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Photosensitizing Activity and Photostability of *Hibiscus sabdariffa* calyces

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ABSTRACT

The vivid red colour of *Hibiscus sabdariffa* calyces (Roselle) extract is due to the presence of anthocyanins, which have found uses in food and beverage colouring and other dyeing-related applications. In the present study, stability of the anthocyanins of the methanol extract of *Hibiscus sabdariffa* calyces was examined by exposing it to light and measuring the decrease in absorption with time. Also, its ability to photosensitize the reaction of a conjugated diene with singlet oxygen was compared to that of methylene blue, which is commonly used. The degradation pattern monitored by absorbance measurement was tested at a significance level of 0.05 and was found to be statistically significant. Its photosensitization was also confirmed by its ability to induce the formation of endoperoxides in the photooxygenation of conjugated cyclohexadiene in ambient light. This was monitored using thin layer chromatography. Thus, *Hibiscus sabdariffa* calyces extract may be used as a photosensitizer in fine chemical synthesis, and as a natural colorant, however with care in storage and handling.

Keywords: Anthocyanin, Endoperoxides, *Hibiscus sabdariffa*, Photooxygenation, Photostability

INTRODUCTION

Photostability studies are conducted to determine how fast materials under test can degrade or bleach when exposed to light. Photo-bleaching is a dynamic, mostly irreversible process in which fluorescent molecules undergo photo-induced chemical reactions upon absorption of light, losing their ability to fluoresce (Rohit *et al.*, 2016). This can be ascribed to the destruction of covalent and non-covalent bonds in the molecule of the dye arising from non-specific binding caused by excitation of light.

Temperature, low pH, and solvency affect the stability of fluorescent molecules. Anthocyanin dyes are more stable at low pH (acidic conditions) - the higher pH value of anthocyanin will provide colour fading of the colour blue (Wahyuningsih *et al.*, 2017). According to Reichardt (1988), the solvent in which the dye is dissolved may have an important influence on the photophysical and photochemical properties of the fluorescent dye, especially on the molecular energy levels of the dye. This is made manifest not only in the non-specific solute-solvent interactions due to dielectric interactions but also on the specific interactions like hydrogen bonding. Since the properties of the electronic states are determined by individual electronic charge distributions, the energies of the states may be shifted differently if the solvent is different. Increasing temperature decreases the quantum efficiency of fluorescent dyes (Suganya *et al.*, 2012). According to Abdou *et al.* (2013), anthocyanin dyes exhibit a certain level of resistance to direct photolysis by UV–Vis irradiation, however, certain anthocyanins groups appear to be more susceptible to photodegradation (Yijun *et al.*, 2018).

High photostability, long triplet state lifetime, a triplet state of appropriate energy and high absorption coefficient are the properties listed by DeRosa and Crutchley (2002) as important for a compound to function as a photosensitizer. A number of other natural and synthetic dyes have been shown to be photosensitizers in biological systems, among which are the xanthenes, porphyrins, quinones, and flavins (Rajendran, 2016). Dyes function as photosensitizers that absorb energy directly from light sources, which they may then transfer to ground-state triplet molecular oxygen to create an activated form called singlet oxygen ($^1O_2$). Ochnner (1997) reported that there are two pathways available for the photosensitizer-excited triplet state to act. It can react directly with a substrate and transfer a proton or an electron to form a radical anion or radical cation respectively and these radicals may further react with oxygen to produce reactive oxygen species. Alternatively, the triplet photosensitizer can transfer its energy directly to molecular oxygen to form excited state singlet oxygen. Anthocyanins are glycosylated polyhydroxy and polymethoxy derivatives of flavylium salts having electron-deficient chemical structures, rendering them susceptible to reaction with reactive oxygen (Chen *et al.*, 2018).

