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การสร้างสารไดเซนทรีนในเซลล์แขวนลอยและรากลอยเพาะเลี้ยงของบอระเพ็ดพุงช้าง

ธฤตา กิติศรีปัญญา[1], จักรพันธ์ โคมัยกุล[1], วราภรณ์ ภูตะลุน[2]*

บทคัดย่อ

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บทนำ: บอระเพ็ดพุงช้าง (*Stephania suberosa*) เป็นพืชสมุนไพรที่มีลักษณะเป็นไม้เถา มีหัวใต้ดินขนาดใหญ่ ส่วนหัวใต้ดินพบสารไดเซนทรีน ซึ่งเป็นอัลคาลอยด์กลุ่มอะโปรฟีน ที่มีฤทธิ์ทางเภสัชวิทยาหลากหลาย อาทิ ฤทธิ์ยับยั้งเอนไซม์อะเซทิลโคลินเอสเทอเรส, ฤทธิ์ต้านการเจริญของเนื้องอก และฤทธิ์ต้านอาการเต้นผิดจังหวะของหัวใจ แต่เนื่องจากส่วนหัวใต้ดินของบอระเพ็ดพุงช้างใช้ระยะเวลานานในการเจริญเติบโต การเพาะเลี้ยงเนื้อเยื่อพืชสมุนไพร จึงเป็นวิธีการหนึ่งเพื่อใช้ในการสร้างสารทุติยภูมิที่มีฤทธิ์ทางชีวภาพจากบอระเพ็ดพุงช้าง โดยลดระยะเวลาจากการปลูกพืชตามธรรมชาติ และสามารถนำไปประยุกต์ใช้ในการผลิตเชิงอุตสาหกรรม **เครื่องมือและวิธีดำเนินการวิจัย:** ในการศึกษานี้ ได้ทำการเหนี่ยวนำเนื้อเยื่อพืชเพาะเลี้ยงบอระเพ็ดพุงช้างในรูปของเซลล์แขวนลอย และรากลอยที่เหนี่ยวนำจากการใช้แบคทีเรีย *Agrobacterium rhizogenes* รวมทั้งเติมสารกระตุ้น ได้แก่ สารสกัดยีสต์ และเมทิลจัสโมเนท เพื่อศึกษาผลของสารกระตุ้นทั้งสองชนิดต่อการสร้างสารไดเซนทรีนในเนื้อเยื่อพืชดังกล่าว **ผลการศึกษา:** ผลการศึกษาพบว่า เซลล์แขวนลอยและรากลอยของบอระเพ็ดพุงช้างอายุประมาณ 1 เดือนหลังเปลี่ยนถ่ายลงสู่อาหารใหม่ สามารถผลิตสารไดเซนทรีนได้เฉลี่ย 0.2 – 1.5 และ 4.5 – 8.9 มก.ต่อกรัมของน้ำหนักแห้ง ตามลำดับ ผลจากการเติมสารกระตุ้นในเซลล์แขวนลอยพบว่า ทั้งสารสกัดยีสต์และเมทิลจัสโมเนทไม่สามารถเพิ่มการสร้างสารไดเซนทรีน ยกเว้น สารสกัดยีสต์ความเข้มข้น 0.1 มก.ต่อมล.ที่เพิ่มการผลิตสารไดเซนทรีนมากกว่ากลุ่มควบคุม 1.2 เท่า ส่วนในรากลอยเมื่อเปรียบเทียบกับกลุ่มควบคุม การเติมสารสกัดยีสต์ไม่มีผลกระทบต่อการสร้างสารไดเซนทรีนอย่างมีนัยสำคัญทางสถิติ ต่างจากเมทิลจัสโมเนทที่พบว่า กดการสร้างสารไดเซนทรีน **สรุปผล:** เพื่อให้ได้การผลิตสารไดเซนทรีนปริมาณสูง การเพาะเลี้ยงเนื้อเยื่อพืชบอระเพ็ดพุงช้างในรูปของรากลอยมีความเหมาะสมกว่าเซลล์แขวนลอย ส่วนการใช้สารกระตุ้นด้วยสารสกัดยีสต์สามารถกระตุ้นการสร้างไดเซนทรีนได้ในเซลล์แขวนลอยเท่านั้น ขณะที่เมทิลจัสโมเนทกดการสร้างไดเซนทรีนทั้งในเซลล์แขวนลอย และรากลอยของบอระเพ็ดพุงช้าง

