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Bioactive Compound Potential of *Brassicaceae* Sprouts and the Effect of UV-B Radiation on their Antioxidant Activity

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Current trend of climate change and depletion of ozone layer may cause the amount of ultraviolet (UV) radiation in the atmosphere to increase and thus, affecting biodiversity and ecosystem. Plants are one of the most affected organisms due their need of sunlight to carry important process of photosynthesis and the presence of UV radiation may result in detrimental effects on them. However, with controlled amount of UV radiation exposure as abiotic stress condition, plants will be able to develop a plant defense chemical and present an alternative source of drug discovery as natural products. Plant secondary metabolites are important compounds largely used in pharmaceutical industry and commonly found in *Brassicaceae* family plants such as broccoli sprouts, red cabbage sprouts and kale sprouts. Previous studies suggested that *Brassicaceae* sprouts is a good source of phytochemicals and reported to exhibit bioactive properties including antioxidative and anti-inflammatory. In this study, an overview of the effect of UV-B radiation exposure as abiotic stress on the amount of *Brassicaceae* sprouts bioactive compound and antioxidant activities are presented and discussed. *Brassicaceae* sprouts are grown and exposed to UV-B radiation under different sets of time and fresh samples of the sprouts are extracted with ethanol before undergoing phytochemical analysis for total phenolic content and then proceed with DPPH assays to study its antioxidant activity. Results suggest that UV-B radiation causes increase in double in the amount of total phenolic content for red cabbage sprout from 0.33 to 0.63 mg gallic acid/g^{-1} and increase in the antioxidant activity of kale sprout from 0.74 to 0.80 mg Trolox/g^{-1} from *Brassicaceae* species at 5^{th}-min of exposure. This study is important to understand the increment of UV radiation from ozone layer depletion for plants particularly from *Brassicaceae* family to adapt to these changes while applying them in different industries including agriculture and health for their bioactive compounds.

1. Introduction

Depletion of protective ozone layer due to environmental issues caused by recent rapid industrialization and technology advancement resulted in research focus on its effect towards biodiversity and ecosystems. This phenomenon causes increment in the amount of harmful ultraviolet (UV) radiation reaching the earth's surface and its potential detrimental effects of the existing ecosystems and the progression of biodiversity (Dwivedi & Ahmad, 2023). Ozone layer depletion occurred mainly from chlorofluorocarbons (CFCs) and hydrochlorofluorocarbons (HCFCs) effluents by the industries and it is reported that the increase in the occurrence of extreme climate change events also increases the depletion of ozone layer, and it will continue to be prevalent in the future. The increase in the levels of UV radiation raise a concern of its detrimental effects on organisms that require light to generate chemical energy. According to Soni et al. (2022), for photosynthesis, plants utilize wavelengths in the range of 400 nm to 700 nm while UV radiation ranging from 100 nm to 400 nm and categorized into three types which are UV-A, UV-B, and UV-C. While the radiation range does not correspond with photosynthesis process, UV-A is characterized as the least harmful radiation, and it does not get absorbed in the ozone layer which ranging from 315 nm to 400 nm due to its low energy. Meanwhile, UV-C is the most harmful range at 100 nm to 280 nm, but it is absorbed by the atmosphere and will not reach the

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Earth's surface. Next is UV-B, in the range of 280 nm to 315 nm, at high levels it will be detrimental towards living things and ozone layer acts as a protection layer by absorbing it from reaching the Earth's surface (Soni et al., 2022). As UV-B is the only type of UV radiation that can threaten the environment while reaches the Earth's surface resulted from the depletion of the ozone layer, it is important to understand how this will affect plants biodiversity and its adaptation towards this abiotic stress. With the current trend of ozone layer depletion, it is expected to continue rising in the future and some of the factors associating with this trend are climate change, emission of greenhouse gases and volcanic eruptions. *Brassicaceae* family or formerly known as *Cruciferae* has a wide range of species with rich contents of phytochemical for health promotion and is considered as one of the earliest groups of cultivated plant (Cámara-Martos et al., 2021). As one of the sources of important nutrition in food, species from *Brassicaceae* family is also ranked in top 10 of the most economically important plant families in the world due to its rich source of bioactive contents such as vitamin C, phenolic compounds, and carotenoids (Cámara-Martos et al., 2021). Broccoli sprout, radish sprout, kale sprout and red cabbage sprout are some of the examples categorized under *Brassicaceae* family are also known as superfood for the health benefit they can offer which commonly found in traditional medicine and culinary (Šamec et al., 2018). With good environmental adaptations, *Brassicaceae* sprouts are grown worldwide for food consumption. Bioactive compounds available in plants from *Brassicaceae* family has also been extensively analyzed by previous research and known for their antioxidant activity, anti-inflammatory activity and gastro protective associated with their presence of different types of phytochemicals (Šamec et al., 2018). Simple and cost-effective approaches of cultivating *Brassicaceae* sprouts makes them commercially attractive in obtaining their high-value phytochemical yields for health application. According to Soni et al. (2022), responses in plants from an increase in the level of UV-B is particularly important on its growth and development where the responses vary depending on how UV-B light is perceived, how signals are transduced, and how gene expression is altered. In addition to that, the application of UV-B radiation is also associated with simple manipulation, leaves no residue, terminates instantly and most importantly, cost-effective for an ideal elicitation method which help to increase the accumulation of bioactive secondary metabolites of plants via *in vitro* cultures (Gai et al., 2022). The bioactivity potential of *Brassicaceae* sprouts under environmental stress (UV-B stress) with enhanced accumulation of phytochemicals may benefit the consumer in providing alternative sources of nutrition other than synthetic ones. The main objective of this research is to evaluate the effect of UV-B radiation at different exposure times as abiotic stress on total phenolic content and antioxidant activity of *Brassicaceae* sprouts.

