PHYSICOCHEMICAL AND RHEOLOGICAL CHARACTERIZATION OF ANTHOCYANIN DERIVED BUTTERFLY PEA FLOWERS

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ABSTRACT

In the realm of cosmetology, anthocyanin has recently been used as one of the essential ingredients. Anthocyanin has been demonstrated to lessen irritability and aid in moisture retention when applied. However, adding a natural ingredient to a skin care product formulation might alter the consumer's experience when the product is applied since it increases the actual contact area and adhesion, which alters friction. In this study, the chemical and flow characteristics of anthocyanin are examined in order to evaluate the rheological characteristics of butterfly pea flowers that contain anthocyanin. Anthocyanins were derived from standard liquid, powder and extracted raw and the characteristic have been compared. Total Anthocyanin Content (TAC) of extracted raw anthocyanin was on par with standard liquid at 102.14 mg/L and 136.89 mg/L respectively. Shear rheological tests of extracted raw anthocyanin illustrate that the flow behaviour was altered from shear thinning to shear thickening at higher temperature. The results of the present study indicate that standard liquids offer a promising approach for use in cosmetics able to maintain shear-thinning flow behaviour at increasing temperatures and 25.38% higher total anthocyanin content than other sources of anthocyanin.

Keywords: *Anthocyanin, Butterfly Pea Flower, Rheological properties*

1.0 INTRODUCTION

Over time, the significance of skincare products, improve the quality and health of the skin and provide nourishment arise. The global market for skincare is anticipated to grow from $136.4 billion in 2020 to $187.68 billion in 2026 [1]. These products are used daily for a variety of functions including moisturizing and hydrating. Organic and herbal skin care has recently acquired popularity. Ingredients originating from natural sources such as flower extracts are non-irritating and therefore suitable for use on the skin. According to Ferreira *et al*.[2], the extraction of anti-ageing compounds from natural sources soared from 2011 onwards as a result of their enormous benefits in preserving a young appearance.

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Anthocyanin is one of the proven natural antioxidants able to restrain the oxidation of scavenging radicals when skin is exposed to UV light [3]. Anthocyanin from butterfly pea flower has proven to have anti-inflammatory and anti-oxidative content that inhibit the formation of wrinkles [4,5]. The antioxidant content helps skin self-repair and reduce inflammation, correcting visible damage to skin. In addition to its biological benefits, the anthocyanin-derived butterfly pea flower has a longer shelf-life and greater stability compared with other anthocyanin resources [5–7]

Lee et al. [8] has stated in their research that perceived quality become the primary factor in consumer skincare selection. The perceived quality is generally affected by the rheological, mechanical and also physicochemical of the ingredients as it alters the consumer perception in the application of products [9,10]. Thus, this makes the rheological properties of its ingredients need to acknowledge first to estimate its quality in skincare formulation. However, the application of anthocyanin extracted from butterfly pea flower is new in skincare formulation made its rheological properties are still undisclosed.

This study aims to acknowledge and compare the physicochemical and rheological properties of extracted raw, standard liquid and powder anthocyanin. In this regard, utilizing UV-visible spectrophotometer and Fourier-transform infrared spectroscopy (FTIR), anthocyanins' chemical structure, class, and total anthocyanin content (TAC) are identified. Rheological properties of each sample were then described by using a shear rate scan test rheometer at temperatures ranging from 25°C to 85°C.

2.0 EXPERIMENTAL PROCEDURE

Three samples of anthocyanin from standard liquid, powder and extracted raw have been analysed in order to comprehend the crucial behavior of anthocyanin. While the dried, raw butterfly pea was obtained from a local farmer in Melaka, the standard liquid and powder were purchased from Bionutricia Manufacturing. The standard liquid anthocyanin is compared physically, chemically and rheologically to two different extracted liquids made from powder and raw, dried butterfly pea.

