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Abstract

The experiments were purposed to investigate the effect of chitosan and yeast extract on Stemona alkaloid production in Stemona sp. culture. Both elicitors enhanced Stemona alkaloid production over the control. Treatment with chitosan at a concentration of 25 mg/L for 1 week resulted in the highest production of Stemona alkaloids. It was found that 1', 2'-didehydrostemofoline and stemofoline production was 2.65 fold and 2.95 fold higher than the control, respectively.

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Response of *Stemona alkaloid* Production in *Stemona* sp. to Chitosan and Yeast Extract Elicitors

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Abstract: The experiments were purposed to investigate the effect of chitosan and yeast extract on *Stemona alkaloid* production in *Stemona* sp. culture. Both elicitors enhanced *Stemona alkaloid* production over the control. Treatment with chitosan at a concentration of 25 mg/L for 1 week resulted in the highest production of *Stemona alkaloids*. It was found that 1', 2'-didehydrostemofoline and stemofoline production was 2.65 fold and 2.95 fold higher than the control, respectively.

Keywords: 1', 2'-didehydrostemofoline, chitosan, stemofoline, *Stemona* sp., yeast extract

INTRODUCTION

Stemona alkaloids are valuable secondary metabolites produced from *Stemona* spp. The pure alkaloids and extracts derived from the leaves and roots of *Stemona* species have been shown to have insect toxicity (Tang *et al*., 2007), antioxidant (Brem *et al*., 2004) and antitussive activities (Lin *et al*., 2008). Many *Stemona* alkaloids from *Stemona* species in Thailand have been reported, such as stemocurtisine, stemocurtisinol, oxyprotostemonine and stemokerrin (Pyne *et al*., 2007). An unidentified *Stemona* sp. was collected from Mae Moh District, Lampang, Thailand (This voucher specimen was deposited at the Herbarium (number 25375) of the Department of Biology, Chiang Mai University) from which the isolation of 1', 2'-didehydrostemofoline has been reported (Sastraruji *et al*., 2005). It has been shown that 1', 2'-didehydrostemofoline, when compared to the other *Stemona* alkaloids, has the highest inhibitory activity against acetylcholinesterase. Thus indicating this compound as a potential therapeutic to treat the initial symptoms of Alzheimer's disease (Baird *et al*., 2009). Stemofoline, another *Stemona* alkaloids found in this *Stemona* sp., also has inhibitory activity against acetylcholinesterase (Sastraruji *et al*., 2010). Further, stemofoline was able to significantly increase the sensitivity of the anticancer drugs, vinblastine, paclitaxel and doxorubicin, to drug resistant cancer cells (Chanmahasathien *et al*., 2010). For these reasons, these valuable *Stemona* alkaloids were of interest to produce using plant tissue culture techniques because alkaloid production from plants grown in nature was not always reliable. Plant tissue cultures techniques employing elicitors has been widely used to enhance the production of secondary metabolites such as *Stemona* alkaloids from *Stemona curtisii* Hook. (Chotikadachanarong *et al*., 2011). Chitosan and yeast extract have been shown to be effective biotic elicitors for many plants. For example, the feeding of chitosan to *Cistanche deserticola* cell suspension cultures increased production of phenylethanoid glycosides by 3.4-fold higher than the control without elicitation (Cheng *et al*., 2006). In addition, yeast extract also induced accumulation of xanthones in *Centaurium erythraea* cell cultures (Beerhues and Berger, 1995). For this research, the effect of chitosan and yeast extract on *Stemona* alkaloid production was investigated.

MATERIALS AND METHODS

Plant material: Shoot tips and auxiliary buds of *Stemona* sp. were surface sterilized with 15% clorox solution for 20 min followed by three times washing with sterilized distilled water. After sterilization, shoot tips and auxiliary buds were cultured on MS (Murashige and Skoog, 1962) agar medium supplemented with 3 mg/L benzyladenine for multiple shoot induction. For root induction, single shoots were transferred to half-MS medium supplemented with 2 mg/L indolebutyric acid (Chaichana *et al*., 2011). The cultures were placed in a growth room at 25±2ºC under a 16 h per day photo period.