คำสำคัญ: ไดเซนทรีน, บอระเพ็ดพุงช้าง, รากลอย, เซลล์แขวนลอย, สารกระตุ้น

Abstract

Dicentrine production in cell suspensions and hairy root cultures of *Stephania suberosa*

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Introduction: *Stephania suberosa* Forman is the herbal climber plants with very large tuber. Dicentrine, aporphine alkaloids, is the bioactive compound that was found in tuberous root of *S. suberosa* with various pharmacological activities like acetylcholinesterase inhibition, antitumor effect and antiarrhythmic effect. However, *S. suberosa* tuber take a long time to growth therefore plant tissue culture technique is the optional approach for bioactive secondary metabolites production from *S. suberosa* with shorter than natural cultivated period and might be apply to the industrial scale production. **Materials and Methods:** In this study, *S. suberosa* cell suspensions were induced and hairy root cultures were established by *Agrobacterium rhizogenes*. Both cultures were elicited by yeast extract and methyl jasmonate to investigate their effect on dicentrine production. **Results:** After subculture into fresh media, approximately one month, cell suspensions and hairy root cultures of *S. suberosa* produced dicentrine average range 0.2 – 1.5 and 4.5 – 8.9 mg/g dry weight, respectively. In cell suspensions, yeast

extract and methyl jasmonate did not improve dicentrine production except yeast extract at concentration 0.1 mg/ml in day 9 which increased dicentrine more 1.2 time than control group. To compare with the control group in hairy root cultures of *S. suberosa,* yeast extract did not significantly affected dicentrine content but methyl jasmonate suppressed dicentrine production. **Conclusion:** To obtain the high dicentrine production, hairy root cultures of *S. suberosa* was suitable than cell suspension cultures. And elicitation with yeast extract only increase dicentrine in cell suspensions of *S. suberosa* while methyl jasmonate suppressed dicentrine production in both cell suspensions and hairy root cultures of *S. suberosa*.

Keywords: dicentrine, *Stephania suberosa*, hairy root culture, cell suspension culture, elicitor

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Introduction

Stephania suberosa Forman is the herbal climber plants with very large tuber and found in mixed deciduous forest, limestone, 400 m in semi-endemic area (Santisuk *et al*., 2006). In Thai traditional remedy, the tuber of this plant has been used as longevity and neurotonic while methanolic extract from tuber at concentration of 0.1 mg ml-1 has been reported 92% AChE inhibitory activities (Ingkaninan *et al*., 2003). Dicentrine, aporphine alkaloids, is the bioactive compound that was found in tuberous root of *S. suberosa* with various pharmacological activities like acetylcholinesterase inhibition (Ingkaninan *et al*., 2003; Sriprang *et al*., 2006), antitumor effect (Woo *et al*., 1999; Stevigny *et al*., 2005) and antiarrhythmic effect (Young *et al*., 1994). However, *S. suberosa* tuber take a long time to growth therefore plant tissue culture technique is the optional approach for bioactive secondary metabolites production from *S. suberosa* with shorter than natural cultivated period and might be apply to the industrial scale production.

Yeast extract and methyl jasmonate have used as elicitors in many plant species for several years. These biotic elicitors are organism derived substances which stimulate the defense mechanism in plant cells and the consequential secondary metabolite or phytoalexin formation. In this study, we produced cell suspensions and hairy root cultures of *S. suberosa* for investigation of dicentrine content. And the effects of yeast extract and methyl jasmonate on dicentrine production were investigated.

Materials and Methods

Cell suspension cultures were induced by callus of *S. suberosa* that was cultivated in Murashige and Skoog (MS) medium with thidiazuron (TDZ) and Naphthaleneacetic acid (NAA) supplement at concentration 0.1 and 0.5 mg/l, respectively. The cultures were suspended into liquid MS media with above supplement ratio. Hairy root cultures of *S. suberosa* were established with *Agrobacterium rhizogenes* ATCC15834 and cultured in half strength Murashige and Skoog (MS) liquid medium. Both types of cultures were agitated on a rotary shaker that operated at 100 rpm with temperature at 25°C, light for 16 hours per day. Twenty-five days old of the cultures were used to elicit with methyl jasmonate (50, 100 and 200 µM) and yeast extract (0.1, 0.2 and 0.5 mg/ml) that counted as day 0. After the elicitation, the cultures were harvested and dried at 50°C on day 3, 6 and 9.