2.1 Extraction of anthocyanin

A probe ultrasonicator with a power output of 750 watts and a frequency of 20 kHz was used to extract 10 g of dried butterfly pea flowers from distilled water that had been ground up with a mortar and pestle. The supernatant was then filtered, and it was concentrated at 50°C using a rotary evaporator. While anthocyanin from standard powder was prepared by dissolving it in distilled water at ratio 1:1.

2.2 Determination of Functional group

The functional group was identified using Fourier- Transform Infrared (FTIR) Spectroscopy. 1 ml of anthocyanin was placed in IR chamber for analysis.

2.3 Determination of Total Anthocyanin content (TAC)

Total anthocyanin content was determined using pH differential method [11] . At pH 1.0, anthocyanins exist in the oxonium form, while at pH 4.5, they exist in the hemiketal form. Under varying pH conditions, the structurally reversible trans-formations of anthocyanins exhibit distinct UV–Visible absorptions. At 520 nm (A_{520}) and 700 nm (A_{700}), the absorbance of butterfly pea flower anthocyanins was measured using a 0.025 M potassium chloride buffer of pH 1.0 and a 0.4 M sodium acetate buffer of pH 4.5.
Absorbance (A) was determined via Equation (1):

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$$A = (A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH4.5} \quad (1)$$

TAC of Butterfly Pea flower, expressed in mg cyanidin-3-glucoside/ L was calculated according to Equation (2):

$$\text{TAC}=\frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \quad (2)$$

Where MW is 449.1 g/mol and represents the molecular weight of cyanidin-3-glucoside (Cy3gl), ε represents the extinction coefficient equal to 26900 L/mol/cm, DF represents the dilution factor, and l is the path length (l).

2.4 Rheological test of Anthocyanin

The rheological behaviour of pure anthocyanin determined with a Modular Compact Rheometer (Anton Parr, MCR 302) with a 50 mm spindle and a 1 mm gap at temperatures ranging from 25 to 85 °C. The samples were placed evenly enough between the dishes to fill the space entirely. This device enables the assessment of sample flow and deformation by monitoring the fluids' increasing viscosity and elasticity. To assess the quality and stability of semisolid formulations, it is crucial to examine their rheological characteristics**.**

2.5 Moisture Content

The moisture content of sample was determined based on the AOAC oven-drying method at 105 °C for 5 hours. The moisture content (%) values were calculated using equation (3).

$$\text{Moisture Content (\%)} = \frac{M_1 - M_2}{M_1} \times 100 \quad (3)$$

Where M_1 is the weight before drying (g) and M_2 is the weight after drying (g).

3.0 RESULTS AND DISCUSSION

3.1 FTIR analysis of anthocyanin.

Figure 1 displays the FTIR spectra range in the 4000–600 cm^{-1} wave band. The FTIR spectra from all samples showed the presence of phenols with a peak at range 3230.16 cm^{-1} to 3328 cm^{-1}, which corresponded to the stretching frequency of hydroxyl and O-H, respectively [12]. This was detected from the functional groups of anthocyanin. While the presence of carboxylic acids is indicated by the peak at 1638.72 cm^{-1} to 1649.23 cm^{-1}. FTIR spectra range in the 4000–600 cm^{-1} [13]. Last but not least, the peak between 700 and 1000 cm^{-1} indicates the presence of the aromatic rings [14]. Anthocyanin is present since the three peaks are present in all three samples. The remaining peaks at the typical liquid and powder anthocyanins show that the sample contains stabilizer.