Feeding elicitor: Eight-week-old *in vitro* *Stemona* plantlets were cultured in liquid MS medium supplemented with various concentrations of chitosan or yeast extract for one and two weeks at concentrations of

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25, 50, 100 and 200 mg/L. The control treatment was cultured in liquid MS medium without adding any elicitor. Yeast extract was dissolved in distilled water and chitosan was dissolved in 1% acetic acid. All culture experiments were performed in triplicate. Roots from all treatments, as well as the cultured medium, were extracted and analyzed for *Stemona* alkaloid production. The total *Stemona* alkaloid production was expressed as the sum of the amount of alkaloid in the root and medium.

Extraction method and HPLC conditions: Dry roots of *Stemona* sp. were ground and extracted three times with methanol (Merck, HPLC grade, Germany). The solution was filtered and evaporated to give the crude extract which was extracted again with Dichloromethane (DCM) (Merck, HPLC grade Germany). The crude DCM extract was dissolved and filtered with 0.45 μM membrane filter. Finally, *Stemona* alkaloids were quantified by HPLC (Agilent 1200 series, Palo Atto, CA, USA). Data acquisition and analysis were performed by the Agilent chemstation software. HPLC condition development was performed to investigate the appropriate column, mobile phase, flow rate and UV detection wavelength. Pure samples of 1, 2 -didehydrostemofoline and stemofoline were used as standards.

Research location: This research was studied at Plant Tissue Culture Laboratory, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand for 1 year (September 2010-September 2011).

Statistical analysis: Statistical significance was determined by Analysis of Variance (ANOVA) with adjustments for multiple comparisons with Turkey test. Differences referred to as significant had a P-value less than 0.05. Data were expressed as average of three separate experiments.

RESULTS AND DISCUSSION

HPLC conditions for analysis of *stemona* alkaloids: HPLC conditions were developed to find the appropriate column, mobile phase, flow rate and UV detection wavelength. An Inertsil C18 ODS-3 5 μm particle size, 4.6×150 mm column (GL sciences Inc., Japan) was used. A mixture of methanol and water was used as the mobile phase under gradient elution conditions with a flow a rate of 0.5 mL/min. The gradient conditions are shown in Table 1. Detection of compounds was made at 297 nm. A 20 μL of sample was injected into the chromatography system. The analysis was achieved within 27 min. *Stemona* alkaloids were identified by comparing retention times of the standards. The chromatogram of the *Stemona* alkaloid standards and the cultured root extract are shown in Fig. 1. The retention times of the 1 , 2 -didehy drostemofoline and stemofoline standards were 11.5 and 18.2 min, respectively. Quantification was then carried out by comparing the areas of the corresponding peaks of standard curves. The standard curves were prepared in the concentration range of 25-1000 mg/L.

Table 1: The HPLC gradient flow conditions for *stemona* alkaloids separation

Time (min)	Methanol (%)	Water (%)	Flow rate (mL/min)
7	70	30	0.5
7.5	10	90	0.5
12	10	90	0.5
13	70	30	0.5
20	70	30	0.5

Effect of elicitors on *stemona* alkaloid production: The effect of chitosan on the growth rate of *Stemona* root is shown in Fig. 2a. This compound slightly decreased root growth. The effect of chitosan on the growth of *Panax ginseng* has been investigated. It was found that chitosan slightly inhibited hairy root growth (Jeong and Park, 2005). On the otherhand, chitosan had a positive effective of *Stemona* alkaloid production. The highest *Stemona* alkaloid production was found using 25 mg/L chitosan over a one week culture period. 1, 2 -Didehy drostemo foline and stemofoline production were 2.65 and 2.95 fold higher than the control, respectively (Fig. 2b-c). Chitosan, a polycationic polysaccharide, has been confirmed as

