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Comparison of Anthocyanin and Phenolic Contents between Tuber and Callus of *Ipomoea batatas* (L.)

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ABSTRACT

This study was aimed to investigate and compare the anthocyanin and phenolic contents between the tuber and callus of *Ipomoea batatas* (L.). Callus induction was performed *in vitro* using the Murashige and Skoog media supplemented with 0.5 mg/L 2,4-D and 0.1 mg/L kinetin. Meanwhile, the anthocyanin content was measured by pH differential method using cyanidin-3-glucoside as a standard. The total phenolic content was measured using the Folin-Ciocalteu method with gallic acid as a standard. Based on the results obtained, the anthocyanin content of the tuber *I. batatas* was 1.04 ± 0.12 mg cyanidin-3-glucoside/g fresh weight, while the anthocyanin content of the callus was 0.50 ± 0.07 mg cyanidin-3-glucoside/g fresh weight. The total phenolic content of the tuber *I. batatas* was 0.46 ± 0.01 mg gallic acid/g fresh weight and the phenolic content of the callus was 0.20 ± 0.01 mg gallic acid/g fresh weight. Both the anthocyanin and phenolic contents of the tuber were found to be higher than those of the callus of *I. batatas*.

Keywords: *Ipomoea batatas* (L.), tuber culture, callus, determination of anthocyanin, phenolic contents

LIST OF ABBREVIATIONS

MS : Murashige and Skoog
2,4-D : 2,4-dichlorophenoxy acetic acid

INTRODUCTION

Plant tissue culture is a process where cells, tissues or organs of a plant species are isolated, surface sterilized and cultured in an aseptic environment (Rout *et al*., 2006). Tissue culture techniques are capable to be used in minimizing the time needed for propagation of new plantlets and to increase the availability of plants with improved horticultural characteristics. Meanwhile, plant tissue cultures can be used for mutant selection, gene transfer, artificial seed and secondary metabolite production. Callus is undifferentiated cell that emerges from structural tissues in response to the wounding effect. Besides, callus induction and physical disorganization of cells happen when there is a breakdown of intercellular physical and chemical communication. Therefore, callus can be induced by changing the composition

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of media and the concentration of plant growth regulators (Dennis & Sreejesh, 2004).

Ipomoea batatas (L.) belongs to the family *Convolvulaceae* and it is widely grown in the tropical, sub-tropical and warm temperature regions. Jayasinghe *et al*. (2003) reported that *I. batatas* was able to sustain the populations during time of crisis even after the Second World War in Japan, the earthquake in Northern Luzon and the civil disturbances in Rwanda. *I. batatas* is also rich in dietary fibre, minerals, vitamins, and antioxidants, such as phenolic acids, anthocyanin, tocopherol and β-carotene. In addition, *I. batatas* contains β-carotene and vitamin C and these two components are able to eliminate free radicals from the human body. The tuber of this plant is also recognized as an anti-inflammatory food because it can reduce the severity of asthma, rheumatoid arthritis and osteoarthritis. Besides, anthocyanin, calcium and dietary fibre of sweet potatoes can reduce cardiovascular diseases (Huang *et al*., 2007).

Secondary metabolites are compounds produced by organisms which are not directly needed for the cell's survival but required for the plant's survival in the ecosystem. Plants produce secondary metabolites for protection against bacteria, fungi and viruses (Kliebenstein *et al*., 2005). Anthocyanin was known to be non-toxic and non-mutagenic. Previous research revealed that anthocyanin exhibited anti-inflammatory, anti-proliferative, vasoprotective and hepatoprotective activities (Wang *et al*., 2000; Lazzé *et al*., 2003). Phenolic compounds are able to protect cells, food and organs from oxidative degeneration. In human diets, phenolic compounds can prevent diseases, such as cancer, cardiovascular and neurodegenerative diseases (Manach *et al*., 2004). This study was aimed at comparing the anthoycanin and phenolic contents in both the tuber and the callus of *I. batatas*, in order to determine if *in vitro* culture would result in a reduction of the total phenolics and anthocyanins.

MATERIALS AND METHODS

In vitro Callus Induction

Purple sweet potato, *Ipomoea batatas* was obtained from the central market of Kepong, Selangor. The tuber of *I. batatas* was cut into 1 cm × 1 cm × 2 mm size and used as explant materials. The surface-sterilized explants were cultured on the MS medium (Murashige & Skoog, 1962) supplemented with 0.5 mg/L 2,4-D and 0.1 mg/L kinetin for callus induction.

