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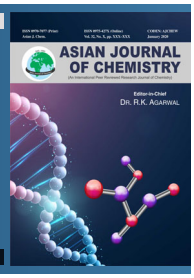

Anthocyanin in *Flacourtia inermis* Peel: Analysis and Electronic Transition Study

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This study aimed to determine the anthocyanin content present in *Flacourtia inermis* peel and its ultraviolet-visible absorption potential as a dye agent. This work focused on developing dye-sensitized materials that require natural eco-friendly dyes which are easily reproducible. The red to purple colour of *Flacourtia inermis* peel indicates the presence of anthocyanin contents, which were derived from flavonoids. Hence, anthocyanin analysis, UV-vis absorption test and electronic transition study were conducted for information regarding the potential use *Flacourtia inermis* fruit peel in natural dyes. To determine anthocyanin and absorption ability, UV-visible spectrophotometry was used. Furthermore, the electronic transition of anthocyanin was determined using the semi-empirical method of ZINDO/s. The results indicate that total anthocyanin content of *Flacourtia inermis* peel was 10.35 mg/100 g, with UV absorption occurring at erythema transmission percent of 0.7553; the pigmentation transmission percent was 0.78696, whereas the electronic transitions of molecular orbitals were observed at an optimum wavelength 425.5 nm visible area, with intensity 1.1233. Molecular orbital levels were six, with two electronic transition shifts, namely $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$.

Keywords: Anthocyanin, *Flacourtia inermis*, Semi-empirical method.

INTRODUCTION

Dye-sensitised solar cell (DSSC) is one of the third generation solar cells [1] and consist of a colouring agent (dyes) generally used between different electrodes. This solar cell has semi-conductor electrodes, light-sensitive dyes, redox mediators and counter electrodes [2]. Unlike other solar cells, DSSC uses dyes, which absorb a certain range of visible light [3]. One of the natural pigments used as dyes in DSSC is anthocyanin, which are widely used in food, medicine and textile industries [4].

Anthocyanin is used as a dye because it absorbs visible light. Anthocyanin structure consists of a double bond conjugated to the chromophore group, which leads to absorption of visible light [5]. It is a flavonoid derivative and commonly found in fruits and vegetables with strong red to purple colour [6]. Anthocyanin derivatives include aurantinidin, cyanidin, delphinidin, europinidin, europinidin, malvidin, pelargonidin, peonidin, petunidin and rosinidin. The charac-teristics of each anthocyanin derivative varies based on their functional groups [7]. Anthocyanidin-3-glycoside is one of the most common anthocyanin derivatives and used as a natural pigment source from plants [8-11]. The placement of -H, -OH and -OCH_3 groups among the seven functional groups in the basic structure of anthocyanins determine the characteristics of anthocyanidin. *Flacourtia inermis* or the tome-tome fruit contains anthocyanin and rich in antioxidants. The antioxidant activities and phenolic compound contents of *Flacourtia inermis* extract are reported in the literature [12,13].

The anthocyanin content of *Flacourtia inermis* peel makes it a source of dye for its potential application in DSSC. The electromagnetic radiation interacts with the dye in ultraviolet (190-400 nm) and visible light (400-800 nm) to provide a visible colour contraction. The anthocyanidin activity can be measured and analyzed by shifting the wavelength of UV-visible spectrophotometer through a electromagnetic radiation absorbance [14-16]. Through determination of UV-visible absorption and electronic transition of *Flacourtia inermis* peel, its potential as a dye agent can be studied.

EXPERIMENTAL

Flacourtia inermis fruits were collected from Ternate Island, Indonesia. In this study, entire chemicals and reagents of analytical grade of highest purities were procured from Sigma-Aldrich and used without additional purification. UV-Vis

(Shimadzu UV 1800) spectrophotometer and the initial calculations were optimized using HyperChem 8.03 software [17].

Sample preparation: *Flacourtia inermis* fruits (~ 25 g) was washed, peeled and macerated with ethanol. The extract obtained was filtered and concentrated using a rotary evaporator.