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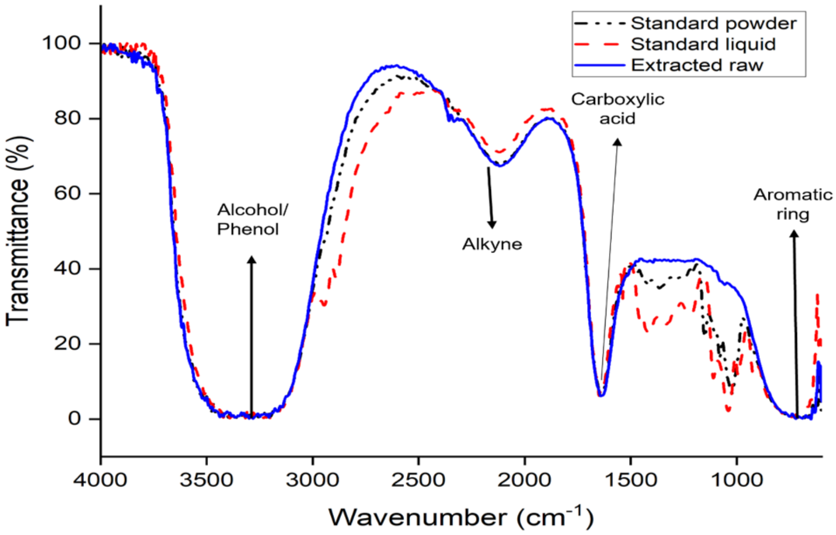

Figure 1: Comparison of FTIR spectra of liquid, powder and raw anthocyanin.

3.2 Total Anthocyanin Content (TAC).

Referring to Figure 2, the anthocyanin derived from pure liquid anthocyanin has the highest anthocyanin content (136.89 ± 0.97 mg/L), followed by raw extracted anthocyanin (101.3 mg/L), while powder anthocyanin exhibits the lowest TAC (66.87 ± 3.86 mg/L). Comparing TAC from the three sources, the conventional liquid anthocyanin has the greatest TAC level (136.89 mg/L). On the other hand, according to the product data sheet, the TAC gradually decreases from 200 mg/l to 136.89 mg/L. Rashid et al. [15]demonstrated this as the encapsulated anthocyanin will deteriorate over time.

As for the raw anthocyanin, it is extracted from dried butterfly pea flower by using probe sonicator with acidified distilled water as extraction solvent. The anthocyanin extracted has lower TAC compared with anthocyanin extracted from Pham et al. [16] which is 121 mg/L. This is due to the solvent factor used which is ethanol. However, the extraction using distilled water is favorable as it is green solvent, non-toxic and safe to be used. The fact that the TAC of standard powder anthocyanin is only 62.61 mg/L makes standard liquid and extracted raw anthocyanin more desirable for utilisation in skin care formulations.

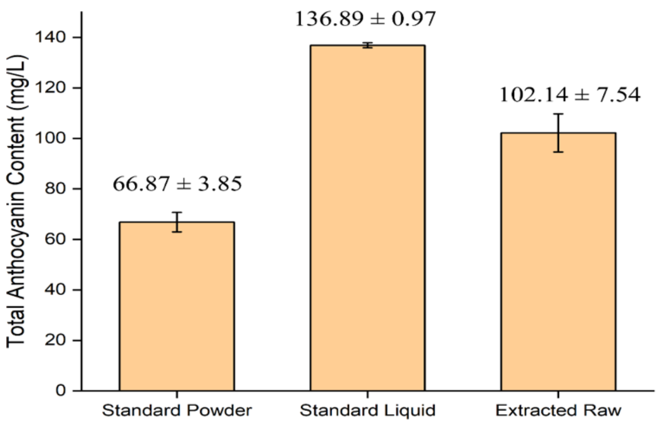

Figure 2: TAC from different type sources of Butterfly Pea Flower.

3.3 Moisture Content

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Moisture content is commonly stated as a percentage, denoting the amount of water present in a substance. According to Zhang et al. [17] , the moisture content of a product is an important experimental parameter for assessing its viscosity, shelf life, texture, and rheological properties. Typically, commercial products are encapsulated with a stabilising agent to ensure their long-term stability. As water has a lower boiling point than the other compounds (stabilizer) in the sample, each sample was allowed to dry at 105 °C to entirely eliminate water in order to evaluate the differences in physical appearance between each sample (Figure 3).

Table 1: Moisture Content of Anthocyanin.

