Glucosinolates Content in Non-elicited Plant Culture, Elicited Plant Culture and Wild Plant of Watercress (*Nasturtium officinale*)

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ABSTRACT Watercress (*Nasturtium officinale*), a green vegetable belongs to the Brassicaceae, contains considerable amount of vitamins, minerals and secondary metabolites such as glucosinolates (GS). Watercress contains phenyl ethyl glucosinolate (gluconasturtiin), a precursor of phenyl ethyl isothiocyanate, which is widely reported to restrain the growth of cancer cells. The content of secondary metabolites and other compounds in plants is affected by different growth conditions such as pH, temperature, light intensity and nutrient supply. Thus, the aim of the current study is to evaluate the concentration of gluconasturtiin (and other GS) from *in vitro* grown watercress under non-elicited and elicited plant culture with wild plant of *N. officinale*. The samples were collected from watercress growing wild in a spring in Kundasang area, Ranau Sabah and subjected to sterilization to establish *N. officinale in vitro* culture under laboratory condition. The sterilization was done by using Chlorox® solution (5% v/v) containing Tween 20 to obtain 90% survival rate of the plants. Explants were grown in glass jar containing hormone-free with 30 g/L of sucrose and pH was adjusted to 5.7 - 5.8. The medium was solidified with 4 g/L^{-1} of agar and sterilized at 121°C for 20 minutes. All cultures were kept inside growth chamber at 25°C under 16 hours photoperiod for 30 days before sub-cultured into fresh medium treated with elicitors. Different concentration of elicitors tested in this study were chitosan (10, 20, 40, 60, 100 mg/mL), casein hydrolysate (0.5, 1.0, 1.5, 2.0 g/L) and coconut water (5, 10, 15, 20, 25 % v/v). The results showed that gluconasturtiin and benzyl glucosinolate (glucotropaeolin) increased over five-fold and six-fold, respectively, in non-elicited plant culture compared to the wild *N. officinale*. Compared to the non-elicited, the concentrations of these GS were significantly lower by 52 – 76 % for gluconasturtiin and 33 – 55 % for glucotropaeolin in all the *in vitro N. officinale* treated with the elicitors. Nonetheless, the GS concentrations in all the *in vitro N. officinale* were higher compared to the matured wild plant. Tissue culture method could be a valuable alternative for higher production of GS in *N. officinale* with short period of plant development (30 days in this study).

KEYWORDS: *Nasturtium officinale*; *in vitro*, glucosinolates, gluconasturtiin, glucotropaeolin

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INTRODUCTION

Gluconasturtiin (PEGSL), a major GS in watercress, produces phenyl ethyl isothiocyanate (PEITC) upon hydrolysis (Aripin & Surugau, 2016). Many previous studies revealed that PEITC restrain the growth of cancer cell (Powolny *et al*., 2011), possess a strong chemopreventive effect on colorectal cancers in mice model (Khor *et al*, 2008) and as antitumor compound for oral cancer therapy (Chen *et al* 2012). However, despite the promising benefits of PEITC, the production of GS in wild growing watercress is limited to ecological conditions and nutrient supply yet advancement in research allows us to exploit cell, tissue, organs or entire organism through *in vitro* method to enhance the production of secondary metabolites or specific compounds (Rao & Ravishankar, 2002). Treatment using elicitors such as salicyclic and methyl jasmonate in turnip (*B. rapa ssp. rapifera*) were reported to increase aromatic gluconasturtiin and indole GS in secondary roots and exudates (Smetanska *et al*., 2007). Therefore, the aim of this study is to evaluate the concentration of GS in *in vitro* grown watercress under various growth media. Similar studies on the wild watercress were also carried out for comparison.

METHODOLOGY

Plant Materials and Explants Preparation

The samples were collected from watercress (*N. officinale*) growing wild in a spring in Kundasang area, Ranau Sabah. The young healthy shoots were cut about 1 - 1.5 cm and used as explants. The explants were cleaned under running tap water for 5 minutes before sterilized for 15 minutes in 15 % of NaOCl solution containing Tween 20. Finally they were rinsed three times with sterile distilled water before soaked into sterile distilled water containing plant preservative mixture (PPM).