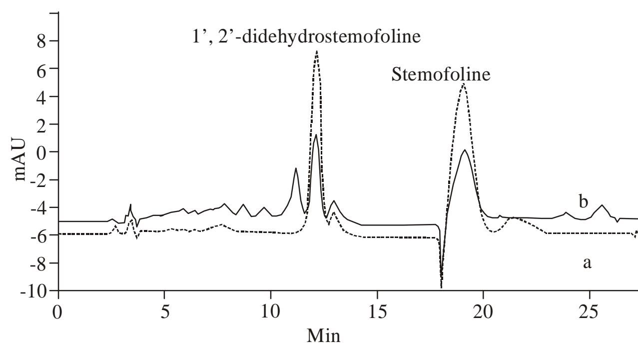

Fig. 1: The chromatogram of (a) 1', 2'-didehydrostemofoline and stemofoline standards and (b) the root extract of chitosan cultured

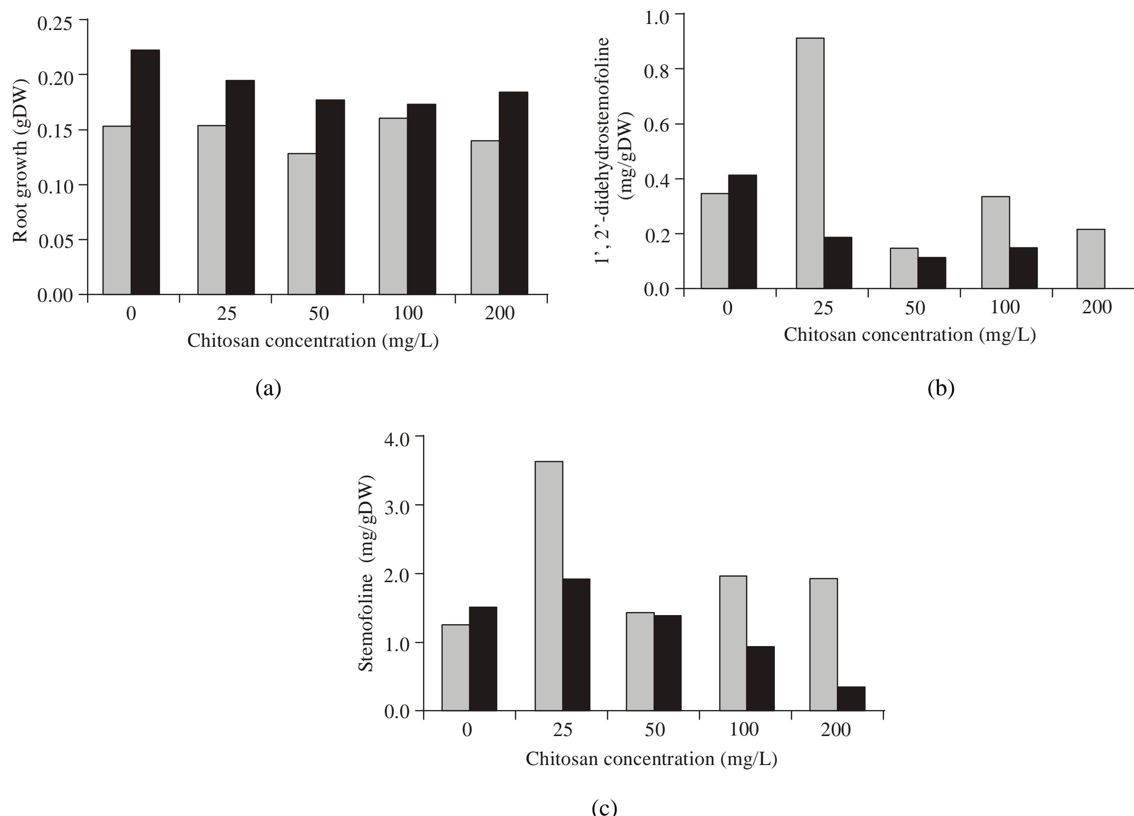

Fig. 2: The effects of various concentrations of chitosan on root growth (a), total alkaloid production of 1', 2'-didehydrostemofoline (b) and total alkaloid production of stemofoline (c). The data were shown mean of triplicates. (The light bar and the dark bar represented one week and two weeks cultured period, respectively