Extracts of *Hibiscus sabdariffa* calyces (Roselle), which belong to the Malvaceae family, have been shown to contain anthocyanins (Cahlíkováa *et al.*, 2015). These anthocyanins are water-soluble and the dry calyces, when extracted,

are consumed as herbal tea, jams, jellies, and wines (Xiaowei *et al.*, 2021). They have also been used in ethno-medicine to treat urinary tract infections, colds, diarrhea, and dysentery as well as an antipyretic (Ghazala & Rajni, 2018). They are also found to be effective photo-larvicides (Ugboaja *et al.*, 2022). These anthocyanin dyes are among the chief colourants that have been used in the food industry for ages, especially as natural colours in yeast (Nguyen *et al.*, 2018). Several research have also demonstrated that *H. sabdariffa* exhibits bioactivities such as antioxidant, antibacterial, anti-cholesterol, nephron and hepato-protective, renal and diuretic, and anti-diabetic effects (Xiaowei *et al.*, 2021).

This study evaluates the photostability, as well as provides insight into the photosensitizing action of the methanol extract of *Hibiscus sabdariffa* anthocyanins.

MATERIALS AND METHODS

Reagents

The methanol (99%) used for extraction of the plant material was a product of Guangdong Guanghua Sci-Tech Co. Ltd, China. Distilled water was used to dissolve the extract. The cyclohexa-1,3-diene was purchased from Sigma Aldrich.

Extraction

Dried calyces of *H. sabdariffa* (909g) were pulverized and extracted in sequence with dichloromethane (DCM, 1500 mL), ethyl acetate (1500 mL), and 1500 mL of methanol (Ndukwe *et al.*, 2020). The methanol extract (HSME)was filtered through cotton wool and concentrated to 96 g using a rotary evaporator at 40 °C.

Photostability Test

The procedure used was adapted from the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 1996.

In conducting the photostability test, the sample prepared by dissolving 200 mg of the concentrated methanol extract (HSME) in 100mL of water, was filtered and irradiated in capped glass bottles at a distance of 40cm with a 500 W sodium-tungsten lamp at room temperature. Photodegradation of the dye was monitored hourly for six hours, by UV absorbance measurement using Hach-Lange DR3900 spectrophotometer maintained at single wavelength of 520 nm, corresponding to the measured maximum absorption wavelength.

Photosensitization Test

The photosensitization test of HSME was done by dissolving approximately 5 mg of it in 10mL of a mixture of 10% methanol in DCM and adding 5 drops of cyclohexa-1,3-diene in the solution. The solution was then exposed to ambient light and air and allowed to stand for one hour. The reaction was monitored using TLC (silica gel TLC plate purchased from Merck, UK), spotting against a methylene blue-sensitized reaction mixture of the same concentration (and setup) and a control experiment (without any photosensitizer). After elution, the products on the TLC plate were detected by charring as they were not visible under UV light. Solvent system used for TLC was 10% methanol in DCM.

RESULTS AND DISCUSSION

The maximum absorption wavelength of *H. sabdariffa* methanol extract (HSME) was found to be 520 nm as shown in Figure 1. This value agrees with values recorded in literature by Galera-Moreno *et al.* (2015) and that reported for the aqueous and ethanol extracts of *H. sabdariffa* by Izonfuo *et al.* (2006). The value of 520 nm obtained, which is the region of visible absorption wavelength indicates that the methanol extract is predominantly coloured anthocyanins. This corroborates what Abdel-Aal *et al.* (2006) had reported when they opined that UV-Vis data has the ability to confirm some of the anthocyanins. They outlined the typical UV-Vis absorption maxima of some anthocyanins; specifically, that cyanidin-based compounds are observed around 512-520nm while delphinidins are observed at 525nm. The anthocyanins of *H. sabdariffa* calyces had previously been identified by Grajeda-Iglesias *et al*. (2016), Owoade *et al*. (2019) and Cahlíkováa *et al*. (2015) to be majorly delphinidin-3-O-sambubioside, cyanidin-3-O-sambubioside, delphinidin-3-glucoside and cyanidin-3-glucoside as the major anthocyanins of *H. sabdariffa*. That *H. sabdariffa* extract absorbs at this wavelength makes it useful as a colouring matter, especially in the food and beverage industry.