Anthocyanin Sources	Moisture Content (%)	Physical Appearance
Standard liquid	61.87 ± 1.46	Gel like
Standard powder	78.16 ± 2.82	Hard pieces
Extracted from raw	97.05 ± 2.29	Crispy powder

Table 1 indicates the standard liquid had the lowest moisture content at 62.39%. After being dried at 105 °C, the dried standard liquid anthocyanin takes on a gel-like appearance (Figure 3a). This is a result of the anthocyanin solution also including additional compounds that serve as stabilizers.

As for standard powder anthocyanin, after the sample is dried, the anthocyanin is burnt like, and the texture is hard (Figure 3b) indicating there is presence of stabilizer in the powder. The raw extracted anthocyanin formed crispy powder (Figure 3c) after being dried at 105°C. The high moisture content for extracted anthocyanin indicates that it is purely anthocyanin, and all solvent is completely evaporated. The moisture content also explained the rheology behaviour of various anthocyanin sources, in which the addition of a stabiliser agent decreased the moisture content of the product, increased its viscosity by its complex structure, and stabilised the rheological behaviour of the base solvent structure [18].

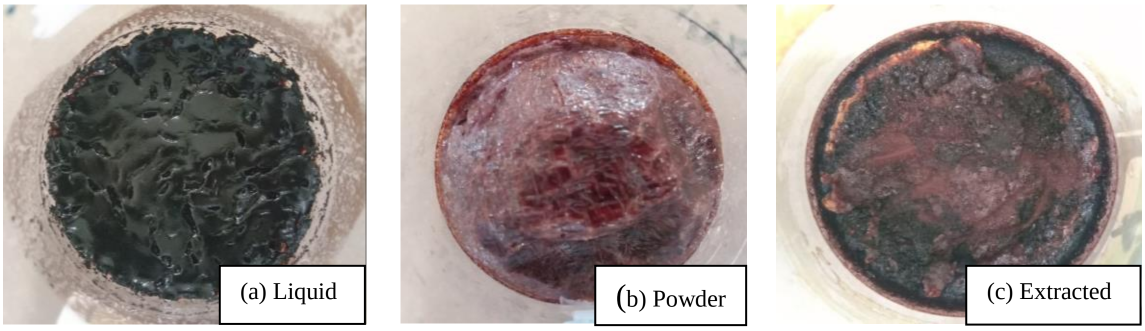

Figure 3: Condition of Anthocyanin after being dried at 105° C.

3.4 Rheological behaviour of different sources of anthocyanin.

The rheological behaviour was determined at temperature ranging from 25 °C to 85 °C. Based on Figure 4 (A), liquid standard anthocyanin exhibits shear thinning, non-Newtonian behaviour. The viscosity of anthocyanin reduces as the shear rate increases consistently at 25 °C to 85 °C. The shear stress of standard anthocyanin increase with the increase of shear rate depicts the sample's pseudoplastic behaviour. This rheology property indicates a high shear rate, decreases viscosity of standard liquid anthocyanin, and facilitates flow. In terms of conditioning and dissemination on the skin, as well as the establishment of a continuous film at the application site, it is ideal for topical application systems to exhibit pseudoplastic behaviour. At high shear rates, such as when dispensing from the conditioning vessel, the material will flow quickly, thereby facilitating administration, whereas at low shear rates, such as when the product is being spread on the skin, the material will adopt a high viscosity by regaining the rheological properties it possessed prior to administration [19]. According