Culture Medium and Incubation Conditions

Explants were grown in glass jars containing hormone-free Murashige and Skoog (Murashige & Skoog, 1962) with 30 g/L of sucrose and the pH was adjusted to 5.7 - 5.8. The medium was solidified with 4 g/L of agar and sterilized at 121°C for 20 minutes. All cultures were kept in a growth chamber at 25°C under 16 hours photoperiod for 30 days before sub-cultured into fresh medium treated with elicitor. Both the non-elicited (control) and elicited plant cultures were grown for 30 days before harvested. Elicitors tested with different concentration in this study were chitosan (10, 20, 40, 60, 100 mg/mL), casein hydrolysate (0.5, 1.0, 1.5, 2.0 g/L) and coconut water (5, 10, 15, 20, 25 % v/v).

Chemical Analysis

The method for extraction of GS was adopted from Rossetto *et al.* (2013) with modification. About 1.00 g of the fresh samples was homogenized in a porcelain mortar containing 5 ml of 70 % of methanol. The extract was transferred into conical flask and placed into water bath for 30 minutes at 70°C. The crude extracts were then centrifuged at 8000 ×g for 20 minutes. The supernatants was collected prior to HPLC analysis. All samples were prepared in triplicates.

HPLC Conditions

The HPLC conditions were in accordance to method reported by Aires *et al.* (2013) with slight modifications. The GS in extracts of *N. officinale* were analyzed using reversed-phase chromatography at 30°C with flow rate of 1 ml/min for 10 minutes. Isocratic elution was performed with mobile phase consisting of 0.1 % trifluoroacetic acid (TFA) aqueous solution and 0.1 % TFA acetonitrile. The injection volume was 10 µl and the UV detection was at 229 nm.

RESULTS AND DISCUSSION

Effects of Elicitors on the GS Content of in vitro N. officinale Culture

The effects of the chitosan, casein hydrolysate and coconut water on the *in vitro* watercress were evaluated in terms of gluconasturtiin and glucotropaeolin contents. Each treatment was replicated thrice and the values are mean ± standard deviation as shown in Table 1. A control was prepared i.e. without the addition of elicitor and wild plants were harvested at matured stage.

Table 1 shows that gluconasturtiin and glucotropaeolin increased over five-fold and six-fold, respectively, in non-elicited plant culture compared to wild plant. Kopsell *et al.* (2007) reported that gluconasturtiin content in watercress is affected by nitrogen and sulphur treatments. *In vitro* plant culture obtained nitrogen and sulphur from MS medium but nutrient supply are limited to wild plants. The concentration of the gluconasturtiin was reduced to 0.680 ± 0.030 mg/g FW and 0.670 ± 0.002 mg/g FW when it was treated with casein hydrolysate and coconut water, respectively. Gansau *et al.* (2016) have reported that addition of coconut water increased protocorms proliferation in

Dendrobium lowii, however they did not studied its effect on the secondary metabolites in the orchid. Glucotropaeolin concentrations from *in vitro* plant elicited with casein hdrolysate are higher compared to other elicitors in this study. However, elicitation using chitosan reduced the production of gluconasturtiin to 0.370 ± 0.004 mg/g FW. Elicitors were used to imitate the effect of stresses to activate the biological system in plant to increase the production of secondary metabolites. However, for the elicitor concentration range used in this current study, the GS contents in all the elicited plant culture were lower than the non-elicited. Nevertheless, all the *in vitro* grown watercress in this study showed higher concentrations of gluconasturtiin and glucotropaeolin compared to the matured wild plants.

Table 1. Glucosinolates content of *in vitro* plant cultures (non-elicited and elicited) in comparison with wild *N. officinale.*

Treatments	Concentration	Glucosinolates content (mg/g FW)	
		Gluconasturtiin	glucotropaeolin
Wild plant	-	0.240 ± 0.040	0.080 ± 0.020
Control (non-elicited)	-	1.404 ± 0.030	0.490 ± 0.020
Chitosan (mg/L)	10	0.370 ± 0.002	0.302 ± 0.001
	20	0.404 ± 0.003	0.240 ± 0.002
	40	0.330 ± 0.002	0.250 ± 0.005
	60	0.330 ± 0.004	0.240 ± 0.003
	100	0.370 ± 0.004	0.220 ± 0.004
Casein hydrolysate (g/L)	0.5	0.680 ± 0.030	0.330 ± 0.005
	1.0	0.620 ± 0.080	0.330 ± 0.010
	1.5	0.650 ± 0.010	0.330 ± 0.005
	2.0	0.504 ± 0.010	0.310 ± 0.003
Coconut water (%, v/v)	5	0.602 ± 0.004	0.270 ± 0.005
	10	0.560 ± 0.100	0.240 ± 0.003
	15	0.630 ± 0.030	0.260 ± 0.003
	20	0.670 ± 0.002	0.250 ± 0.004
	25	0.670 ± 0.003	0.250 ± 0.002