Using the absorption maxima of 520 nm, the change in photostability of the dye as a function of absorptivity was evaluated as presented in Figure 2. The graph shows a downward trend, a continuous degradation with time of exposure to light. The calculated p-value of 0.001421789 at a significance level of 0.05 provides statistical evidence that the degradation of methanol extract of *H. sabdariffa* calyces with continuing irradiation time is statistically significant. This model has also been reported previously by Contreras-Lopez *et al.* (2014) and Albarici & Pessoa (2012) for the degradation of ethanolic extract of blackberry anthocyanins and non-pasteurized Acai pulp respectively. This implies that in handling either of itself or used as colourant in food or beverage, care should be taken to avoid exposure to photo-irradiation which will lead to colour degradation. The decrease in the absorption and by extension the concentration of monomeric anthocyanins, which give the red colour to fruit drinks, according to Wang & Xu (2007), is a loss of food quality.

Table 1 shows the R_f values obtained from the TLC of the reaction mixtures of cyclohexa-1,3-

diene and singlet oxygen without a photosensitizer (a), with HSME (b) and with methylene blue (c) as photosensitizers (Scheme 1). The spots with R_f value of 0.426 corresponds to the product formed from the reactions which are endoperoxides. Gollnick & Griesbeck (1984), quoted in Sevin & McKee (2001), concluded that the only product of singlet oxygen ($^1O_2$) plus cyclohexa-1,3-diene is endoperoxide. The R_f value of 0.852 observed for the reaction mixture with HSME photosensitizer is suspected to be a reaction product of the endoperoxide formed or other unreacted components of the HSME. It has been shown that due to homolytic cleavage of the O-O bond, endoperoxides are versatile starting materials for further reactions (Sevin & McKee, 2001). This spot was not seen for the methylene blue sensitized reaction mixture. It can be inferred from the above that HSME may function as a photosensitizer though may not be as effective as the universally acknowledged methylene blue.

