Exploiting the photoprotection capabilities of anthocyanin for human use

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***Abstract* — Leaves produce anthocyanin that provides effective photoprotective capabilities. Anthocyanin can intercept high-energy quanta to prevent important photo-labile molecules from degradation by intense light. These properties make anthocyanin a potential natural sunblock that could replace current commercial sunblock, which may contain hormone disrupting chemicals and carcinogens, and are also environmental pollutants. The objective of this study is to show the photoprotective capabilities of anthocyanin in plants. Results supported the hypothesis that a greater concentration of anthocyanin reflects greater photoprotective capabilities. By placing anthocyanin extracts on pig's skin and leaving this set-up under the sun, this study also showed that extracted anthocyanin has better UV absorption capabilities compared to when using commercial spf30 sunblock. It can be concluded that anthocyanin has photoprotective properties and has potential to be incorporated into commercial sunblock.**

***Keywords* — Anthocyanin; Photoprotection; Plant pigments; Ultraviolet rays; Sunblock; Extraction; Human application**

I. INTRODUCTION

Anthocyanin is a plant pigment found in almost all tissues of the higher plants. They, however, occur most in leaves, flower and fruits. Anthocyanin is a pH dependent pigment that changes its color when exposed to different acidity levels in the cell sap of a plant tissue, this results in the plants being able to change the color of their leaf, flower and fruits. For example, leaves of some trees turn red during autumn due to the build-up of anthocyanin content in the leaves of these plants. Anthocyanin in these leaves are being used to provide an effective photoprotection during the critical period of foliar nutrient resorption [1]. Also, anthocyanin has been shown to intercept high- energy quanta to prevent important photolabile molecules from degradation by green light [2]. In anthocyanin, there are plenty of double bonds, implying a large number of Pi orbitals. There are also some oxygen lone pairs, hence a few non-bonding orbitals. Pi orbitals and non-bonding orbitals can absorb a lot of light energy; hence this explains why it has such a high absorption of UV light. This research shows anthocyanin plays a major role in plant photoprotection.

Commercial sunblock currently contains a potential hormone disrupting chemical (oxybenzone) and a potential carcinogen (retinyl palmitate). A research study has also shown that malignant melanoma was found more frequently in sunscreen users compared to non-users, although the cause and effect cannot be determined [3]. Commercial sunblock has also been shown to be an environmental pollutant, with a significant concentration of chemical UV filters included in the formulation of sunscreens such as TiO_2 and ZnO, detected in nearshore waters and on the surface of the water, posing a threat to the planktonic population [4]. Because of these potential harms, a natural yet effective way of sunblock formulation should be sought. This research seeks to find an alternative means of photoprotection by extracting anthocyanin from plants and testing its suitability.

This paper seeks specifically to confirm various aspects – whether anthocyanin's photoprotective capabilities function outside the plant, so as to show viability of use. Whether there is a positive correlation between concentration of anthocyanin and absorbance of UV, to show that anthocyanin is involved in UV absorbance. And whether UV absorbance by anthocyanin can be translated to a practical application of preventing burns on skin surfaces.

II. MATERIALS AND METHODS

A. Measurement of anthocyanin concentration

Anthocyanin extraction procedures were carried out [5]. Three plants were chosen for extraction. They are chosen based on the colors of their leaves since anthocyanin is a colored pigment. These are the purple-leaved *Strobilanthes dyeriana*, the green-leaved *Epipremnum aureum* and the light-green-leaved *Foeniculum vulgare*. First, leaves from each of the three plants were picked, weighed and frozen at -60 degree Celsius. 1.8 ml of the extraction solvent, methanol 1% HCl, was added to the frozen leaves. The leaves were crushed to allow the anthocyanin trapped within the plant cell to be dissolved within the extraction solvent. 3.0ml of non-polar organic chloroform and 1.2ml of polar inorganic pure water is then added to the mixture. This is then centrifuged to separate the chloroform components and the water components. Non-polar molecules such as chlorophyll dissolves in chloroform whereas polar molecules such as our target molecule anthocyanin will dissolve in the pure water. Chloroform is a denser molecule which will sink below pure water, therefore in order to obtain the polar anthocyanin, the supernatant will be extracted.

