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Effect of various factors on extraction efficiency of total anthocyanins from Butterfly pea (*Clitoria ternatea L.* Flowers) in Southern Vietnam

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Abstract. Nowadays, anthocyanins have become an interesting topic of much scientific research and act as potential sources of eco-friendly natural food dyes and their helpful effects on human health.. The purpose of the present article was to apply conventional extraction methods of pigments from Butterfly pea (Clitoria ternatea L.Flowers) in Southern Vietnam were tested to extract anthocyanins. The effects of various factors, including the solvent concentration, extraction time, temperature, and solvent/material ratio on the extraction total anthocyanins from Butterfly pea were investigated. In this study, the concentration of monomeric anthocyanins was completed by the spectrophotometric pH differential method.The highest recovered anthocyanin level was achieved with 50 % ethanol (143.49 mg/L), while the lowest one was obtained with water (90.9 mg/L). The extraction yield was significantly affected by temperature and the excellent condition was 60 ℃. The extraction yield raised with increasing of solvent/material ratio until equilibrium has arrived at the optimal ratio of 25:1 (mL/g). The extraction yield increased first and then decreased with an extension in time values.

1. Introduction

Nowadays, Herbs become more and more popular in many topic of scientific researches as natural products due to their chemical composition, following with two main reasons: applications as natural options for food ingredients and helpful influences on human health based on their antioxidant characteristics [1-6]. Anthocyanins play a vital function on pharmacological against difference human diseases, such as allergies, cancer, cardiovascular disease and so on. [7,8]. Extraction is the first major step in the purification and recovery of active ingredients from plant materials. the purpose of an extraction method in order to provide for the maximum yield which consists of the high concentration of target compounds.

There are many developed techniques which applying to extract anthocyanins, such as microwave-assisted, conventional solvent extraction, supercritical fluid extraction and ultrasound-assisted, however, the solvent extraction technique (liquid-liquid and solid-liquid extraction techniques) is the most generally applied, and has demonstrated to be an efficient and reliable method [9-11]. The difference extraction conditions affect on extraction yields of anthocyanins from plant tissues. The efficacy of solvent extraction depended on many factors such as solvent concentration, temperature, time, pH and so on. Each single factor analyses can provide vital information on the varieties for major extraction parameters on the extraction of anthocyanins compounds from plant materials [12,13]. Clitoria ternatea belong to the family Fabaceae and sub-family Papilionaceae, also known as Butterfly pea.,. Butterfly Pea flower contain high amount of anthocyanins compared to Roselle and dragon fruit [14]. The anthocyanins base found in this flower is delphinidinas. Anthocyanins found in natural sources were useful to food colorant industry when it could give vibrant colors to the food product [15]. Clitoria ternatea (L.) native come from tropical Asia acts as a perennial, twining herbal medicinal plant, has a long history of applying as anxiolytic agent and a memory enhancer. In considering the increasing interest in estimating the antioxidant potential of herbal medicine. Therefore, this research was proposed to determine the best conditions including solvent concentration, type of solvent, liquid-to-solid ratio, extraction time and temperature in order to maximize the yield of total anthocyanins level from Butterfly pea by individual factor experiments.This research contributes a chance to evaluate the potential of Butterfly pea (Clitoria ternatea L. Flowers) in Southern Vietnam as an available source of natural antioxidants and a potential supplement in the pharmaceutical and food industry.

2. Material and methods
Dried petals of butterfly pea were ground using a commercial grinder and were weighed to 10g, put in the two neck round bottom flask and was extracted by Ethanol 50° solutions. Ethanol (C_2H_5OH) is purchased from Sigma Aldrich (US). For extraction parameter study, 10g of butterfly pea powder was placed in the two neck round bottom flask as and was extracted by ethanol with concentration at 50. The liquid/solid ratio in this experiment ranges from 10:1 to 30:1 (mL/g) and. The extraction temperature is adjusted from 40 to 80 (^{o}C) and time varies from 15to 75 (min). Then, centrifugation took place at 4000 rpm for 15 min by high speed centrifuge Model LACE16 (from COLO lab expert). The supernatant was collected and the extract, after being filtered with filter paper, was transferred into plastic bottle for yield estimation. The pH scanning of supernatant ranges from 400 nm to 700 nm. Monomeric anthocyanin was calculated as cyanidin-3-glycoside using a pH differential method [16].