2. Material and methods

Methods used in this study were divided into three parts, plants cultivation, UV-B treatment, and analysis of bioactive activities.

2.1 Plant material and sprouting conditions

Three organic *Brassicaceae* sprouts seeds originated from Italy were purchased (broccoli sprout, red cabbage sprout and kale sprout). Each type of the seeds was prepared in three containers with wet cotton. The seeds were left to germinate in the dark for 3 days in a growth chamber at room temperature. After germination, the sprouts were exposed to light in growth chamber.

2.2 UV-B treatment

UV-B lamp was placed in a closed box and the distance between lamps and sprout cultures was set to obtain a low radiation intensity (3 W/m^2) monitored using a UV sensor. The sprouts cultures were exposed to the UV-B radiation on the 7^{th} day with different sets of exposure time (1 min, 2 min, 5 min and 10 min), where according to, high radiation intensity and long UV-B exposure time may cause a reduce in sprouts quality due to overexposure. The sprouts were left to react for three days in the growth chamber at room temperature.
No UV-B exposure was used as control in this study.

2.3 Preparation of *Brassicaceae* sprouts extracts

On 10^{th} day, the crude extract of *Brassicaceae* sprouts was extracted using ethanol. 0.1 g of bagasse was weighed, and 1 mL of ethanol was then added to the sample. The mixture was left to react for 48 h and prepared for analysis on the 12^{th} day.

2.4 Phytochemical analysis of *Brassicaceae* sprouts extracts

2.4.1 Total Phenolic Content (TPC)

Folin-Ciocalteu method was used in this analysis. Diluted extract of Brassicaceae sprouts (50 μl sample and 50 μl extraction solution) of each sample (crude extracts) was added to a test tube and then 100 μl of Milli-Q was added to the sample. After that, 200 μl of phenol reagent (dilution 1:1) was then added to the extracted samples

and prepared standard solutions and were left in the dark for 3 min before 200 µl of sodium carbonate (10%) was added and left in the dark for 30 min. Then, the absorbance was measured at 750 nm using UV-V spectrophotometer (Sanarat & Srihanam, 2019).
Gallic acid was used as a standard curve to calculate TPC and the results were expressed as mg gallic acid/g sample (Sanarat & Srihanam, 2019). Standard curve is a parameter that is used to compare the results of unknown samples concentration with known concentration of standard this study.

2.5 Antioxidant activity by DPPH assay

DPPH assay was carried out to measure the free radical scavenging activity where a 10 µL of prepared *Brassicaceae* sprouts extracts followed by mixing with 190 µL of DPPH working solutions. After incubation in the dark at room temperature for 10 min, by using a spectrophotometer, the absorbance was read at 520 nm. Trolox was used as standard solution.

3. Results and discussions

3.1 Red cabbage sprout

3.2.1 TPC

Red cabbage sprout is reportedly to have rich amount of vitamin c and this presence of interfering substance may affect the measurement of TPC with Folin-Ciocalteu absorbance. Based on Figure 1, the results show that the amount of phenolic compound available in red cabbage sprout varies based on the amount of exposure time towards UV-B. By comparing to sprouts condition without exposure to UV-B radiation, at minute 5 of UV-B radiation, red cabbage sprouts show the highest increase in phenolic compound compared to 1st-minute, 2nd-minute and 10th-minute.
Increases at the 5th-minute of exposure agrees with studies carried out by Martínez-Zamora et al., (2021) on red cabbage sprout using different doses of UV-B (5, 10 and 15 kJ m^{-2}) compared to untreated plant (control). This shows a protective response of the plants toward the changes in environmental conditions as abiotic stressor and helps to improve its resistance for future exposure.