Table 2: The effects of various concentrations of chitosan on 1', 2'-didehydrostemofoline (a) and stemofoline (b) production. The data were shown mean of triplicates and±S.D. values were presented.

	1 week cultured period				2 week cultured period			
Chitosan	Growth (gDW)	Root extract	Medium extract	Total	Growth (gDW)	Root extract	Medium extract	Total
(a)								
Control	0.152±0.065[a]	0.311±0.063[bc]	0.030±0.014[a]	0.341±0.060[b]	0.221±0.119[a]	0.404±0.084[a]	0.002±0.001[a]	0.406±0.083[a]
25 mg/L	0.154±0.004[a]	0.904±0.201[a]	0[b]	0.904±0.201[a]	0.193±0.004[a]	0.183±0.052[b]	0[a]	0.183±0.052[b]
50 mg/L	0.130±0.007[a]	0.136±0.064[cd]	0[b]	0.136±0.064[bc]	0.177±0.006[a]	0.107±0.035[b]	0[a]	0.107±0.035[b]
100 mg/L	0.157±0.009[a]	0.325±0.032[bc]	0[b]	0.325±0.032[bc]	0.173±0.007[a]	0.141±0.025[b]	0[a]	0.141±0.025[b]
200 mg/L	0.138±0.010[a]	0.192±0.064[cd]	0[b]	0.192±0.064[bc]	0.181±0.014[a]	0[b]	0[a]	0[b]
(b)								
Control	0.152±0.065[a]	0.759±0.087[d]	0.451±0.090[a]	1.210±0.170[d]	0.221±0.119[a]	1.402±0.136[ab]	0.027±0.008[a]	1.429±0.134[ab]
25 mg/L	0.154±0.004[a]	3.504±0.322[a]	0.049±0.003[bc]	3.554±0.324[a]	0.193±0.004[a]	1.842±0.086[a]	0.020±0.003[a]	1.862±0.089[a]
50 mg/L	0.130±0.007[a]	1.340±0.354[c]	0.058±0.007[bc]	1.398±0.357[c]	0.177±0.006[a]	1.357±0.118[ab]	0.025±0.007[a]	1.382±0.125[ab]
100 mg/L	0.157±0.009[a]	1.907±0.274[b]	0.023±0.011[c]	1.930±0.281[b]	0.173±0.007[a]	0.871±0.598[bc]	0.038±0.011[a]	0.909±0.598[bc]
200 mg/L	0.138±0.010[a]	1.826±0.344[b]	0.107±0.012[b]	1.933±0.342[b]	0.181±0.014[a]	0.344±0.125[c]	0.016±0.001[a]	0.360±0.125[c]

an effective biotic elicitor for improving biosynthesis of secondary metabolites and the stimulation was found to associate with the key enzyme in the biosynthesis (Cheng *et al*., 2006). In several other plant species, chitosan has been shown to be a powerful elicitor to produce secondary metabolites. A study of effect of chitosan on Rubia akane Nakai cell culture indicated that 25 mg/L of chitosan was the optimal concentration for anthraquinone production. The total anthraquinone production was approximately increased two fold in a one week culture when compared to that in the unelicited *Rubia akane* Nakai cell culture (Jin *et al*., 1999). In addition, chitosan elicitor was also found to enhance secondary metabolite production in *Sorbus aucuparia* cell cultures (Gaid *et al*., 2009) and *Brugmansia candida* hairy root cultures (Pitta-Alvarez and Giulietti, 1999). In the present study alkaloid accumulation in the media culture was also investigated (Table 2a-b). It was found that chitosan forced the