3. Result and discussion

Figure 1. The process of extraction anthocyanins from Butterfly pea (Clitoria ternatea L.Flowers)
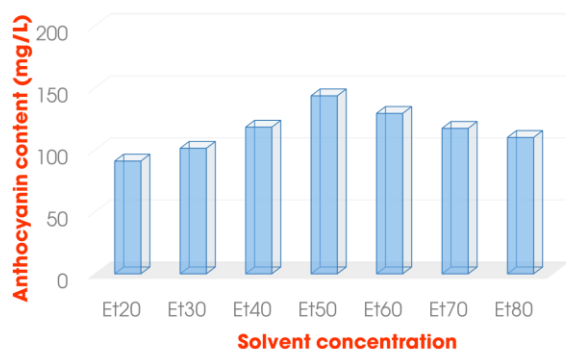
Figure 2. Effect of solvent concentration on anthocyanin content from Butterfly pea
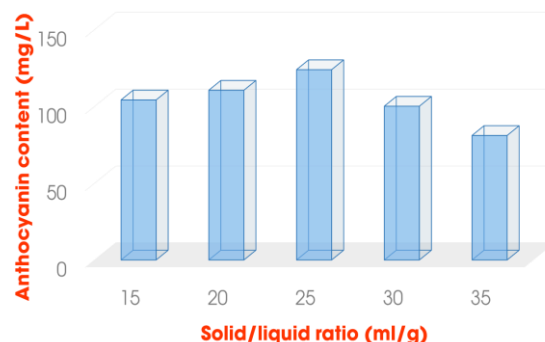

Figure 3. Effect of liquid-solid ratio on anthocyanin content from Butterfly pea
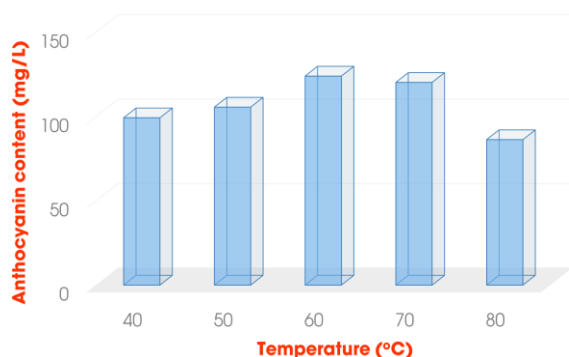

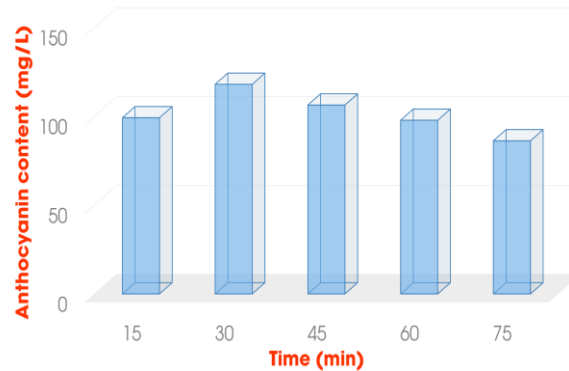**Figure 4.** Effect of temperature on anthocyanin content from Butterfly pea

Figure 5. Effect of time on anthocyanin content from Butterfly pea

3.1. Effect of solvent concentration on anthocyanin content

Solvent extraction plays an important common method of extraction of anthocyanins compounds. In overall, the larger the dielectric loss and dielectric constant, the higher is the ability of the disseminate microwave energy and solvent to absorb.. Anthocyanins, which have hydrophobic hydrocarbons, are only slightly soluble in organic solvents, but have functional polyphenols well soluble in polar solvent. Therefore, to extract anthocyanin, organic solvent or polarizer (usually water) are suitable solvent systems. Alike Figure 2, the anthocyanin content increased steadily from 90.9 mg/L to critical point at 143.49 mg/L when exchange sequence solvent concentration. Therefore, we selected ethanol/water with the ratio of 50:50 as the solvent used in the extraction for this investigation.