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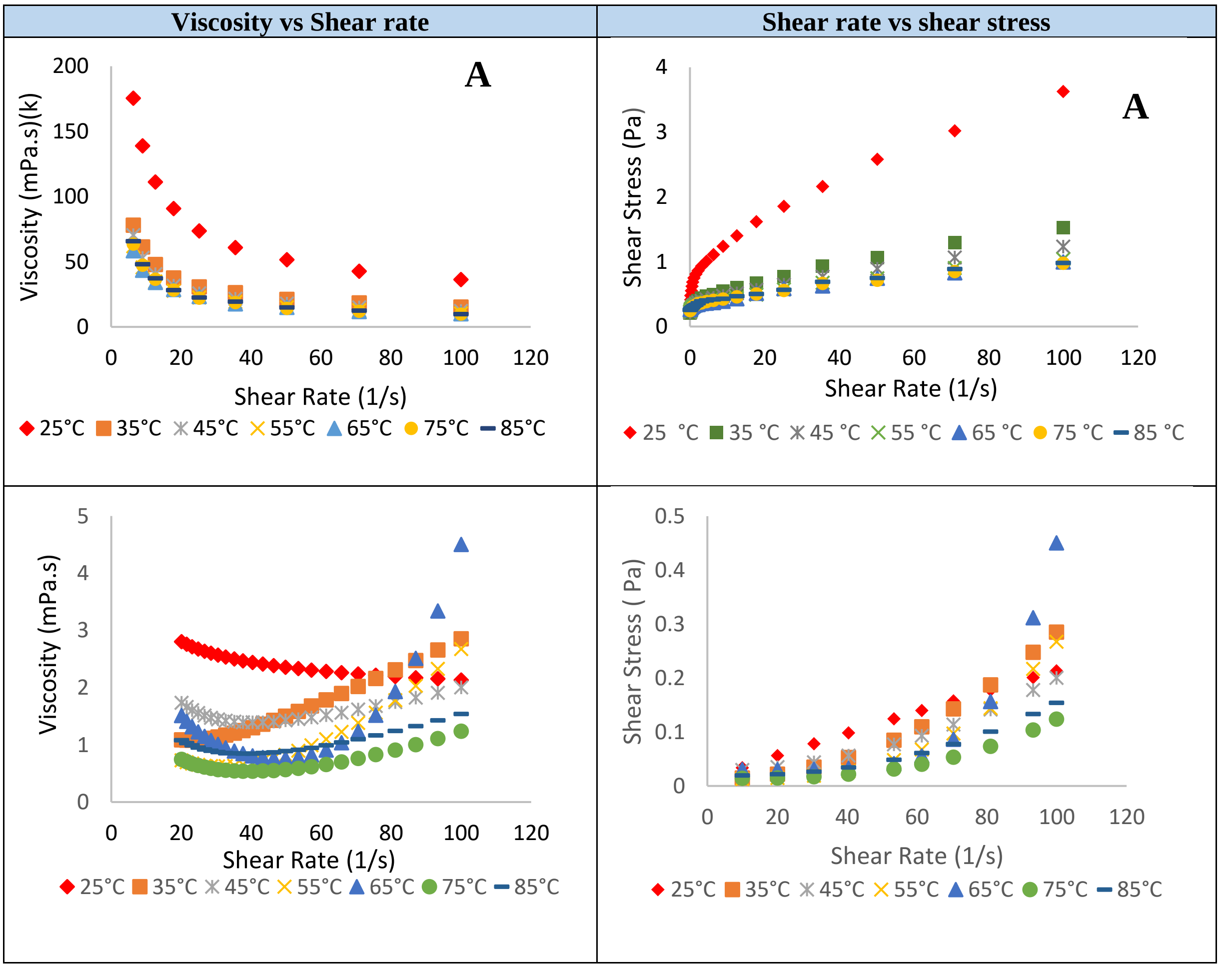
to Martinez et al., [20], the most essential usage sense in these circumstances is spread ability and perceptiveness hence, products displaying shear-thinning, non-Newtonian flow are chosen due to their superior spread ability and perceivability.

Figure 4 (B) shows that an increase in shear rate reduces the viscosity of standard powder anthocyanin indicating the shear thinning behaviour at 25 °C. However, the increase in temperature at 35°C to 85°C alters the flow behaviour of the standard powder anthocyanin from shear thinning to shear thickening after shear rate 20 s^{-1} onwards. This is due to the gradual evaporation of solvent as temperature increase which causes the particles in the anthocyanin solution to become suspended. The increase in the shear rate causing occlusion between particle in the solvent. This can be demonstrated by the formation of dense particles beneath the spindle when the solution is cooled to 25 °C.