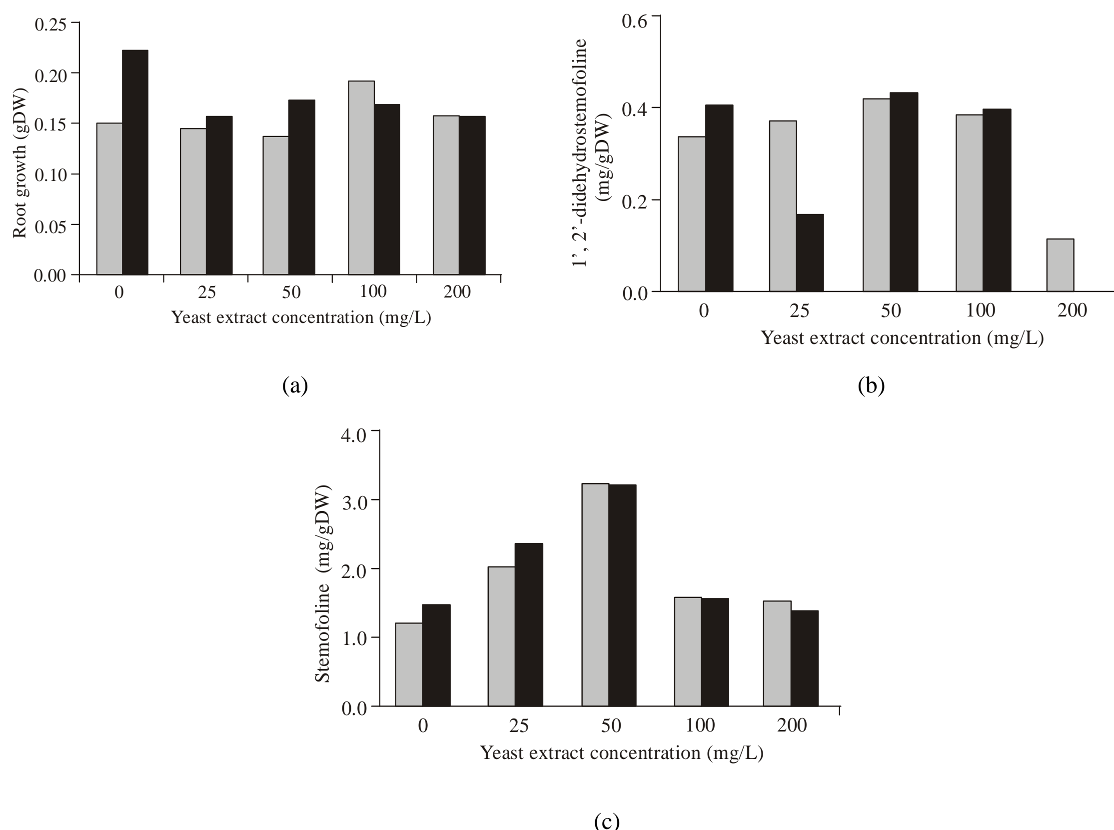

Fig. 3: The effects of various concentrations of yeast extract on root growth (a), total alkaloid production of 1', 2'-didehydrostemofoline (b) and total alkaloid production of stemofoline (c). The data were shown mean of triplicates. (The light bar and the dark bar represented one week and two weeks cultured period, respectively.)

Table 3: The effects of various concentrations of yeast extract on 1', 2'-didehydrostemofoline (a) and stemofoline (b) production. The data were shown mean of triplicates and ±S.D. values were presented.