The *in vitro N. officinale* grown under long photoperiod (16 hours) resulted in high concentration of GS compared to the matured wild plant. These findings are in agreement with previous report by Engelen-Eigles *et al*., (2006) where *N. officinale* grown under 16 hours photoperiod showed high concentration of gluconasturtiin compared to short photoperiod (8 hours) grown plant. The plant was harvested at 10 days interval from 20 days to 60 days. The different stages of plant growth are shown in Figure 1 (a – e). Plant development such as height and weight were tabulated in Table 2. Higher concentration of GS were found in 30 days grown *in vitro* plant as shown in Figure 2. The growth of plant does not stopped at 30 days but the GS content decreased after 30 days. Thus, all *in vitro* plants were harvested at 30 days.

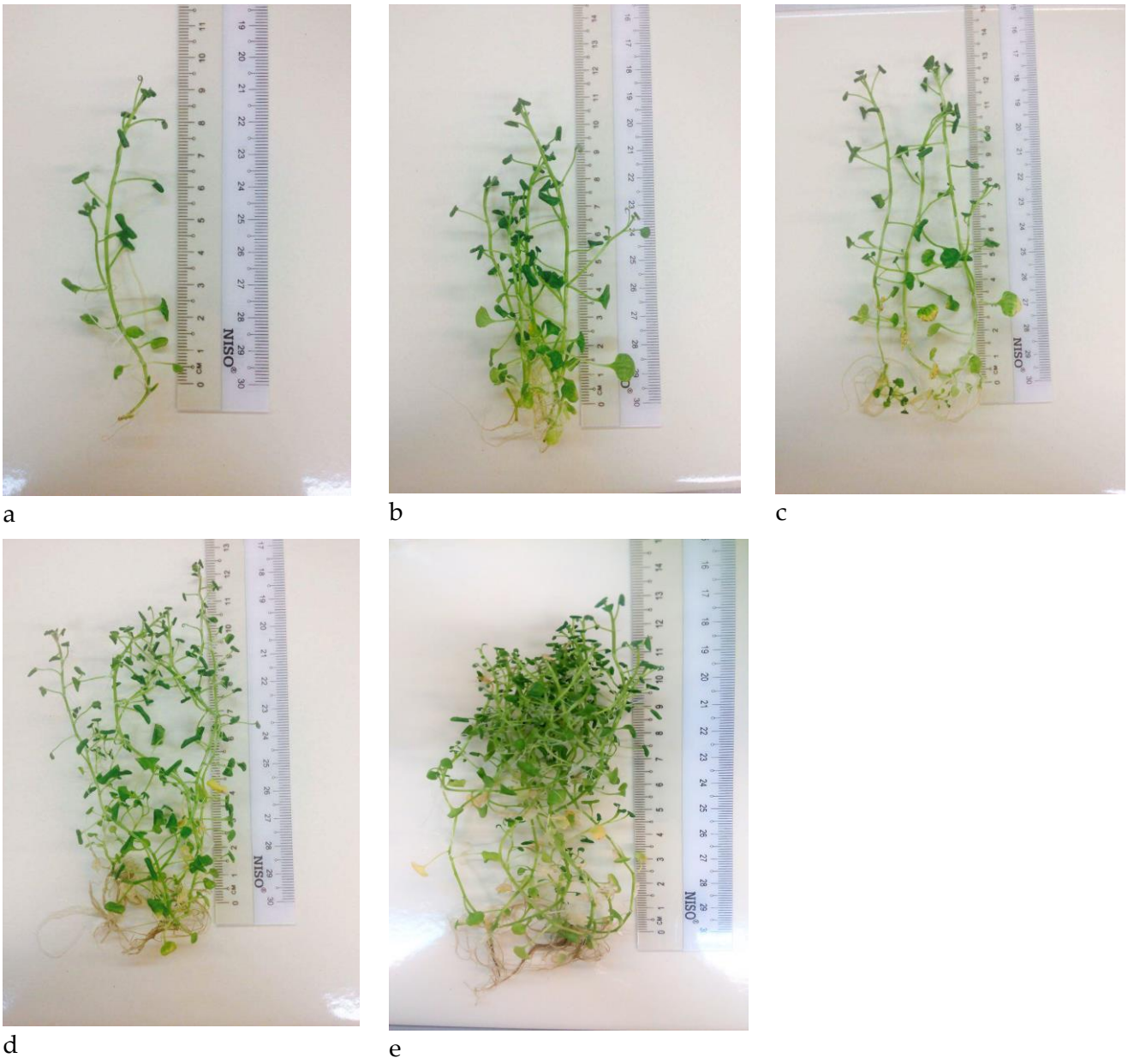

Figure 1. The *in vitro* culture of non-elicited *N. officinale* at different stage of harvest: 20 days (a), 30 days (b), 40 days (c), 50 days (d), 60 days (e).