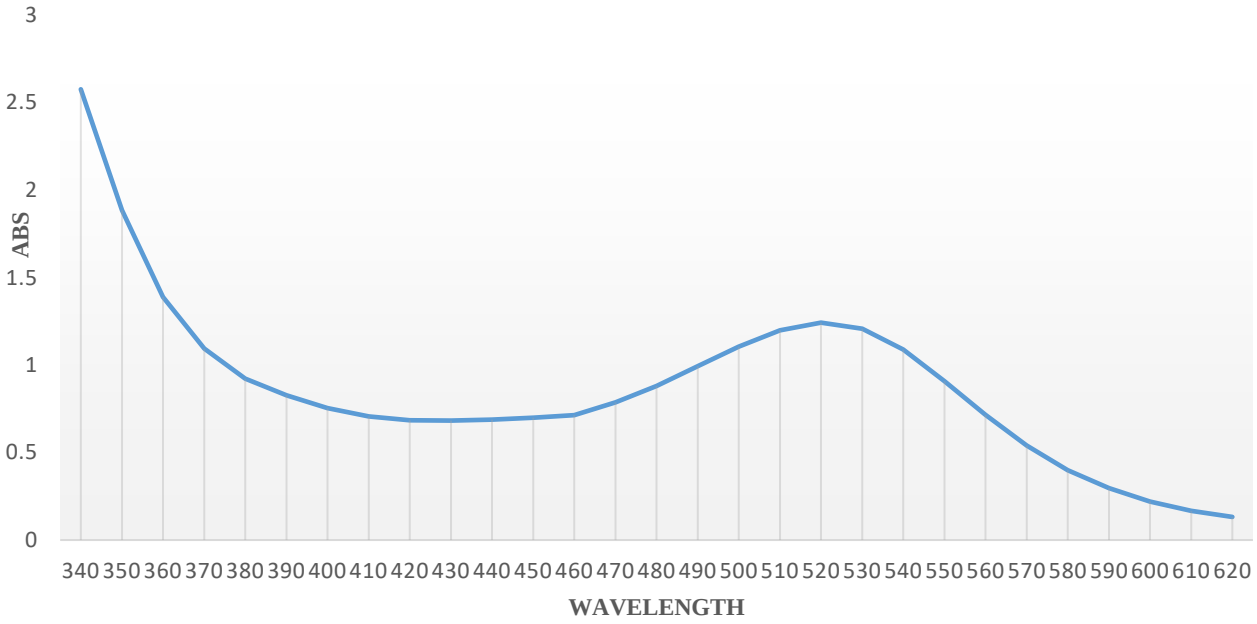


Figure 1: Wavelength Absorption Spectrum of HSME

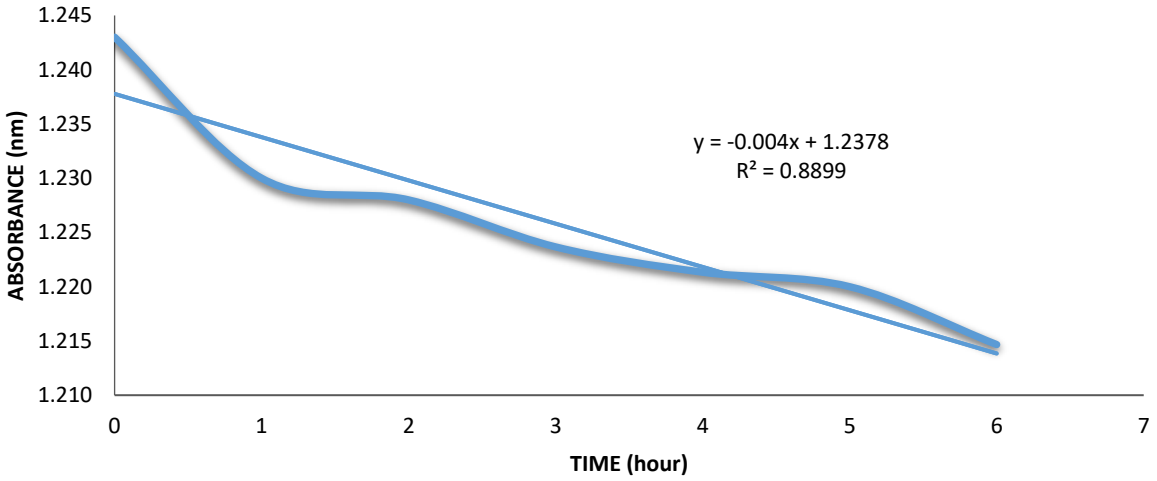
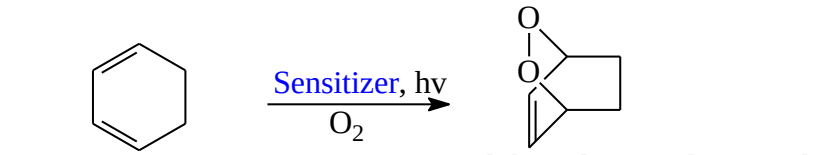


Figure 2: Change in Absorbance with Irradiation Time of HSME

Table 1: TLC of the Photooxygenation Reaction Mixture of Cyclohexa-1,3-diene

Identifier	Photosensitizer	R_f value
a	Reaction mixture with no Photosensitizer	-
b	Reaction mixture with HSME as photosensitizer	0.426, 0.852
c	Reaction mixture with methylene blue as photosensitizer	0.426

HSME -*Hibiscus sabdariffa* Methanol Extract



Cyclohexa-1,3-diene Cyclohexadiene endoperoxide

Scheme 1: Photooxygenation of Cyclohexa-1,3-diene

CONCLUSION

The significant level of degradation found in the photostability assay result gives indication of the reactive nature of HSME. This calls for caution in storage and handling of the extract and product in which they function as colourants. The chromatography information on the reaction of cyclohexa-1,3-diene and singlet oxygen to form endoperoxides with methanol extract of *H. sabdariffa* calyces as photosensitizer in comparison to the widely utilized methylene blue has shown that HSME can function as photosensitizer. Being readily available, *H. sabdariffa* calyces can find application in photodynamic therapy (PDT) for the treatment of malignant lesions or as photo-insecticides. It can also find application in fine chemical synthesis.

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