	1 week cultured period				2 week cultured period			
Yeast extract	Growth (gDW)	Root extract	Medium extract	Total	Growth (gDW)	Root extract	Medium extract	Total
(a)								
Control	0.152 ± 0.065^{a}	0.311 ± 0.063^{bcd}	0.030 ± 0.014^{a}	0.341 ± 0.060^{abc}	0.221 ± 0.119^{a}	0.404 ± 0.0843^{a}	0.002 ± 0.001^{a}	0.406 ± 0.083^{a}
25 mg/L	0.144 ± 0.072^{a}	0.342 ± 0.059^{bc}	0^{b}	0.342 ± 0.059^{ab}	0.154 ± 0.080^{a}	0.164 ± 0.0473^{b}	0^{a}	0.164 ± 0.047^{b}
50 mg/L	0.138 ± 0.087^{a}	0.420 ± 0.066^{a}	0^{b}	0.420 ± 0.066^{a}	0.174 ± 0.093^{a}	0.428 ± 0.1743^{a}	0^{a}	0.428 ± 0.174^{a}
100 mg/L	0.192 ± 0.078^{a}	0.381 ± 0.102^{ab}	0^{b}	0.381 ± 0.102^{ab}	0.169 ± 0.086^{a}	0.110 ± 0.0123^{b}	0^{a}	0.110 ± 0.012^{b}
200 mg/L	0.157 ± 0.061^{a}	0.117 ± 0.028^{de}	0^{b}	0.117 ± 0.028^{cd}	0.157 ± 0.081^{a}	0^{b}	0^{a}	0^{b}
(b)								
Control	0.152 ± 0.065^{a}	0.759 ± 0.087^{c}	0.451 ± 0.090^{a}	1.210 ± 0.170^{b}	0.221 ± 0.119^{a}	1.402 ± 0.136^{cd}	0.027 ± 0.008^{b}	1.429 ± 0.134^{c}
25 mg/L	0.144 ± 0.072^{a}	1.875 ± 0.123^{b}	0.084 ± 0.005^{b}	1.959 ± 0.120^{b}	0.154 ± 0.080^{a}	2.208 ± 0.070^{b}	0.057 ± 0.001^{ab}	2.265 ± 0.070^{b}
50 mg/L	0.138 ± 0.087^{a}	3.073 ± 0.658^{a}	0.077 ± 0.003^{b}	3.150 ± 0.658^{a}	0.174 ± 0.093^{a}	3.034 ± 0.160^{a}	0.074 ± 0.014^{ab}	3.108 ± 0.147^{a}
100 mg/L	0.192 ± 0.078^{a}	1.501 ± 0.104^{bc}	0.027 ± 0.009^{b}	1.528 ± 0.101^{b}	0.169 ± 0.086^{a}	1.441 ± 0.179^{bcd}	0.082 ± 0.011^{ab}	1.523 ± 0.190^{bc}
200 mg/L	0.157 ± 0.061^{a}	1.445 ± 0.434^{bc}	0.070 ± 0.015^{b}	1.515 ± 0.441^{b}	0.157 ± 0.081^{a}	1.272 ± 0.157^{cd}	0.118 ± 0.017^{a}	1.391 ± 0.174^{c}

secretion of stemofoline into the medium, while 1 , 2 -didehydrostemofoline was not found to be released into the medium. In an earlier study, the extracellular accumulation of plumbagin was found in chitosan treated cell cultures of *Plumbago rosea* L. These results indicated that chitosan enhanced the permeability of plumbagin from the cell to the exterior (Komaraiah *et al*., 2002).For the yeast extract experiments, the effect of this elicitor on root growth is shown in Fig. 3a. Yeast extract similarly had a negative effect on root growth (Fig. 3a). A similar study revealed that yeast extract decreased the biomass production of *Salvia miltiorrhiza* cell cultures (Zhao *et al*., 2010). However, some of yeast extract treatments, such as that at 100 mg/L concentration, showed higher root growth than the control treatment. A previous study on *Silybum marianum* L. hairy root cultures showed that yeast extract also had a positive effect on the biomass of the culture (Hasanloo *et al*., 2009). In our study it seemed

