Effect of pH on Natural Pigment Betacyanin Extraction From *Bougainvillea* Bracts

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Abstract—Natural dyes derived from plant sources were used in textile industries. However, there are limitation involved with natural dye including laborious extraction of colour component of the raw material, low colour value and long dyeing time promote the cost of dyeing with natural dyes considerably higher compare to synthetic dyes. Besides, the parameter of the extraction process like medium pH is still ambiguous. Solid-liquid extraction that allows the removal of soluble component from solids using solvent has been applied as the method used in this research. In this work, *Bougainvillea* was chosen as a source of plant base, red natural dye which rich in betalain pigments The present study reports on the extraction of natural dye pigment where one-factor- at a time has been studied that is the pH, range from 1.0 to 10.0 using solid-liquid extraction process. The color strength of dye was then analyzed using UV- Vis Spectrophotometer. The results showed that the best extraction condition was obtained with pH 4.0. In these conditions, it shows the highest absorbance reading was 2.92 and the dye concentration was 10.67 g/L**.** It is suggested that solid-liquid extraction with water as the solvent at acidic medium was an efficient process to extract the betacyanin.

Keywords— betacyanin; extraction; pH

1. INTRODUCTION

Dye produced from natural material can minimize the damage to human health and the environment. It has increased the worldwide interest and growing new market due to increased perception of health and environmental risks associated with the synthesis and processing of synthetic dye. Moreover, synthetic dye is a source of pollution to the environment from the wastewater of textile dyeing and finishing factories because of their loss in the dyeing process since the fixation efficiency ranges from 60-90%. Hence, high amounts of unfixed dye were released in the waste water displaying high organic loads as indicated by high Chemical Oxygen Demand (COD). Although available at low-cost, use of synthetic dyes led to such consequences as carcinogenicity and inhibition of biosynthesis photosynthesis. One of the alternatives, is to construct sufficiently large and highly effective effluent treatment plants, or other way is to use dyes and chemicals produced that was environment friendly [3]. Since, natural dyes are clinically safer than the synthetic dye analogues in handling and use because of non-carcinogenic and biodegradable properties it have, a new tendency has raised mainly due to their environmentally characteristics and gain more importance in food and textile industries.

In Malaysia, the society generates abundant agricultural wastes with the volume of approximately 5 million tons annually and is expected to double by the year 2010 [10]. Some of these wastes include oil palm trunks and fronds, palm kernel cake, sugar cane baggase, rice husk, rice straws, coconut fibres and meal, cocoa pods, rubber wood dusts, fruit peels and many other waste materials [8], [10],[14],[16]. These abundant of waste have been used as animal feed for the poultry industries, fertilizers for agricultures and recycle to make a new product. Apart from that, natural dye also can be extracted from that waste and plant without any chemical which is by water extraction in most cases. This extraction of dye from plant can be extracted from waste such as from the peel or small portion of plant which will not give effect to the plant. However, the problem arise with the natural dye is the solubility and stability of the dye that depends on solvent medium pH that are uncertain. Hence, this research investigate the effect of the pH on the stability and appearance of the betacyanin dye.

Bougainvillea was chosen as the representative source of plant base red natural dye in this research, because the floral bracts are rich in betalain pigments which have the potential to be a natural dye. According to [4] betalains are water-soluble nitrogenous vacuolar pigments present in flowers and fruits of many caryophyllales with potent 17 antioxidant properties. Betacyanin extracted from the flower bracts of this tree ia a natural colour. Betalins are another class of natural pigments with reddish purple (betacyanins) or yellow (betaxanthins) nitrogenous vacuolar part that are widely used as food colorants. Dark pink colored bracts of Bougainvillea glabra are betacyanin structures with different acyl-linked oligosaccharide substitution pattern are pigments of chemotaxonomical [18].