3.2. Effect of liquid-solid ratio on anthocyanin content

In many cases, a large volume of solvent is also used for extraction and recuperation of extracts, which can be a major problem in terms of environmental considerations. Figure 3 demonstrated the effect of liquid-solid ratio on anthocyanin content. By increasing the liquid-solid ratio from 15:1 to 25:1, the extracted anthocyanin content increased from 103.526 mg/L to 121.58 mg/L. However, from 30:1 to 35:1 of liquid-solid ratio, the anthocyanin content decreased sharply. This can be explained as follows. When the ratio of materials/solvent is insufficient to fill up the material, the hypertonic environment could not be created, detaining the color in the vacuole of the material. However, when the solvent/material ratio reaches a certain value (depending on the characteristics of the material), the cell, owing to its capability to rapidly absorb water, swells to the maximum and bursts out simultaneously, releasing the color within the vacuole. Therefore, based on the graph we choose the liquid/solid ratio of 25/1.

3.3. Effect of temperature on anthocyanin content
High extraction temperature generally also leads to increased solubility. This can be demonstrated by the fact that higher temperature leads to reduced intermolecular interactions within the solvent, giving increase to higher molecular motion, and rose solubility of the target compounds in the extraction solvent. The increasing temperature can also cause destruction of cell matrix, resulting in increased availability of components for extraction. Moreover, at higher temperature levels, solvent viscosity decreases, increasing the solubility, and thus it also increases the efficiency of extraction. But in some cases, it has been remarked that the extraction efficiency rises with the increase in temperature up to a certain temperature level and then the extraction yield begins falling with the further increase in temperature levelThis also differences with the type of target compounds to be extracted as the temperature of degradation for every compound. As showed in Fig. 4, the anthocyanin content in the fluid as well as the effect of color separation from the material increased with increasing temperature and reached the highest level at the temperature of 60°C. However, from 60 to 70°C, the anthocyanin content in the extract did not change significantly and rising temperature past 70°C caused the content to diminish due to decomposition. Therefore, in practice, based on the combined effects of the good extraction yield of anthocyanins, an extraction temperature of 60 ℃ could be applied.

3.4. Effect of time on anthocyanin content
As shown in Figure 5, the level of anthocyanin in the fluid tends to decrease with prolonged extraction time. The extraction yield of anthocyanin from Butterfly pea raised as the time values rose from 15 to 30 minute, but it declined as the time values higher than 45 minutes were used, which showed that the extraction time value significantly influenced the extraction yield associated with other factors. Anthocyanin content was highest at 30 minutes, but as the time increased past 45 minutes, the content decreases markedly due to anticyclonic decomposition caused by the long exposure to high temperature. Therefore, the appropriate time to extract anthocyanin is 30 minutes.

4. Conclusion
In this study, the single factor experiments were determined for recognizing the optimum condition of each independent variable influencing the total anthocyanins content extraction efficiency of Butterfly pea (Clitoria ternatea L. Flowers), namely liquid-solid ratio, the solvent concentration, extraction time and temperature.Commonly, high extraction yield was achieved using aqueous acetone (about 50%) as the solvent, the liquid/solid ratio of 25/1 and the extraction yield could further be improved using a prolonged time of 30 min and a higher incubation temperature of 60 ℃. Under these optimized conditions, the experimental maximum yield of total anthocyanins content of 143.49 mg/L was taken. The optimal ranges (minimum and maximum values) acquired for all extraction parameters in the present research can also be served as fundamental information for the scale-up extraction of antioxidant compounds from Butterfly pea.

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