After obtaining the anthocyanin extract from the three plants, quantification of the concentration of anthocyanin is done through the pH differential method [6]. 10 ml of pH 1 buffer is added to 1 ml of all anthocyanin solution. Another 10 ml of pH 4.5 buffer is also added to 1 ml of another anthocyanin solution of the three plants. Each of the pH 1 and pH 4.5 buffered anthocyanin is placed in a quartz cuvette and then into a UV-vis spectrophotometer, where the absorbance is read at both 520nm and 700nm. The equation [7]:

$$\frac{\mathbf{A \times MW \times DF \times 10^3}}{\boldsymbol{\varepsilon \times l}}$$

is used to calculate the concentration of the anthocyanin in mg/l, where:

- **A** = [Absorbance at 520nm (pH 1)- Absorbance at 700nm (pH1)] - [Absorbance at 520nm (pH 4.5)- Absorbance at 700nm (pH4.5)]
- **MW** is the molecular weight of cyanidin-3-glucoside, which is: 484.83 g/mol
- **DF** is the dilution factor, the ratio of Buffer:Anthocyanin is 10:1, thus dilution factor is 11
- $\mathbf{10^3}$ is to convert grams (g) to milligrams (mg)
- **ε** is the molar extinction coefficient (26900 $L \cdot mol^{-1} \cdot cm^{-1}$. for cyanidin-3-glucoside)
- **l** is the path length which the light wave travelled through in the anthocyanin extract

B. Measurement of absorptive capability of anthocyanin

In order to establish a correlation between the concentration of anthocyanin in the extract and the UV absorptive capability of the extract. We obtain an anthocyanin extract once again for all three plant types. Thereafter, dilution of the anthocyanin extracts is carried out, 6.50 ml of pure water is added to a 1.50 ml anthocyanin solution, resulting in a $5\frac{1}{3}$ times dilution. This is to accommodate the UV-vis spectrophotometer which can only detect a limited range of absorption of UV rays. The extracts were placed in a quartz cuvette and placed in a UV-vis spectrophotometer to be read for absorption of UV rays in the spectrum between 200 to 400 nm, which are the range of frequencies of UV rays, this study specifically uses 280nm, 300nm, and 350nm. Before reading, the UV-vis spectrophotometer was zeroed with pure water. The UV-vis will give a reading of 0 to 4, with 0 being the lowest absorbance of UV rays by a sample or in other words when a 100% of the UV rays can pass through the extract. 4 is the highest reading of absorbance.

C. Pig's skin application test

This portion of the study seeks to show the viability of anthocyanin as a sunblock. Extraction was carried out on both *Strobilanthes dyerianus and Epipremnum aureum.* 0.4grams of leaves were removed and extraction was carried out with 27ml of methanol 1% HCl; 18ml of pure water; 45ml of chloroform.

Four petri dishes were prepared together with four pieces of pig's skin from a commercial poultry source. Two of the petri dishes contain the anthocyanin extract and were placed on the rough center of the pig's skin, the remaining petri dishes were used to contain water and spf30 sunblock respectively. Water is used as a negative control to show that anthocyanin is the main factor in the UV protection on the pig's skin. Sunblock was used as a basis of comparison against anthocyanin. The commercial sunblock had a sun protection factor (spf) of 30, which means that $^1/_{30}{}^{th}$ of the burning radiation will reach the skin, assuming the sunscreen is applied evenly at 2 milligrams per square centimeter.

The petri dishes on the pig's skin were left in an open area with direct sunlight for intervals of 1 hour. A total of 6 intervals were recorded and a picture was taken at each interval. Pictures were analyzed with Image J software using its digital color meter function. A ratio was taken between the area covered by the petri dish and the area surrounding that. This is so as to compare the extent of burning of the pig's skin when with protection and when without protection. The ratio of each sample - water, sunblock and both anthocyanin samples- were compared to see which sample provides the highest levels of protection.

D. Heat stability of anthocyanin

Since anthocyanin is a chemical compound, heat may result in the disruption of their structure. When used as a sunblock, the sunblock is placed in conditions with high temperatures. Thus in order to be a practical sunblock, the absorbance ability should not be affected. Pure anthocyanin was heated in 10 degree intervals from 30 to 80 degree Celsius for 5 minutes. This is then placed in a UV-vis spectrophotometer and the spectrum of absorbance from 200 to 800nm was recorded.

III. RESULTS

A. Darker leaves have greatest anthocyanin concentration

Figure 1 shows the concentration of anthocyanin levels in the 3 plants. The most purple *Strobilanthes dyerianus* had the largest anthocyanin concentration, followed by the dark green *Epipremnum aureum*. The light green colored *Foeniculum vulgare* had the lowest anthocyanin concentration. Anthocyanin is a colored pigment thus darker color is probably indicative of higher concentrations of anthocyanin.