Natural dye extraction of betacyanin pigment from *Bougainvillea* flower bract using solid-liquid extraction with the effect of pH has been studied.The extraction procedure has been performed using low cost procedure and equipments. Water has been chosen as the solvent for the extraction process. The absorbance reading and colour strength of dye samples was analyzed using UV-Vis Spectophotometer [2].

2. MATERIALS

A. Materials

The bracts of *Bougainvillea* were picked and collected from the tree in Gambang area. The bracts collected were isolated from the flowers and leaves, allowed to dry under sunlight [9]. The dried bracts were then finely ground into powder form for getting proper extraction result and then stored in the freezer to keep it from wilt [12]. The chemical used in this study included sodium hydroxide (NaOH) and acetic acid (CH_3COOH).

3. METHODS

A. Preparation of Calibration Curve

Calibration curve is a quantitative research tool that the assay was performed with various known concentrations of a substance similar to that being measured. A standard solution is a solution in which the concentration is accurately known. The absorbance of the standard solutions are measured and used to prepare a calibration curve, which is a graph showing how the absorbance varies with the concentration. The concentrations of betacyanins were calculated from the standard curves betacyanin, at five concentrations (namely, 2,4,6,8,and 10 g/L) using a linear regression analysis ($R \geq 0.95$). All determinations were performed in duplicate [13]. The slope, m and intercept,C of that line provide a relationship between absorbance,A and concentration, x in (1) below:

$$A = Mx + C \tag{1}$$

The unknown concentration of dye extract was then analyzed. The absorbance of the dye extract was then used with the slope and intercept from the calibration curve to calculate the concentration of the dye extract.

B. Preparation Raw Materials

Water was chosen as the solvent for the preparation of natural dye, the purpose to make sure that the dye is safe from harmful chemical and substance. 1M of each sodium hydroxide and 1M of acetic acid was prepared to be used for the preparation of solvent, water with pH varied from 1.0 to 10.0. pH meter was used in measuring the pH of the solvent prepared. Flower bracts that have been isolated and dried will be weighed using the weighing scale to be used at different solid-liquid ratio (SLR).

C. Screening Parameter of The Extraction Process

Effect of pH Solution

Experiment set up for extraction process by preparing the pH water solution from 1.0 to 10.0. SLR and extraction time was set as constant parameter. The value of SLR use was 0.25, and extraction time is 2 hours. The temperature was maintained at 28 ^{o}C, room temperature using a water bath. After 2 hours, the dye extract was filtered from the insoluble plant material through a Whatman no.1 filter paper by vacuum suction using a Buchner funnel and then the extracts were centrifuged at 4 ^{o}C, 10,000 RPM for 15 minutes to separate all the residues and the absorbance reading of the dye was measured. The experiment wos conducted in triplicate [15].

D. Photometric Quantification of Betacyanin Content

After 120 minutes, the dye will undergo the same process of determining the optical density of the solution using spectrophotometer. A U-1800 model spectrophotometer was used to measure the λmax and the absorbance of dye extracts of *Bougainvillea* bracts solutions at various pH. The λ_{max} and absorbances of the solutions were measured using the spectrophotometer in the visible light spectra 535nm [2],[6],[12],[19].

4. EXPERIMENTAL RESULT

As stated by [5] pH plays an important role for the stability of the dye extract where pH gave a stable appearance over a broad range from 3 to 7 for betanin. İt is important to have chemical stability when encounter with natural dye. İn this research, the effect of medium pH solution during extraction process was discussed in detailed. The experiment was conducted at constant room temperature, 28 ^{o}C with constant solid-liquid ratio 0.25. The betacyanin content in the extracted dye was calculated by diluting the sample with distilled water and the absorbance reading was recorded. To investigate the effect of pH on the absorbance reading of extracted dye, the absorbance reading was taken by using UV-Vis Spectrophotometer at 535 nm and analyzed.