Anthocyanin Content

For the extraction of anthocyanin, approximately 2 g of homogenized tuber *I. batatas* and 2 g of callus were separately put into 20 mL of methanol, 1% (v/v) hydrochloric acid for 1 hour in dark condition under room temperature. The extracts formed were filtered through Whatman No.1 filter paper. About 1 mL of the extract was used for anthocyanin determination. In addition, the pH differential method was used to measure the anthocyanin content in which the extract was diluted in buffers at pH 1.0 (0.025 M potassium chloride buffer) and at pH 4.5 (0.4 M sodium acetate buffer). The absorbance of the extract was then measured at 510 nm and 700 nm. The absorbance of the extract was calculated using the formula stated below:

$$A = (A510 - A700)_{pH\ 1.0} - (A510 - A700)_{pH\ 4.5}$$

The total anthocyanin content (TA) was calculated as follows:

$$TA = (A \times MW \times DF \times 1000)/(\varepsilon \times 1)$$

The result was calculated as milligram of cyanidin-3-glucoside per gram of fresh weight using a molar absorptivity (ε) of 26,900 and a molecular weight (MW) of 449.2, where DF is the dilution factor.

Total Phenolic Content

For the total phenolic content, about 10 g of the homogenized tuber *I. batatas* and 10 g of

the callus were separately put into 100 mL of methanol for 1 hour, followed by filtration through Whatman No. 1 filter paper. The Folin-Ciocalteu method (Vasco *et al*., 2008) was used to measure the total phenolic content with a standard curve prepared using gallic acid at the concentrations of 20, 40, 60, 80 and 100 mg/L. Meanwhile, a reagent blank was prepared using distilled water. About 1 mL of the extract and the standard solutions were added to a beaker containing 9 mL of distilled water. Approximately 1 mL of Folin-Ciocalteu reagent was added to the extract and standard solutions. After 5 minutes, 10 mL of 7% Na_2CO_3 solution was added and mixed. The solution was then immediately diluted to 25 mL with distilled water and mixed thoroughly. The mixtures were incubated for 90 minutes at 23ºC and finally the absorbance was measured at 750 nm. The result was expressed as milligram of gallic acid per gram of fresh weight.

RESULTS AND DISCUSSION

Anthocyanin Content

The absorbance readings of the anthocyanin content of tuber and callus of *Ipomoea batatas* were repeated three times, while the average readings were taken at the wavelength of 510 nm and 700 nm (see Table 1). Based on the pH differential method (Hosseinian *et al*., 2008), the anthocyanin content for the tuber of *I. batatas* was 1.04 ± 0.12 mg cyanidin-3-glucoside/g fresh weight and the anthocyanin content for the callus of *I. batatas* was 0.50 ± 0.07 mg cyanidin-3-glucoside/g fresh weight. The anthocyanin content of the tuber was found to be higher than the callus of *I. batatas*. One of the reasons for the decline in the content of anthocyanins could be the limitations of the physical conditions adopted *in vitro*, as reported by Meyer *et al*. (2002) for the culture of oheloberry (*Vaccinium pahalae*). However, the results obtained contradict with the previous findings in terms of the ranges of higher plant species including wild carrot (*Daucus carota*), parsley (*Petroselinum crispum*) and parsnip (*Pastinaca sativa*), as reviewed by Bourgaud *et al*. (2001) and Matkowski (2008). They reported that the application of tissue culture approach had significantly enhanced the metabolites production of the plant.

Meanwhile, anthocyanin contributes to the purple colour of the *I. batatas* (Borbalan *et al*., 2003). The absorbance readings of the tuber and callus of *I. batatas* were measured at 510 nm, based on the molar absorption coefficient of the cyanidin-3-glucoside. A previous report showed that the content of anthocyanin in sweet potato clones ranged from 0.017 to 0.531 mg/g fresh weight (Teow *et al*., 2007). At low pH values, however, anthocyanin is more

TABLE 1
Absorbance reading for the anthocyanin contents of the tuber and callus of *Ipomoea batatas* (L.)

Purple Sweet Potato	pH Value	Wavelength (nm)	Absorbance (nm)
Tuber	pH 1.0	510	0.514 ± 0.004
		700	0.123 ± 0.003
	pH 4.5	510	0.484 ± 0.006
		700	0.118 ± 0.006
Callus	pH 1.0	510	0.128 ± 0.002
		700	0.044 ± 0.004
	pH 4.5	510	0.123 ± 0.012
		700	0.051 ± 0.004

stable and highly coloured while at higher pH values, anthocyanin gradually loses its colour and becomes colourless between pH 4.0 and 5.0 (Ferreira *et al*., 2007). The pH differential method is used based on the structural change of the anthocyanin chromophores between pH 1.0 and 4.5. In this method, the difference in the absorbance between the potassium chloride buffer and sodium acetate buffer is caused by the monomeric anthocyanin pigments. Meanwhile, monomeric anthocyanin has little or no absorbance in pH 4.5 buffer, whereas polymeric anthocyanin does not exhibit colour changes with pH (Lee *et al*., 2005).

