR, synthesis of sodium alginate from kelp was carried out in the next section.

6.2 Synthesis of sodium alginate from kelp

Alginate is one of the polysaccharides found in the cell walls of brown algae[37]. It forms a jelly-like viscous solution when dissolved in water.

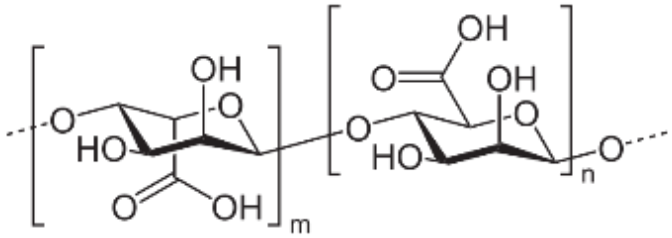


Fig. 47. Chemical structure of alginate in brown algae

It has been often used in food industry to create caviars or used in making hydrocolloids in wound dressing.

To synthesize sodium alginate, the algae powder should be treated with acid to convert any alginate into alginic acid. After filtering off the solution, sodium carbonate solution is added to residue to produce sodium alginate as the salt. 95% ethanol should be added at last to precipitate sodium alginate, which is not soluble in a mixture of ethanol and water[38].

Based on the previous findings in section 3.2, it is suspected that raw alginate produced from kelp could also exhibit UV-absorbing ability. The following experiment was carried out to obtain sodium alginate from kelp in order to create coatings to protect nails or skins during UV nail gel polish.

Methods

1. Add 1 g of kelp powder into 30 mL 0.2 M hydrochloric acid and stand for 24 hours
2. Filter the mixture to obtain the residue by using a muslin cloth
3. Wash the residues with distilled water for three times

4. Immerse the residue in 40 mL 2% sodium carbonate solution for 5 hours
5. Filter the mixture to obtain the filtrate
6. Add 150 mL 95% ethanol into the filtrate
7. Filter the mixture to obtain the residue
8. Press the residue with filter papers and allow it to dry overnight at room temperature
9. Measure the mass of the dried product
10. Repeat the above procedure by using powdered kelp of different masses

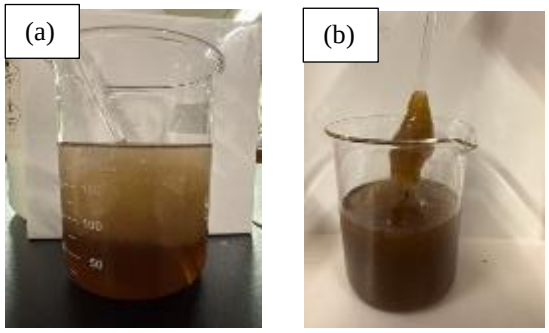


Fig. 48. (a) Formation of semi-solid after adding 95% ethanol; and (b) crude sodium alginate extracted

Results

1. Appearance of dried product
   The dried alginate solid is **dark brown** in colour.

Mass of kelp powder used for synthesis (g)				
1.044	3.037	5.079	7.038	10.038
				

Table 7. Sodium alginate formed after drying

2. Mass of dried product obtained and percentage crude yield
   (for raw data, please refer to Supplementary File P.S-11)