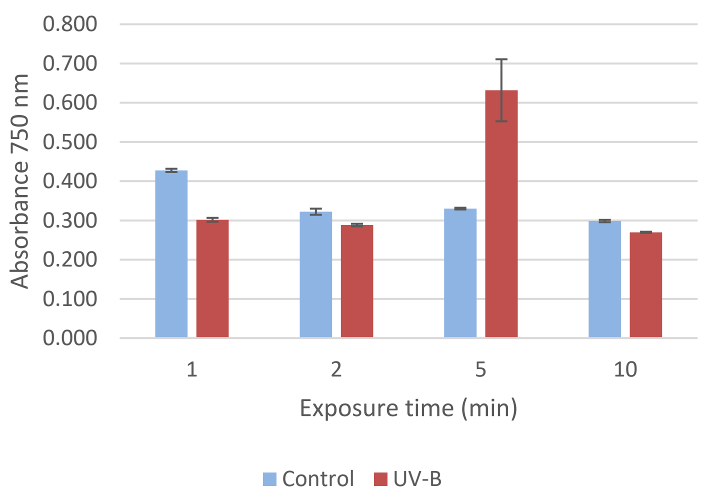

Figure 1: TPC of red cabbage sprout

3.2.2 Antioxidant activity

Antioxidant activity of red cabbage sprout was recorded as follows. At 1st-minute, 2nd-minute, and 5th-minute, there were slight increase in the amount of antioxidant activity of red cabbage spouts compared to the sprouts at controlled condition (no UV-B exposure). While after 10th-minutes of UV-B exposure, the red cabbage sprout shows a decline in antioxidant activity.

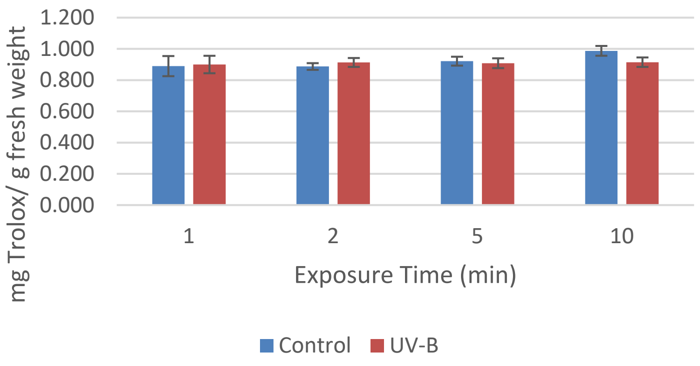

Figure 2: DPPH of red cabbage sprout

As seen in Figure 1 and Figure 2, at higher amount of phenolic content, the antioxidant activity of the sprouts was not increasing respectively. According to Nogueira (2018), different types of phytochemicals present in different type of sprouts may not contribute towards antioxidant activity and thus causes results with high amount of phenolic compound contain lower antioxidant activity. In addition to that, to detect antioxidant activity of every different kind of phytochemical present in various *Brassicaceae* sprouts, DPPH method alone may not be enough for the analysis. Some other option of antioxidant activity analysis includes ABTS assay and FRAP with different principles method which are not focused on this study.

3.2 Kale sprout

3.3.1 TPC

As discussed above for TPC of red cabbage sprouts, kale sprout shows a slight increase in the amount of phenolic compound at 5th-minute of UV-B exposure. While at 1st-minute, 2nd-minute, and 10th-minute, the amount of phenolic compound recorded significant amount of declination compared to the controlled condition. The result that shows increase in the TPC at the 5th-minute of UV-B exposure compared to untreated plant is correlated to results that showed an increase of 22% of TPC at all UV-B doses of 5, 10 and 15 kJ m^{-2} of kale sprouts after 10 days of sprouting (Castillejo et al., 2021).

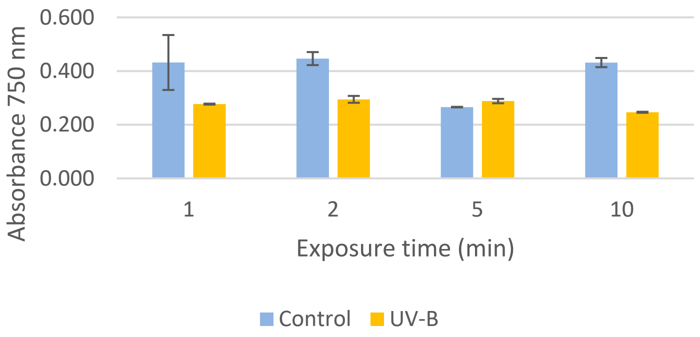

Figure 3: TPC of red kale sprout

3.3.2 Antioxidant activity

As for antioxidant activity of kale sprout, there was slight increase on the 1st-minute, 2nd-minute and 5th-minute of UV-B radiation. Meanwhile at 10th-minute, the antioxidant activity of kale sprout decreased after exposed to UV-B radiation. According to research by Castillejo et al. (2021) at different UV-B doses, the antioxidant activity of kale sprouts changes accordingly where an increase was observed only at 5 kJ m^{-2} compared to untreated plants by 28%.