Fig.1 shows the absorbance reading of the dye extracted at different pH from 1.0 to 10.0 at room temperature 28 ^{o}C from 2 hours extraction process. From the graph it exhibited that among the prominent absorbance reading of the dye was at pH 2.0, 4.0 and 7.0 that was 2.78, 2.92 and 2.85. As this three pH colour in Fig. 2 were exemplified in the work undertaken by [15] where his study showed that the bougainvillea juice display pink colour from pH 1-7, chocolaty at pH 8, greenish colour at pH 9 and yellow colour at pH 10. However, in this study the dye display pink colour at alkaline medium although it should be display yellow-green. This might be because of the betacyanin do not change to betaxanthin in alkaline medium since acid used is a weak acid and not a strong acid such as hydrochloric acid and sulphuric acid.

However, in order to have the red colour of dye, extraction at acidic solution are more encouragable because the dye shows red in colour. From the results the absorbance reading value at pH 4 was higher than the reading at pH 2 about a 2.5% difference. İn addition, in press [1] reported that betacyanin was stable within a pH range of 3 to 8 and the stability is decreased at pH 2 and above pH 9. In his research, [17] stated that the addition of ascorbic acid and make the extraction medium to be slightly acid was recommended during the extraction process of betalain. Moreover, betalain are not susceptible to hydrolytic cleavage as the anthocyanin, upon pH changes and it is relatively stable over broad pH range 3 to 7 [7].

Moreover, the physicochemical properties of betalain pigments that have polarity and ionization, when in aqueous solutions it have dissociation, zwitter-ionic behavior that make it has high solubility in water yet, the stability depends on the pH. According to the stability could be very limited where alkaline solutions produced betalamic acid as the result of cleavage. Furthermore, betacyanin was insoluble in popular organic solvent or semi-polar solvents except for water and mixtures with low-molecular alcohols.

Reference [11] stated that materials with betalain content are generally macerated or ground and can be water extracted because the aqueous extraction promoted better stability of the pigment. Slight acidification of the extraction medium enhances betacyanin stability and avoids oxidation by polyphenoloxidases. The result showed in Fig. 3, betacyanin pigment concentration was found to be the highest at pH 4 and pH 7 that is 10.67 g/L and 10.42g/L. The range of betacyanin concentration for pH 1.0 to 10.0 are about 8 to 10 g/L.