that the root growth either increased or decreased, opposite to that of alkaloid production. The alkaloids production caused by yeast extract is shown in Fig. 3b-c. It was found that 50 mg/L yeast extract over a one week culture period caused the highest production of *Stemona* alkaloids. The production was 1.23 and 2.60 fold higher than the control for 1, 2 -didehydrostemofoline and stemofoline production, respectively. Yeast extract was found to increase the total tanshinone content of *Salvia miltiorrhiza* cell cultures by 10-fold compared to the control and was stimulated the key enzyme activity at the entrance step in the pathway (Zhao *et al*., 2010). Furthermore, yeast extracts stimulated silymarin production in cell cultures of *Silybum marianum* and silymarin accumulation was observed in the culture medium (Sanchez-Sampedro *et al*., 2005). Similar to this research, yeast extract also forced the secretion of stemofoline into the medium but not for 1, 2 -didehydrostemofoline (Table 3a-b). In previous reports, secondary metabolite productions in many plants were stimulated by yeast extract. For example, yeast extract treatment resulted in a large increase of rosmarinic acid accumulation in *Orthosiphon aristatus* cell suspension cultures (Sumaryono *et al*., 1991) and the addition of yeast extract to the cell suspension culture of *Ageratina adenophora* enhanced the accumulation of benzofurans (Monir and Proksch, 1989).

CONCLUSION

Treatment of *Stemona* sp. cultures with chitosan and yeast extract resulted to enhance *Stemona* alkaloid production. The highest *Stemona* alkaloid accumulation was found in the culture using 25 mg/L chitosan. This treatment resulted in the production of 1 , 2 -didehydrostemofoline and stemofoline in 2.65 and 2.95 fold higher amounts than the control, respectively.

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REFERENCES

Baird, M.C., S.G. Pyne, A.T. Ung, W. Lie, T. Sastraruji, A. Jatisatienr, C. Jatisatienr, S. Dheeranupattana, J. Lowlam and S. Boonchalermkit, 2009. Semisynthesis and biological activity of stemofoline alkaloids. J. Nat. Prod., 72: 679-684.

Beerhues, L. and U. Berger, 1995 Differential accumulation of xanthones in methyl-jasmonate-and yeast-extract-treated cell cultures of *Centaurium erythraea* and *Centaurium* littorale. Planta., 197: 608-612.

Brem, B., C. Seger, T. Pacher, M. Hartl, F. Hadacek, O. Hofer, S. Vajrodaya and H. Greger, 2004. Antioxidant dehydrotocopherols as a new chemical character of *Stemona* species. Phytochemistry., 65: 2719-2729.

Chaichana, N., S. Dheeranupattana, A. Jatisatienr and S. Wangkarn, 2011. Micro-propagation and 1, 2 -didehydrostemofoline production from *Stemona* sp. Asian J. Plant Sci., 10: 338-341.

Chanmahasathien, W., C. Ampasavate, H. Greger and P. Limtrakul, 2010. *Stemona* alkaloids, from traditional Thai medicine, increase chemosensitivity via P-glycoprotein-mediated multidrug resistance. Phytomedicine., 18: 199-204.

Cheng, X.Y., H.Y. Zhou, X. Cui, W. Ni and C.Z. Liu, 2006. Improvement of phenylethanoid glycosides biosynthesis in *Cistanche deserticola* cell suspension cultures by chitosan elicitor. J. Biotech., 121: 253-260.

Chotikadachanarong, K., S. Dheeranupattana, A. Jatisatienr, S. Wangkarn, P. Mungkornasawakul, Stephen G. Pyne, Alison T. Ung and T. Sastraruji, 2011. Influence of salicylic acid on alkaloid production by root cultures of *Stemona curtisii* Hook. F. Curr. Res. J. Biol. Sci., 3(4): 322-325.

Gaid, M.M., D. Sircar, T. Beuerle, A. Mitra and L. Beerhues, 2009. Benzaldehyde dehydrogenase from chitosan-treated *Sorbus aucuparia* cell cultures. J. Plant Physiol., 166: 1343-1349.