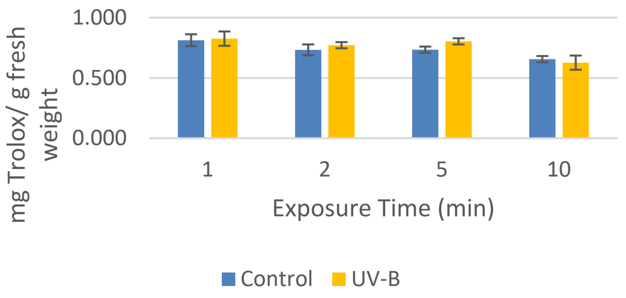

Figure 4: DPPH of red kale sprout

3.3 Broccoli sprout

3.4.1 TPC

Broccoli sprouts show a decrease in phenolic compound for 1st-minute and 2nd-minute compared to controlled condition and increase in phenolic compound on 5th-minute and 10th-minutes of UV-B exposure. The same sprout shows an increase of 160% of untreated sprout after UV-B exposure (Martínez-Zamora et al., 2021).

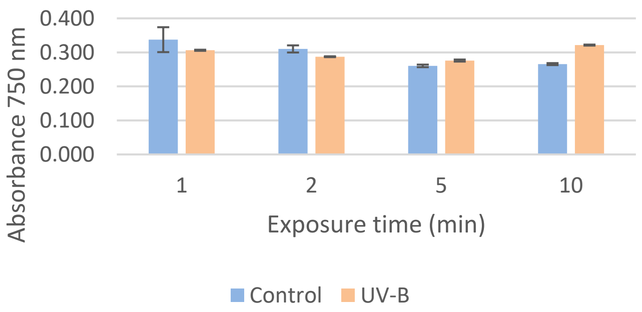

Figure 5: TPC of broccoli sprout

3.4.2 Antioxidant activity

Based on Figure 6, broccoli sprouts show a decrease in every different exposure time of UV-B radiation (1st-minute, 2nd-minute, 5th-minute, and 10th-minute). The decrement in antioxidant activity is more significant as the exposure time increases, while a report on the effect of UV-B, UV-C and UV-B + C showed 55% increase after UV-B exposure (Martínez-Zamora et al., 2021). This showed a varied responses according to UV-B doses.

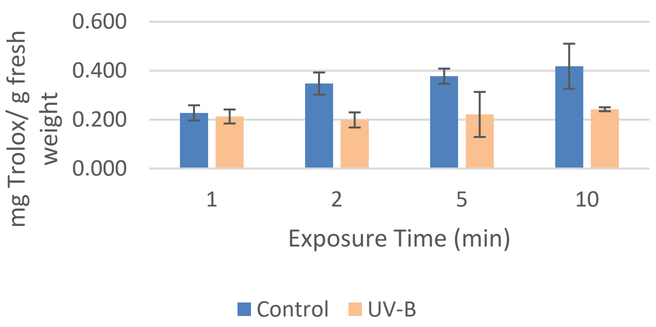

Figure 6: DPPH of broccoli sprout

According to Figure 6, at 10th-minute of UV-B exposure, a significant decrease in the antioxidant activity was observed from 0.42 to 0.24 mg Trolox/g^{-1} due to overexposure to UV-B radiation as seen in Figure 7.

Overexposure to UV-B radiation can occur when *Brassicaceae* sprouts are exposed to the radiation for too long and the sprouts become reduces in size, productivity, and quality (Lemmons, 2023).

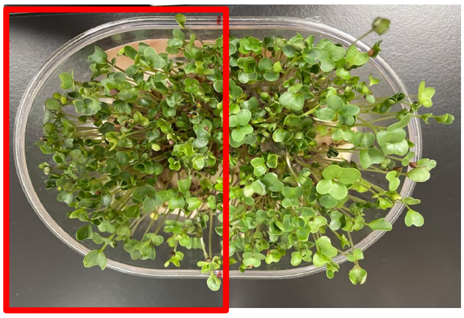

Figure 7: Broccoli sprouts 3 days after 10 min UV-B exposure (in red box)

4. Conclusions

In conclusion, UV-B radiation as abiotic stress affects the sprouts growth both chemically and physically. At certain minute of exposure, the *Brassicaceae*. sprouts reacts positively observed through the increases in phenolic content and antioxidant activity as seen in kale sprout at 5th-minute exposure. There were also some decrements observed in antioxidant activity of broccoli sprout after exposure of UV-B radiation and negative changes on the plant growth. A good understanding on the plants' behaviors towards environmental changes may contribute to agricultural and health industries with their rich source of bioactive compounds.

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