Determination of anthocyanin contents: Each 4.5 mL buffers of pH 1 and 4.5 was each added with 0.5 mL extract of *Flacourtia inermis* peels. Subsequently, using a UV-visible spectrophotometer, the absorbance of solutions at wavelengths 520 and 700 nm were measured. Anthocyanin content in *Flacourtia inermis* peels can be obtained using the following formula:

$$\text{Anthocyanin levels (mg/L)} = \frac{A \times MW \times DF \times 1000}{\varepsilon}$$

Activity of UV light absorption: Using a UV-Vis spectrophotometer, the UV light absorption activity was measured based on erythema transmission percent (%Te) and pigmentation transmission percent (%Tp) on the absorbance of sample extracts at concentrations 1, 10, 100 and 1000 ppm at wavelengths 290-320 and 320-375 nm [18]. The values of %Te and %Tp values were determined as follows:

$$\text{Te (\%)} = \frac{Ee}{\Sigma Fe} = \frac{\Sigma(TxFe)}{\Sigma Fe} \text{ and } \text{Tp (\%)} = \frac{Ep}{\Sigma Fp} = \frac{\Sigma(TxFp)}{\Sigma Fp}$$

Electronic transition of anthocyanin: Using PM3, the electronic transition of anthocyanin was studied preceded by the geometry optimization of anthocyanin-3-glycoside structure. The optimization method was conducted based on the Polak-Ribiere method. A single point was calculated using the ZINDO/s semi-empirical method to obtain electronic transition data. The data obtained were electronic spectrum (nm), intensity, the transition sequence, highest occupied molecular orbitals (HOMO) (eV), lowest unoccupied molecular orbitals (LUMO) (eV), number of electrons and number of orbitals. The data were used to determine the electronic transition and energy gap (ΔE_g) [19].

RESULTS AND DISCUSSION

Analysis of anthocyanin: Aanthocyanin was extracted from *Flacourtia inermis* peel by using the maceration method with a sample ratio of 1:10 v/v. The maceration process was performed thrice and then immersed for 2 h until the pigment from optimally extracted. This method was selected to retain the stability of anthocyanin. Furthermore, using a rotary evaporator, the sample extract was concentrated.

To determine anthocyanin, differential pH method was used and the pH varied from 1 to 4.5. The solution absorbance was measured by using a spectrophotometer UV-visible at 520 and 700 nm (Table-1). On the basis of the measurements, the anthocyanin content of a fresh *Flacourtia inermis* peel was found 10.53 mg/100 g. The anthocyanin content of jenitri fruit peel was lower at 9.58 mg/100 g [20]. Moreover, anthocyanin content of *Flacourtia inermis* peel was lower than mangosteen (59.3 mg/100 g) and red dragon fruit (28.11 mg/100 g) [21,22]. Total anthocyanin of *Flacourtia inermis* peel were obtained from anthocyanidin-3-glycosides [23-25].

TABLE-1
ABSORBANCE OF *Flacourtia inermis* PEEL EXTRACT AT DIFFERENT pH

pH	Absorbance	
	520 nm	700 nm
1.0	0.132	0.070
4.5	0.056	0.056

UV absorption activity: The UV absorption activity by anthocyanin compounds was carried out by observing the amount of UV light transmitted in the erythema/pigmentation spectrum (Fig. 1). The obtained values were categorized according to erythema transmission percent (%Te) and pigmentation transmission percent (%Tp).

The %Te and %Tp of *Flacourtia inermis* peel extract was determined at various concentrations, namely 1, 10, 100, and 1000 ppm. Using UV-visible spectrophotometer from 290 to 370 nm at the intervals of 5 nm, the transmittance value was measured. The %Te and %Tp values were determined using the transmittance value, where %Tp and %Te showed UV-A and UV-B wavelengths, respectively. Then, using the Balsam equation, the values were calculated (Table-2). The transmittance value was inversely proportional to the absorbance and concentration. According to the obtained values of %Te and %Tp, an extract of *Flacourtia inermis* peel was used in sunblock because it absorbs almost all UV-A and UV-B rays [26].

Electronic transition studies: Using the semi-empirical method ZINDO/s, electronic transition studies were conducted on antocyanidin-3-glycosides, which involved 143 orbitals. Wavelength (λ), intensity (oscillator strength), dipole moment, orbital molecular level and HOMO and LUMO energy were the parameters measured in simulation studies. The ZINDO/s simulation results for antocyanidin-3-glycosides showed six