The extract raw anthocyanin's rheological behaviour shows inconsistency as illustrated in Figure 4(C). Shear thinning behaviour is demonstrated by the viscosity decreasing as shear rate increases. According to Figure 4(C), the change in flow behaviour from shear thickening at higher temperatures, such as 35 °C, to shear thinning at lower temperatures, is due to an increase in temperature. It is important to stabilize anthocyanin extract at different temperature from 25°C to 85 °C to preserve the rheological stability as temperature rises.



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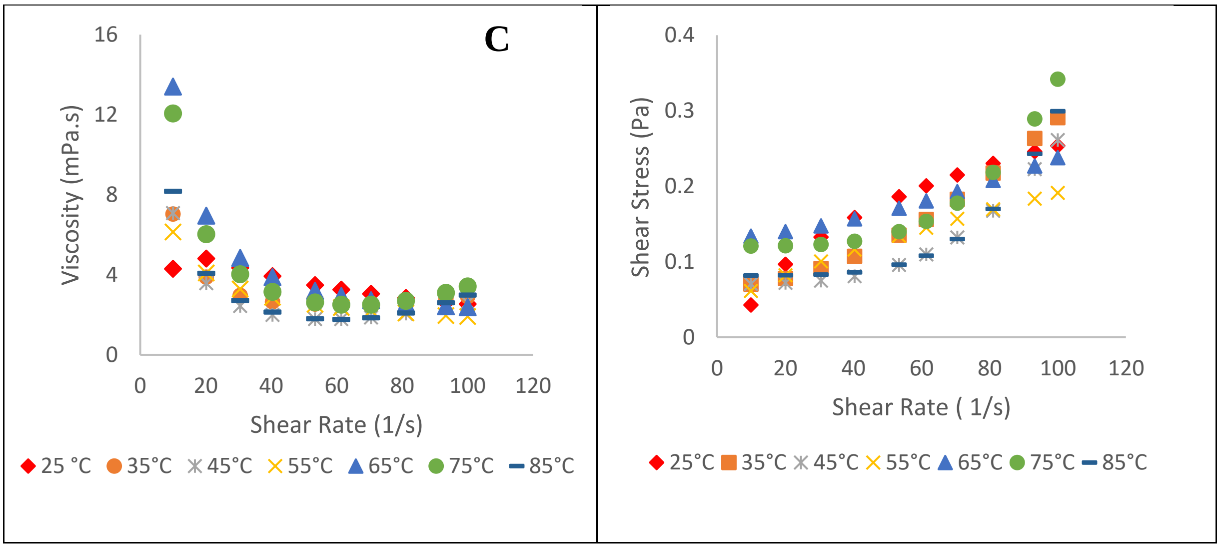

Figure 4: Effect of shear rate on viscosity and shear stress of anthocyanin (A) standard liquid (B) extracted raw (C) standard powder

4.0 CONCLUSION

This study demonstrates the significance difference of anthocyanin derived butterfly pea flower from three sources. This was made apparent to understand the physicochemical of standard liquid, powder and raw, extracted anthocyanin, highlighting total anthocyanin content and the effect of moisture content on the rheological behaviour of anthocyanin. The TAC value of extracted anthocyanin (102.14 mg/L) is comparable with the standard liquid anthocyanin (136.89 mg/L) made the extracted anthocyanin has potential to be utilized in product formulation. However, the rheology data revealed the differences of flow behaviour of standard liquid anthocyanin with powder and extracted anthocyanin. Under strong shear stress, the absence of stabiliser in raw extracted anthocyanin alters the rheological behaviour of anthocyanin from shear thinning to shear thickening, making standard liquid anthocyanin more desirable for usage in skincare formulation. It was obvious that the addition of a stabiliser to the raw extracted anthocyanin is important for ensuring the molecular stability of the molecule in the solvent and maintaining its fluidity at room temperature.

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