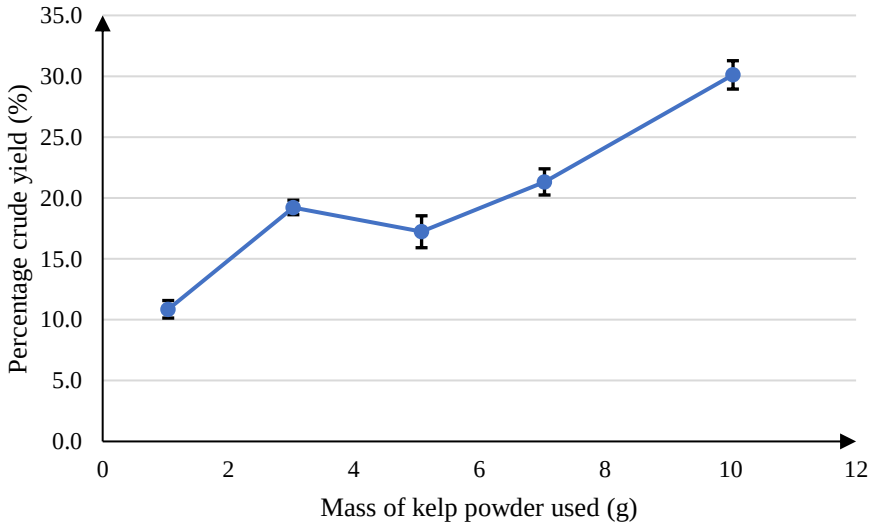


Fig. 49. Product yield of synthesizing sodium alginate from kelp

6.3 Formation of coatings by using the alginate obtained from kelp

When sodium alginate solution is mixed with aqueous calcium ions, an insoluble gel-like calcium alginate solid is formed (Fig. 50).

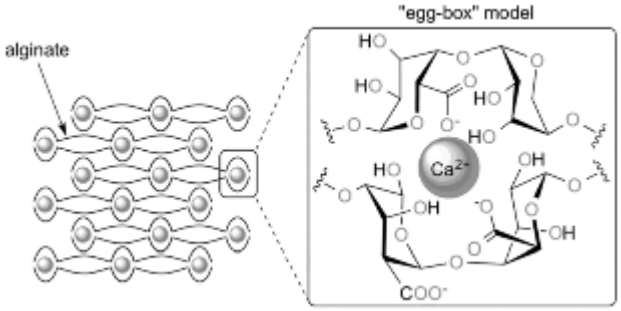


Fig. 50. Formation of calcium alginate from sodium alginate [40]

Therefore, a coating can be made by mixing the alginate solution formed with calcium chloride solution.

In the following experiment, 3 cm x 2 cm plastic slides of 0.5 mm thick were used to mimic the nails. A brush was used to spread the alginate solution evenly on the slides to simulate the brushing action to apply the solution to the nails. At last, calcium chloride solution was spray onto the slides from a spray bottle to form a layer of calcium alginate on the surface.

In order to utilize the anti-oxidizing and UV-absorbing properties of kelp/olive extract prepared in section 5.3, the alginate solutions prepared were mixed with the extract and coatings were formed on the plastic slides accordingly.

<u>Method</u>

1. Grind the dried alginate solid into fine powder
2. Add various masses (about 0.3 g, 0.5 g, 0.7 g, 0.8 g and 1 g) of powder into 50 mL distilled water
3. Stir the mixture well until no more powder remains
4. Add about 1 mL of the alginate solution onto 3 cm x 2 cm plastic slides and spread the solution evenly by using a brush
5. Spray 0.1% calcium chloride solution onto the slides quickly
6. Allow the layer formed to dry in air
7. Repeat the above procedure by mixing 10 mL of each alginate solution with 1 mL kelp/olive oil extract (made from 7.08 g kelp in 30 mL water) obtained from section 5.3

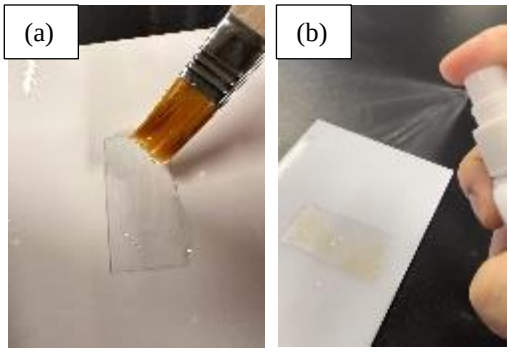


Fig. 51. (a) Applying the sodium alginate solution onto a plastic slide; and (b) spraying calcium chloride solution on the slide with sodium alginate to form a coating of calcium alginate

Results

Coated plastic slides

(a) without kelp/olive extract

Mass concentration of sodium alginate (g/dm^3)				
6.5	10.0	13.6	16.1	20.0
				

Table 8. Plastic slides coated with calcium alginate without adding kelp/olive oil extract

(b) with kelp/olive extract

Mass concentration of sodium alginate (g/dm^3)				
6.5	10.0	13.6	16.1	20.0
				

Table 9. Plastic slides coated with calcium alginate with 1 mL kelp/olive oil extract added

Discussion

During the coating process, it was found that the higher the concentration of the alginate solution used, the more uniform coating could be formed on the plastic slides. It can be explained by the viscosity of the solution. As the solutions of lower concentration was less viscous, the liquid tended to form droplets and did not spread out evenly on the slides (Fig. 52). Therefore, it was hard to have the alginate solution evenly spread on every part of the slides before spraying with calcium chloride solution.