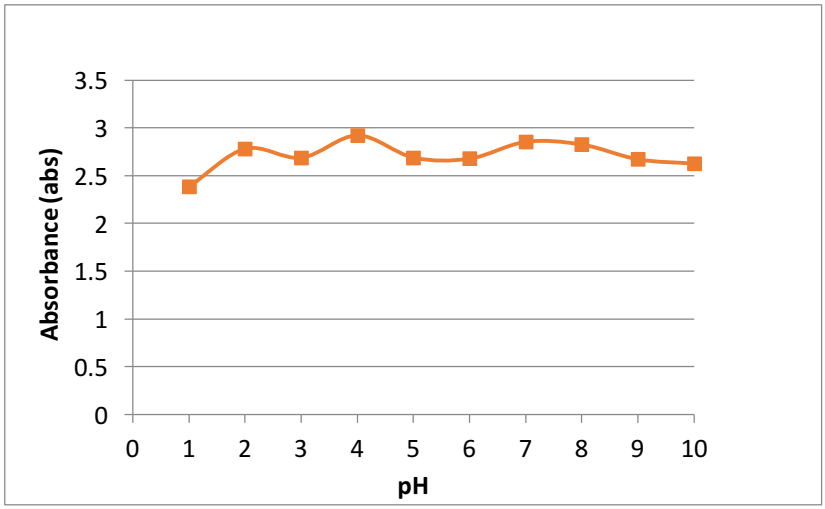

Figure 1: Effect of pH on the optical density reading with SLR 0.25

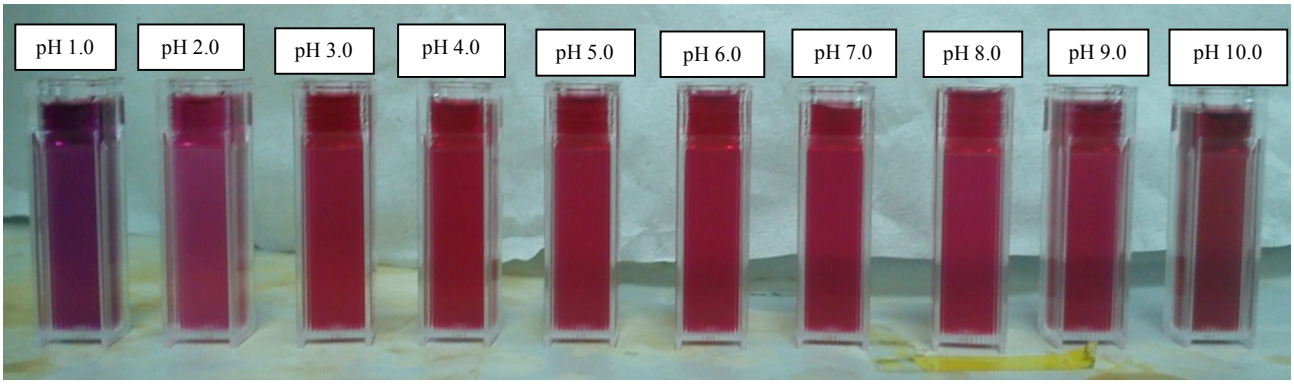

Figure 2: Colour of dye from pH 1.0 to 10.0 (left to right) with SLR 0.25

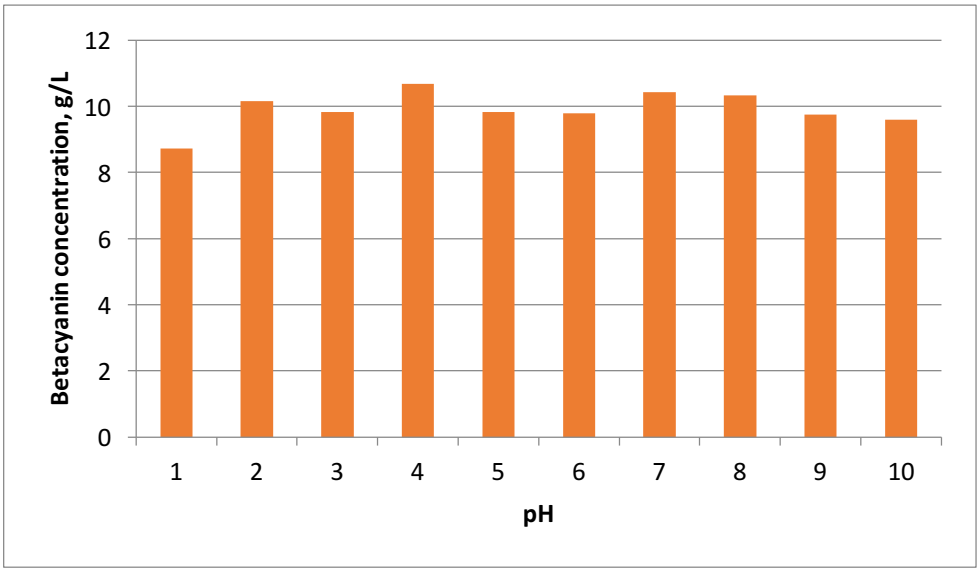

Figure 3: Effect of pH on the betacyanin concentration with SLR 0.25

5. CONCLUSION

The results revealed that betacyanin can extracted from Bougainvillea bract is an alternative source to red natural dye for textiles. This study revealed that the extraction of betacyanin was performed using solid-liquid extraction would produced high concentration of betacyanin dye at acidic condition pH 4. The dye was showing red colour at pH 4 with among the high absorbance reading, 2.92 with concentration of 10.67 g/L. The results clearly suggest that the benefit of this technique include, environmental friendly, low cost process, satisfactory colour obtained in both quantity and quality.

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