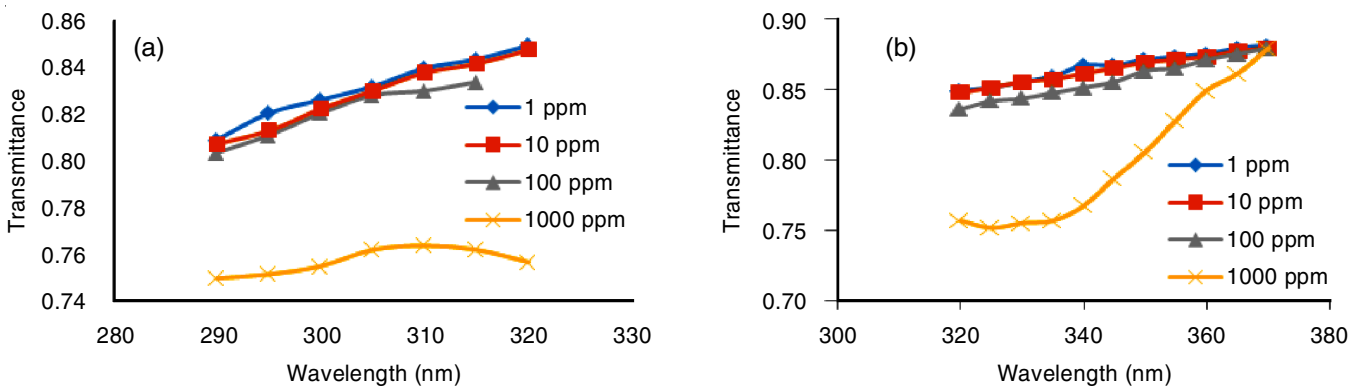

Fig. 1. UV absorption spectra of anthocyanin using (a) UV-B and (b) UV-A

TABLE-2
MEASURED VALUE OF %Tp AND %Te
AT VARIOUS CONCENTRATION

Concentration (ppm)	%Tp	%Te	Category
1	0.86151	0.8257	Sunblock
10	0.85986	0.8212	Sunblock
100	0.85179	0.8186	Sunblock
1000	0.78696	0.7553	Sunblock

peak transitions from the existing eighteen peak transitions. The six peak transitions were selected based on an oscillator strength of more than 0.1. The highest peak of electronic transition has the greatest intensity at a visible wavelength of 425.5 nm (1.1233), whereas the electronic transition in the UV region showed five peaks (Table-3). The dipole moment value shows the polarity level of a dissolved compound [27].

Anthocyanidin-3-glycoside showed two types of electronic transitions, namely n→π* and π→π*. The transition n→π* occurred because anthocyanidin has a simple chromophore group of conjugated bonds in three aromatic rings (C=C). In addition, the transition π→π* occurs because of substitution groups, such as -OH, -H and glucose, which are auxochrome groups that affect the wavelength shift in anthocyanidin-3-glycoside. The orbital molecule levels showed the location of electron excitation from HOMO to LUMO, where the gap energy difference affected excitation of electrons from HOMO to LUMO easily [27]. The ZINDO/s simulations with three limits of HOMO and LUMO each produce six molecular orbital levels, namely -2/80, -1/81 and 0/82 for HOMO and 0/83, +1/84 and + 2/85 for LUMO (Fig. 2). The lowest value of gap energy was at the orbital molecule level 82 → 83 at -5.750408 eV.

TABLE-3
SIMULATION RESULTS OF ANTHOCYANIDIN-3-GLYCOSIDE MOLECULE USING ZINDO/s

λ (nm)	Oscillator strength (Osc)	Dipole moment (BM)	MO level	ΔE_g (eV)	Transition type
452.5	1.1233	3.7461	82→83	-5.750408	n→π*
267.5	0.3305	3.1341	82→84	-7.477716	n→π*
227.3	0.2618	3.1513	82→85 81→84	-8.143935 -8.264815	π→π*
218.1	0.3361	8.9231	81→84 80→84	-8.264815 -8.607446	π→π*
200.4	0.3566	8.7562	81→85 80→85	-8.931034 -9.273665	π→π*
185.7	0.1584	20.3405	81→85 80→85	-8.931034 -9.273665	π→π*