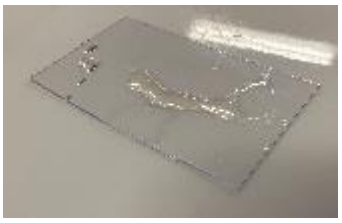


Fig. 52. Alginate solution of lower concentration did not spread easily

Besides, addition of kelp/olive oil extract caused the liquid mixture spread more evenly on the plastic slides. As a result, more uniform coatings were prepared.

6.4 UV-absorbing ability of the coating in UV nail gel machine

In order to study the UV-absorbing ability of the alginate coating, the coated plastic slides were placed under an UV nail gel machine. The following experimental set-up was designed to measure the UV-absorbing ability of the alginate coating.

Design of experimental set-up

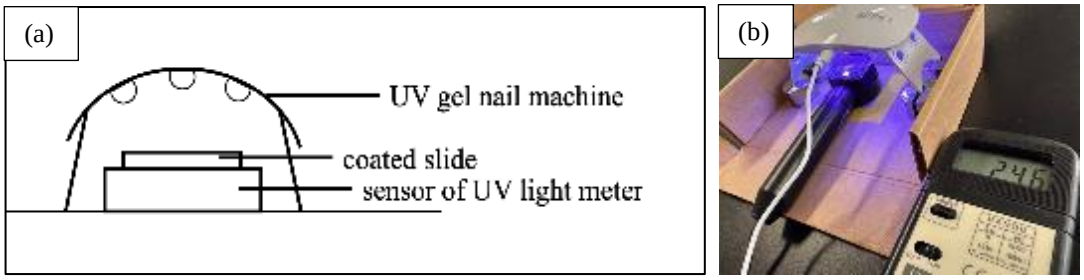


Fig. 53. (a) A design of set-up to study the UV-absorbing ability of alginate layer with a commercial UV nail gel machine; and (b) the actual set-up

The coated slide is placed on top of the sensor of the UV light meter. The distance between the slide and the UV bulbs in the machine is about 1 cm, which simulate the actual distance between the nail and the UV lamp in real scenario (Fig. 53).

Results

(a) The **highest UV intensity** reading from the **commercial UV nail gel machine** : 3200 μW/cm^2

(b) Percentage decrease in UV intensity from the commercial UV nail gel machine *(for raw data, please refer to Supplementary File P.S-11)*

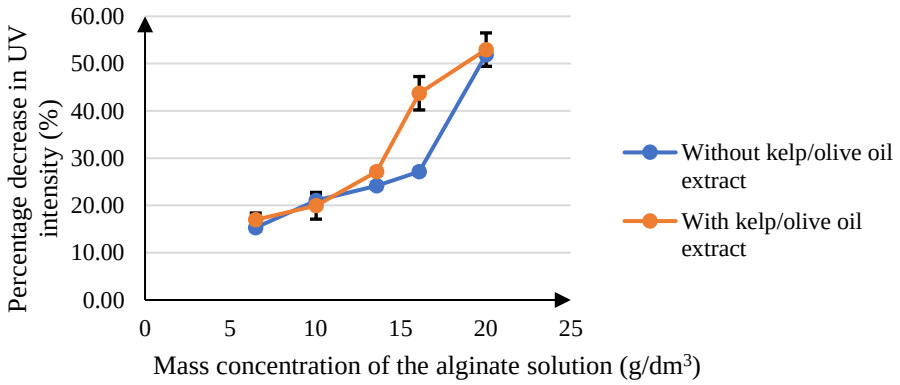


Fig 54. Comparison between UV-absorbing abilities of alginate coating with and without kelp/olive oil extract added

Discussion

A simple test was conducted by measuring the UV intensity on an outdoor sportsground during a sunny day at noon. The highest reading obtain was 2440 $\mu W/cm^2$ (Fig. 55). Comparing with the UV intensity reading from the UV nail gel machine, the machine gave off UV light with intensity 1.3 times higher than the sunlight. One of the reasons is the close proximity of sensor to the UV lamp, which accurately simulate the position of finger during UV nail gel polish.