Table 2: Plant development of *in vitro* culture of *N. officinale.* All results are in average of three replicates and values are mean ± standard deviation.

Days	Plant development	
	Height (cm)	Weight (g)
20	9.77 ± 0.25	0.69 ± 0.02
30	11.30 ± 0.36	1.02 ± 0.10
40	12.80 ± 0.67	1.13 ± 0.07
50	13.10 ± 0.56	1.21 ± 0.04
60	13.40 ± 0.41	1.40 ± 0.05

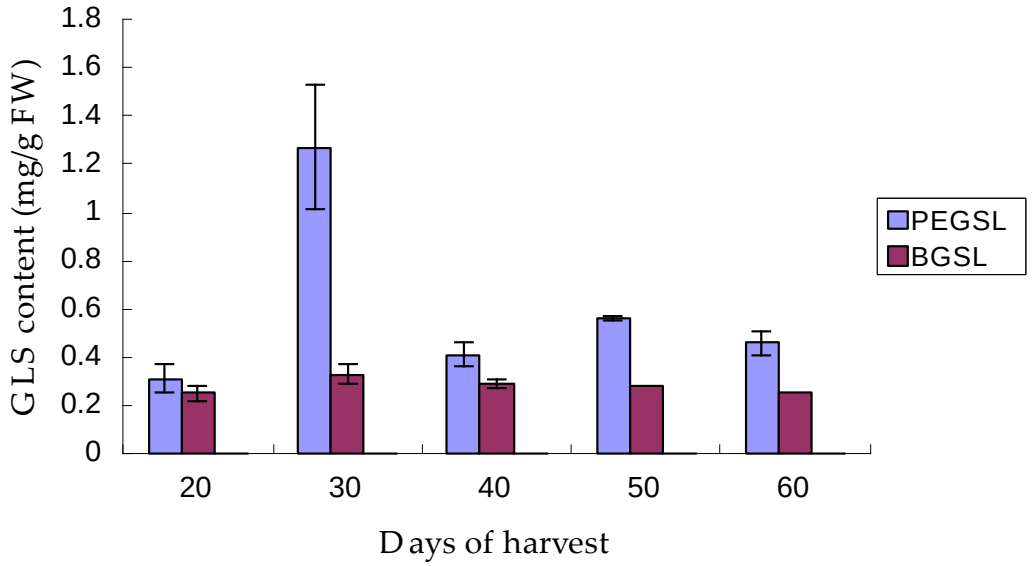

Figure 2. Glucosinolates content of *in vitro N. officinale* at different stages of harvest. All results are in average of three replicates.

CONCLUSION

In summary, a 30 days *in vitro* grown watercress showed much higher GS compared to the matured wild plant. Addition of additives (elicitors) namely chitosan, casein hydrolysate and coconut water have all increased the GS compared to the wild plants. It is shown here that tissue culture method could be a valuable alternative for higher production of GS in *N. officinale* with a short period of plant development (30 days in this study). In addition, the plants produced are healthier because they are not exposed to the common environmental pollutants such as heavy metals and agrochemicals.

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