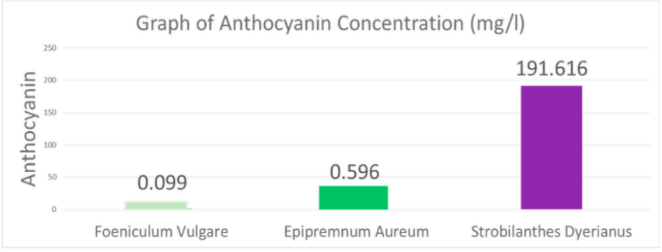

Figure 1. Concentration of anthocyanin in mg/L for each species of plants

B. *Strobilanthes dyerianus offers greatest UV absorption*

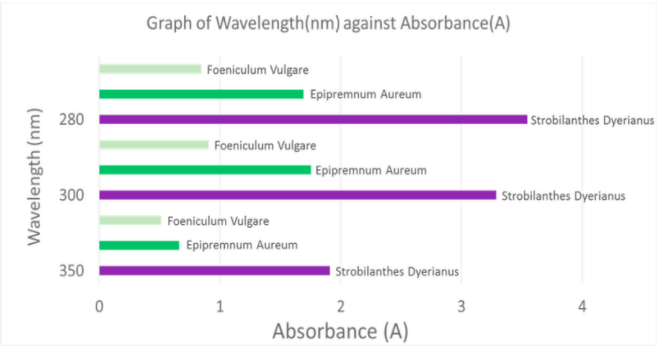

Figure 2. *Wavelength (280, 300, 350nm) against the absorbance in the UV-vis spectrophotometer (in arbituary units).*

Figure 2 shows the absorption of UV rays at various wavelengths by different plants. At 280 nm, most of the plant's solution had the highest UV absorption. At 300nm, there was a dip in absorbance of UV and at 350 nm there was a sharp decrease in the absorption. Thus the peak of anthocyanin absorption lies closer to the 280nm value.

From Figure 1 and Figure 2, it can be seen that *Strobilanthes dyerianus* has the largest concentration and the largest absorbance, *Epipremnum aureum* has the next largest concentration and the second greatest absorbance reading whilst lastly, *Foeniculum vulgare* has the smallest concentration and the least absorbance. There can be made to be a correlation between absorption of UV radiation and the concentration of anthocyanin in the plant.

C. *Strobilanthes dyerianus offers greatest photoprotection*

From Figure 3, the results obtained in the pig's skin experiment can be seen. The circular area is where the petri dish containing the sample is. The remaining areas are not covered. This is to tackle the issue that different pig's skin can be burnt to different extent. When the In/Out ratio is calculated, the same skin is used and thus mitigate the effects when trying to compare between the different samples used.

Similarly, whilst placing a petri dish on top of a pig's skin is dissimilar from direct application, in this study, a gel is unable to be produced for direct application. Thus all the set-ups use the same type of clear petri dish to test how absorbance by the samples reduce the burntness of the skin.

Figure 3. Extent of burntness of Pig's skin with application (left to right) water, sunblock, *Epipremnum aureum* and *Strobilanthes dyerianus.*

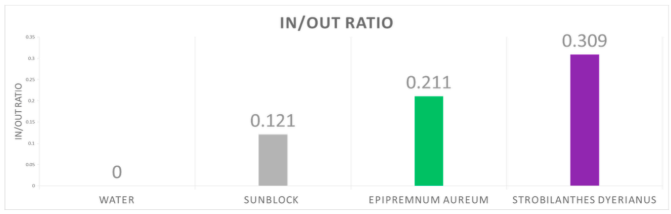

Figure 4. *The In/Out ratio shows the extent of the burns, the closer to 0, the more burnt the skin is.*

The In/Out Ratio was calculated using an image software, Image J. The In reading was divided by the Out reading. The smaller the reading the more similar the Inside is to the burnt Outside. Therefore, a smaller In/Out ratio means that there is the a high burntness and thus the sample's absorbance of UV rays is likely to be the least. The lowest In/Out ratio is the Water sample at 1.434, since it is the negative control, the sample was set that to be 0, and the remaining values were adjusted accordingly, as can be seen from Figure 4.

The performance of anthocyanin in preventing burns is relatively high when compared to current commercial sunblock. This can be seen by the higher In/Out ratio of these anthocyanin samples. Also, the one with a higher concentration of anthocyanin - *Strobilanthes dyerianus*, has a higher photoprotective capability than the one with the lower concentration - *Epipremnum aureum,* showing that anthocyanin does provide this photoprotective abilities. Thus these show anthocyanin's potential viability as a photoprotective device that can be developed into sunblock.