Fig. 55. UV intensity measured outdoor at noon

The result in Fig.63 shows that the protective coating made from 20 g/dm^3 kelp alginate solution could effectively block UV light from the UV nail gel machine by about 53%. Furthermore, with addition of kelp/olive oil extract, the UV-absorbing performance further enhanced by 17% in the case of 16.1 g/dm^3 alginate solution.

7 CONCLUSIONS AND POTENTIAL APPLICATIONS

Harmful effects imposed by ultra-violet radiation (UVR) have been constant concerns in many years. Depletion of ozone layers has significantly increased the health risks related to excessive exposure to UVR, for instance skin cancers and cataract. Not only from the sunlight, daily leisure activities like UV nail gel polish might also lead to unexpected body damages due to UV exposure. Although there are many sunscreen products available in the market, most of them are composed of ingredients that are non-renewable, which makes the use of the product not environmentally friendly and might lead to marine pollution.

In light of these, this research aims to utilize marine living organisms to deal with the issue. In this research, UV-absorbing compounds from kelp, wakame, sea grape and nori were extracted by using three types of solvents. All extracts exhibit a promising UV-absorbing ability. In particular, kelp demonstrated the highest UV-absorbing ability when extracted with distilled water and 95% ethanol. Over 50% of the original UV intensity could be reduced by kelp extract prepared from 3 g kelp in 30 mL distilled water. As for extraction using olive oil, wakame and kelp extracts reduced UVA intensities by 98.9% and 97.9%; while reduced UVB intensities by 93.3% an 86.7%. Apart from kelp, other algae extracts like wakame and nori could absorb a level of UVA and UVB comparable to nano-zinc oxide, which is often used in commercial sunscreens and is toxic to small marine organisms. However, the UV-absorbing performance of green algae like sea grapes was found to be relatively weaker than others.

Apart from UV-absorbing ability, kelp extract also demonstrated an excellent anti-oxidizing property that can help prevent the damage caused by free radicals upon UV exposure. In this research, kelp/water extract could remove up to 94.9% of DPPH compound. Both of the mentioned properties render kelp a potential material for making sunscreen products. In this study, it is also proved that the UV-absorbing and anti-oxidizing abilities of kelp extract retained upon at least 30-day storage under room conditions. Moreover, cost of preparing kelp/extract was also lower than that of zinc oxide suspension.

From the result in this project, it is hoped that algae, especially kelp, could be better utilized as a renewable and sustainable material against UVR and free radicals.

*** Project Continuation (Future work), References and Raw Data are included in the Supplementary File.**



【評語】080017

This research extracted UV-absorbing and anti-oxidizing materials form algae including kelp, wakame, sea grape and nori. Then, three applications of algae were explored in details. They found kelp, wakame and nori extracts by using over 2 g of algae in 30 mL olive oil could absorb UVA and UVB by over 90%, comparable to the performance of zinc oxide, and the cost of using kelp and nori was cheaper. The kelp extract was stable for at least 30 days at room temperature storage. Sodium alginate (海藻酸鈉) was successfully extracted from kelp with a product yield up to 30%.

Strength：

The research requires much efforts with patience. The experiments were well executed and the results have practical application.

Suggestion：

1. Sodium alginate (海藻酸鈉) has been marketed already as dressings. In 1947, George Blaine first extracted sodium alginate

from algae. By missing with Ca ion to form Calcium alginate fiber, the product can form non-adherent hydrogel to cover the wound area for recovery. However, for UV protection could be novel.

2. Mycosporine-like amino acids (MAAs) and fucoidan have been found to have UV-absorbing and anti-oxidizing properties [20-23]. Whether they extracted were MAAs, fucoidan, and/or Sodium alginate was not characterized and not clearly described.