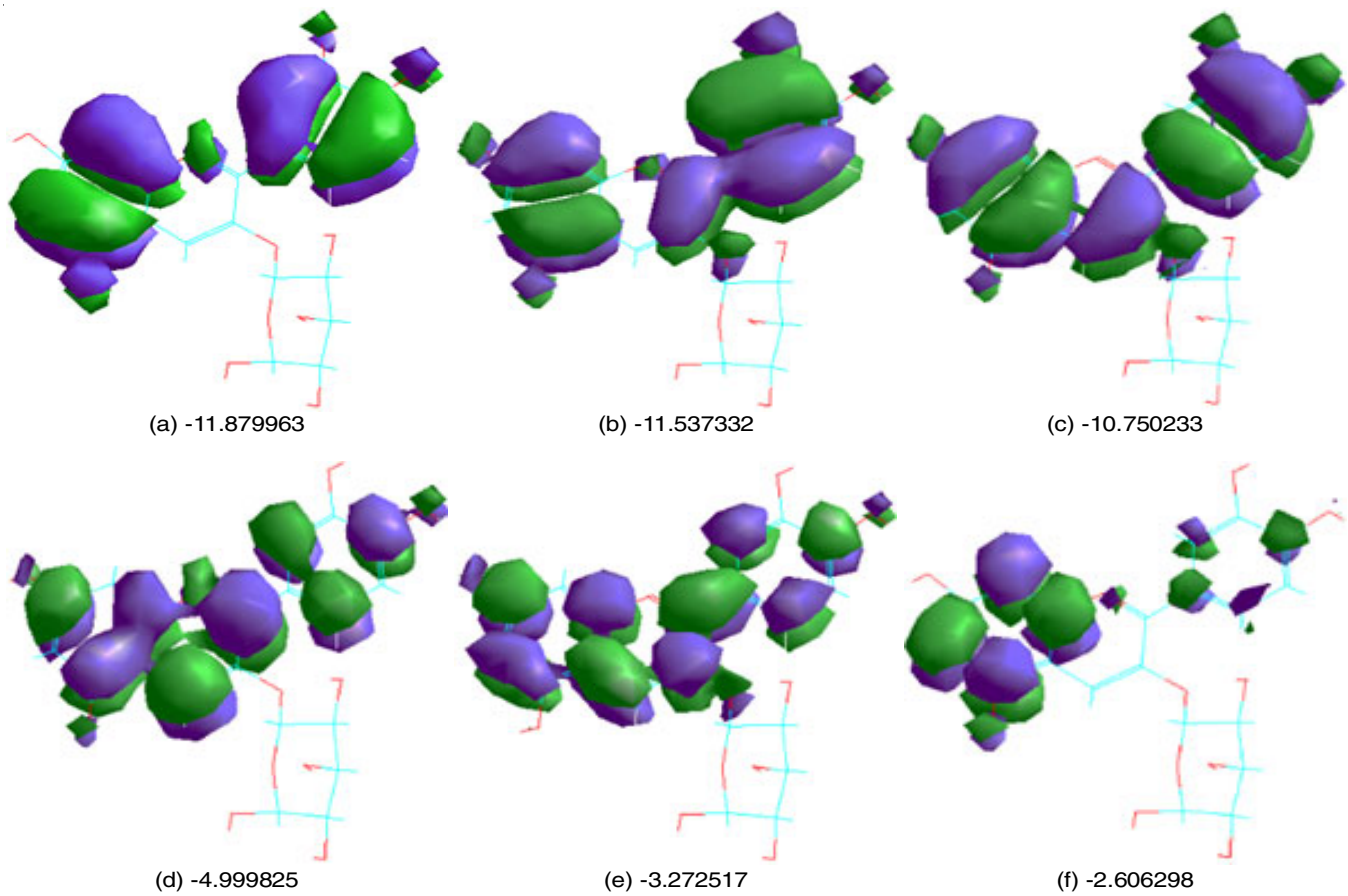

Fig. 2. Molecular orbital levels of anthocyanidin-3-glycoside having HOMO 80 (a), 81 (b), 82 (c) and LUMO 83 (d), 84 (e), 85 (f)

Thus, electron excitation was easy (reactive) at the molecular level because lesser energy is required here than at the other orbital molecule levels [28].

Anthocyanin as a dye agent: Anthocyanidin-3-glycoside as a potential natural dye was investigated experimentally and through computational simulation using a semi-empirical method ZINDO/s (Table-4). The spectrum measurement simulation of anthocyanidin-3-glycoside obtained a λ_{max} value of 452.50 nm.

TABLE-4
COMPARISON OF UV-VISIBLE SPECTRA WITH ZINDO/s

Wavelength (nm)	Zindo/s	Oscillator strength	Pigment colour
520	452.5	1.1233	Blue-Purple
290	267.5	0.3305	Colourless
220	218.1	0.3361	Colourless

Using ZINDO/s method with computational simulation might aid in the prediction of the peak as experimentally measured using a UV-vis spectrophotometer. However, several peaks were noted with low oscillator strength, which may not be measured or read experimentally [29]. The ZINDO/s simulation results showed that three peaks were experimentally measured in the UV and visible area wavelengths. The measurement in the visible area was obtained at a wavelength of 400-800 nm, which has a blue-red character [30]. Anthocyanidin-3-glycoside was measured in this wavelength range both experimentally and comparatively and showed a blue pigment character. Thus, anthocyanidin-3-glycoside is a potential natural dye obtained from plants [30,31].

Conclusion

The anthocyanin content (10.35 mg/100 g) from *Flacourtia inermis* peel was determined using UV absorption at concentrations of 1, 10, 100, and 1000 ppm being, respectively, 0.85257, 0.8212, 0.8186, and 0.7553 for %Te and 0.86151, 0.85986, 0.85179, and 0.78696 for %Tp. Electronic transitions in molecular orbitals were optimum at a wavelength of 425.5 nm in the visible light area with an oscillator strength of 1.1233. Molecular orbital levels were six, with two electronic transition shifts, namely $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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