Hasanloo, T., R. Sepehrifar, H. Rahnama and M.R. Shams, 2009. Evaluation of the yeast-extract signaling pathway leading to silymarin biosynthesis in milk thistle hairy root culture. World J. Microbiol. Biotechnol., 25: 1901-1909.

Jeong, G.T. and D.H. Park, 2005. Enhancement of growth and secondary metabolite biosynthesis: Effect of elicitors derived from plants and insects. Biotechnol. Bioprocess Eng., 10: 73-77.

Jin, J.H., J.H. Shin, J.H. Kim, I.S. Chung and H.J. Lee, 1999. Effect of chitosan elicitation and media components on the production of anthraquinone colorants in Madder (*Rubia akane* Nakai) cell culture. Biotechnol. Bioprocess Eng., 4: 300-304.

Komaraiah, P., R. Naga Amrutha, P.B. Kavi Kishor and S.V. Ramakrishna, 2002. Elicitor enhanced production of plumbagin in suspension cultures of *Plumbago rosea* L. Enzyme Microb. Technol., 31: 634-639.

Lin, L.G., K.M. Li, C.P. Tang, C.Q. Ke, J.A. Rudd, G. Lin and Y. Ye, 2008. Antitussive stemoninine alkaloids from the roots of *Stemona tuberose*. J. Nat. Prod., 71: 1107-1110.

Monir, H. and P. Proksch, 1989. Yeast extract induced accumulation of benzofurans in cell suspension cultures of *Ageratina adenophora*. Phytochemistry, 28: 2999-3002.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant, 15: 473-497.

Pitta-Alvarez, S.I. and A.M. Giulietti, 1999. Influence of chitosan, acetic acid and citric acid on growth and tropane alkaloid production in transformed roots of *Brugmansia candida* Effect of medium pH and growth phase. Plant Cell Tiss. Org., 59: 31-38.

Pyne, S.G., A.T. Ung, A. Jatisatienr and P. Mungkornasawakul, 2007. The pyrido[1, 2-*a*]azepine *Stemona* alkaloids. Maejo Int. J. Sci. Technol., 01: 157-165.

Sanchez-Sampedro, M., J. Fernandez-Tarrago and P. Corchete, 2005. Yeast extract and methyl jasmonate-induced silymarin production in cell cultures of *Silybum marianum* (L.) Gaertn. J. Biotech., 119: 60-69.

Sastraruji, T., A. Jatisatienr, S.G. Pyne, A.T. Ung, W. Lie and M.C. Williams, 2005. Phytochemical studies on *Stemona* plants: Isolation of stemofoline alkaloids. J. Nat. Prod., 68: 1763-1767.

Sastraruji, T., S. Chaiyong, A. Jatisatienr, S.G. Pyne, A.T. Ung and W. Lie, 2010. Phytochemical studies on *Stemona aphylla*: Isolation of a new stemofoline alkaloid and six new stemofurans. J. Nat. Prod., 74: 60-64.

Sumaryono, W., P. Proksch, T. Hartmann, M. Nimtz and V. Wray, 1991. Induction of rosmarinic acid accumulation in cell suspension cultures of *Orthosiphon aristatus* after treatment with yeast extract. Phytochemistry, 30: 3267-3271.

Tang, C.P., T. Chen, R. Velten, P. Jeschke, U. Ebbinghaus-Kintscher, S. Geibel and Y. Ye, 2007. Alkaloids from stems and leaves of *Stemona japonica* and their insecticidal activities. J. Nat. Prod., 71: 112-116.

Zhao, J.L., L.G. Zhou and J.Y. Wu, 2010. Effects of biotic and abiotic elicitors on cell growth and tanshinone accumulation in *Salvia miltiorrhiza* cell cultures. Appl. Microbiol. Biotechnol., 87: 137-144.