Total Phenolic Content

The standard curve for gallic acid was plotted (see *Fig. 1*), while the average absorbance readings of the total phenolic contents of tuber and callus of *I. batatas* were taken at the wavelength of 750 nm (Table 2). Based on the standard curve, the total phenolic content of the tuber of *I. batatas* was 0.46 ± 0.01 mg gallic acid/g fresh weight and the phenolic content for callus was 0.20 ± 0.01 mg gallic acid/g fresh weight. The total phenolic content for the tuber of *I. batatas* was higher than the callus culture and the difference was about 0.26 mg. Once again, the results obtained contradict with the previous findings by Bourgaud *et al*. (2001) and Matkowski (2008) on various plant species, as discussed above.

TABLE 2
Absorbance reading for the total phenolic contents of the tuber and callus of *Ipomoea batatas* (L.)

Purple Sweet Potato	Absorbance (750nm)
Tuber	0.255 ± 0.006
Callus	0.113 ± 0.007

Phenolic compounds are located in different parts of plant's tissues and cells, such as vacuoles, cell walls and cell nuclei. In particular, phenolics also protect plants from biotic stresses, such as fungi and parasitic plant invasion. Genetic factors and growing conditions might play an important role in the formation of secondary metabolites in plants, such as anthocyanin and

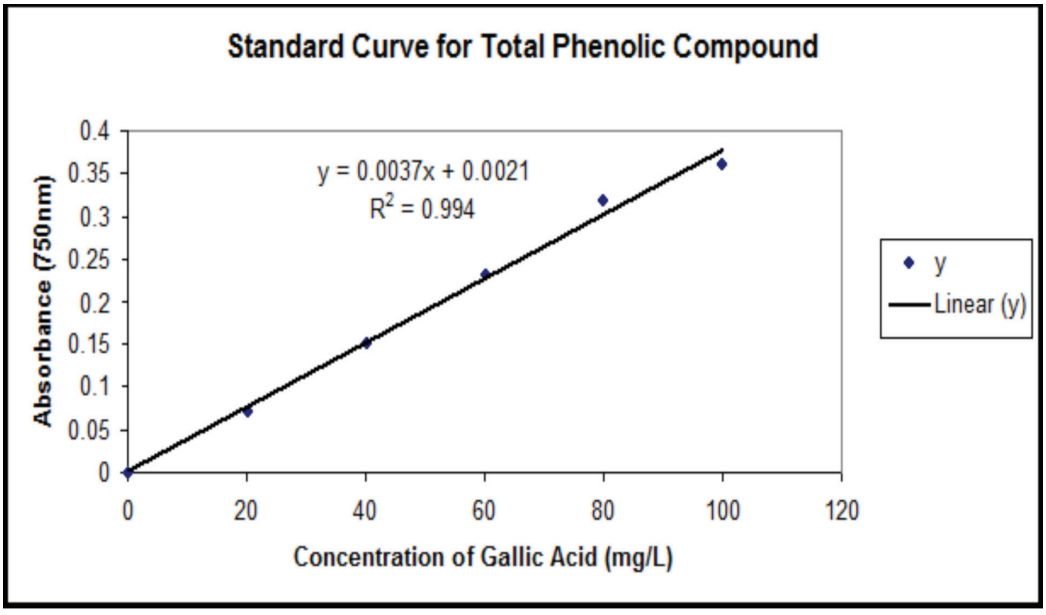

Fig. 1: Standard curve for the total phenolic compound

phenolic compounds. However, the results of the total phenolic content obtained might be affected by other non-phenolic compounds such as ascorbic acid and reducing sugar (Escarpa & Gonzalez, 2001). In the Folin-Ciocalteu method, methanol, acetone, ethanol and boiling water could be used to extract phenolic compounds from plants. However, methanol and acetone were found to be more effective than water for the extraction of phenolic compounds from plants (Yao *et al*., 2004; Zhou & Yu, 2004). Reyes *et al*. (2004) pointed out that the weight of the tuber sweet potato increased with the decrease of its total phenolic content. Meanwhile, fresh cut in the sweet potato would result in an increase in the phenolic compounds and those phenolic compounds would act as a defence against pathogen after the tissue damage (Báidez *et al*., 2006). The total phenolic content varied among different sweet potato cultivars and these differences ranged from 192.7 to 1159.0 mg gallic acid equivalent/100 g dry sample between the cultivars grown in the Philippines (Rumbaoa *et al*., 2009).

CONCLUSION

The anthocyanin content of the tuber of *Ipomoea batatas* was 1.04 ± 0.12 mg/g fresh weight, while the anthocyanin content of the callus was 0.50 ± 0.07 mg/g fresh weight. The total phenolic content of the tuber was 0.46 ± 0.01 mg/g fresh weight and this was 0.20 ± 0.01 mg/g fresh weight for the callus. The results indicated that *in vitro* conditions could have significant impacts on the production of phenolics and anthocyanins. This could be attributed to the interaction of environmental parameters under field conditions, which eventually influenced and induced important metabolic pathways. Although both the anthocyanin and total phenolic contents of the tuber were higher compared to the callus of the *I. batatas*, *in vitro* culture and mass propagation of *I. batatas* continued to serve as an alternative source of secondary metabolite production.

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