D. *Anthocyanin's absorbance is not affected by heat*

Figure 5 shows the plot spectrum amongst the various temperatures from 30 to 80 degree Celsius. There is not much real deviation in these plotted spectrum, thus temperature does not affect the absorbance capabilities of anthocyanin significantly within these range of values and thus can be used to absorb UV rays under high temperatures as well. Thus, anthocyanin is useful when used in real world situations requiring photoprotection.

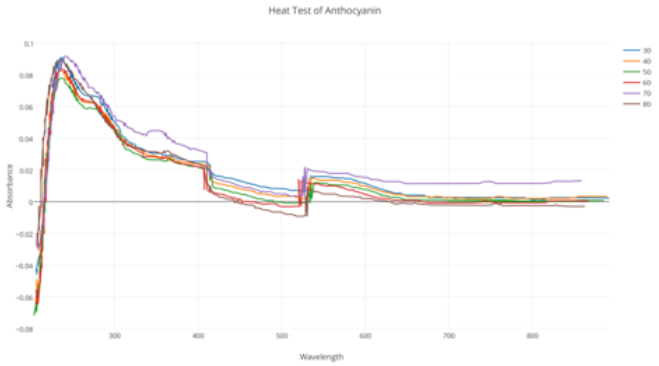

Figure 5. *The absorbance value versus wavelength in UV-vis spectrophotometer at temperatures (30-80 degree Celsius).*

IV. Discussion

Plants are sessile living things that are confined to one fixed position in their environment, this means that there has to be some form of mechanism that allows the plants to prevent its photolabile molecules from being degraded by direct sunlight and its associated UV rays. By having many double bonds and oxygen lone pairs in its molecular structure, anthocyanin has the ability to absorb large amounts of UV rays due to the Pi and non-bonding orbitals being able to absorb large quanta of energy, thus providing protection for the plants [1]. By a similar logic, the anthocyanin can be used to intercept UV rays that would otherwise hit the skin. In order to prove that anthocyanin can effectively intercept and absorb UV rays, this study subjected a sample of anthocyanin extract with UV rays and calculated its absorptivity. The results effectively show that anthocyanin is capable of UV absorption even outside a living plant body and is a first step towards sunblock application [2].

The capability of anthocyanin to intercept UV rays goes to show a strong link that anthocyanin has a very large potential to prevent sunburns. In order to test how well anthocyanin can prevent burns from occurring, a novel method of testing photoprotection on pig's skin was carried out. The anthocyanin sample was placed in a petri dish above the pig's skin and in direct sunlight. The associated burns on the pig's skin would determine the protection level that the anthocyanin has provided. The results from the Pig's skin experiment shows that there is a significant protection, and goes even further to show that anthocyanin from the *Strobilanthes dyerianus* as well as that from the *Epipremnum aureum* serves as a better protection source than commercial spf 30 sunblock. Also because a direct correlation can be established between the concentration of anthocyanin (*Epipremnum aureum* having lower concentration; *Strobilanthes dyerianus* having a higher concentration) and the burntness of the pig's skin. Therefore, anthocyanin may serve as an alternative photoprotection agent to the skin and has great potential to be incorporated into commercial sunblock.

This can greatly reduce environmental effects that are currently inherent in commercial sunblock due to the use of synthetic chemicals [4]. The long term healthcare risks of these photoprotective synthetic chemicals are also being put into the spotlight [3]. Anthocyanin on the other hand is a natural anti-oxidant that has established healthcare benefits [8]. The wide availability of anthocyanin in nature may also mean that less developed nations can begin to self develop such forms of skin protection product at a low cost so as to reduce the risks that are associated with the lack of UV protection including higher risks of skin cancer [9].

However, current methods of extracting anthocyanin from the leaves are limited to having to use methanol 1% HCl and chloroform. These extracting agents are considered to be hazardous if contacted with the skin, therefore other extraction methods have to be established that do not make use of potentially hazardous chemical. Possible alternatives to the extraction methodology would be glycerol. Glycerol is a polar substance more polar than water and thus is capable of dissolving the polar anthocyanin. Chlorophyll and non-target components are non-polar and therefore are not dissolved by the glycerol [10]. This allows us to carry out a similar separation technique as with this study to separate anthocyanin and thus extract it for use. Such a method will reduce the hazard of anthocyanin extraction but carry over the other advantages.

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