Dried powder samples (30 mg) were extracted four times with 0.5 ml of methanol with sonicated for 15 minutes. The combined extracts were evaporated and adjusted final volume to 1 ml with methanol. The HPLC system was used RP-18 column (LiChroCART, 125 mm × 4 mm, 5 µm particle size, Merck, Germany) and Hewlett

Packard series 1100 with UV/VIS detector (HP 3396 series III integrator, 308 nm) at flow rate 1.0 ml/min by solvent system contained 35% v/v acetonitrile and 0.1% v/v aqueous trifluoroacetic acid to determine the dicetrine contents. Each sample was examined in triplicate. The data were analyzed statistically by one-way analysis of variance (ANOVA) and comparison with Tukey's HSD at probability level of 0.01.

Results and Discussion

Approximately, one month of cell suspensions and hairy root cultures of *S. suberosa* could produce dicentrine average range 0.2 – 1.5 and 4.5 – 8.9 mg/g dry weight, respectively. The data were shown as growth curve in figure 1a and 2a, respectively. In natural, dicentrine content was founded in tuberous root of *S. suberosa* more than other parts (data not shown). This result might explain why the hairy root cultures of *S. suberosa* could produce dicentrine level more than cell suspension cultures. Although, dicentrine production in hairy root cultures are less than in natural tuberous root (16.6 mg/g dry weight) but the cultures are more high growth rate which can be the alternative source for improve dicentrine production in a short time.

In *S. suberosa* cell suspensions (Fig.1b), all varied concentrations of yeast extract suppressed dicentrine production except at concentration 0.1 mg/ml in day 9 which significantly increased dicentrine level more (1.84±0.17 mg/g dry weight) than control group. Hairy root cultures which also elicited by yeast extract does not enhanced dicentrine production (Fig.2b). On the contrary, the sanguinarine and stemofoline production were increased more than 2 times after added yeast extract as elicitor in *Eschscholtzia californica* suspension cultures (Cho *et al*., 2008) and *Stemona* sp. root cultures (Chaichana *et al*., 2012), respectively. This could be suggest that long exposure with yeast extract at low concentration (0.1 mg/ml) would rather increase dicentrine content in cell suspension cultures but not appropriate for improve dicentrine production in hairy root cultures of *S. suberosa.*

Methyl jasmonate, the volatile derivative of jasmonic acid, is the hormone regulating the defense mechanism in plant cell that act as signal transducer to induce secondary metabolites formation (Memelink *et al*., 2001) and has stimulated the expression of some common methyltransferases in benzylisoquinoline alkaloids pathway lead to increase its production (Frick and Kutchan, 1999; Cho *et al*., 2008). Although it has reviewed to enhance alkaloids production in many plant species (El-Sayed and Verpoorte, 2002; Zayed and Wink, 2004; Cho *et al*., 2007; Eskandari Samet *et al*., 2012) but treatment with all varied concentration of methyl jasmonate in *S. suberosa* cell suspensions and hairy root cultures have decreased dicentrine production in all culture periods (Fig.1c and 2c, respectively). This result might caused by methyl jasmonate could not be induced specific enzymes in dicentrine (aporphine alkaloids) biosynthesis such as (*S*)-corytuberine synthase; CYP80G2 (Ziegler *et al*., 2009) and different in plant species are also different affect.

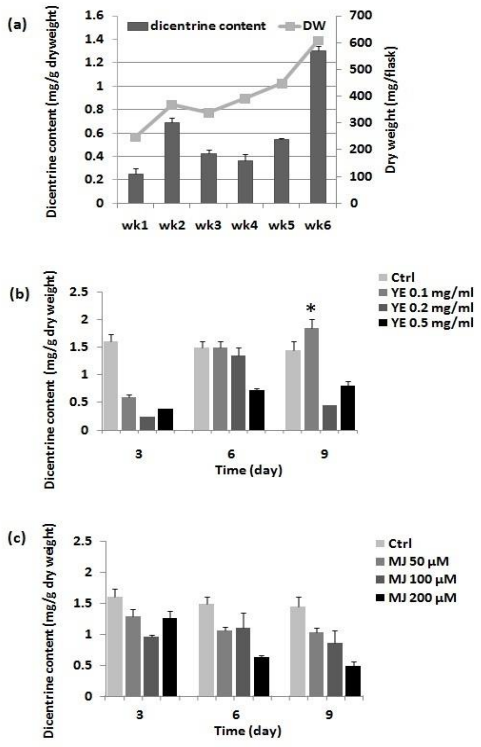

Figure 1 Cell suspension cultures of *S. suberosa*; (a) growth curve (b) effect of yeast extract and (c) methyl jasmonate on dicentrine production at day 3, 6 and 9. The data are presented as the mean ± SD and * indicates statistical significance at the P <0.01 vs control group.

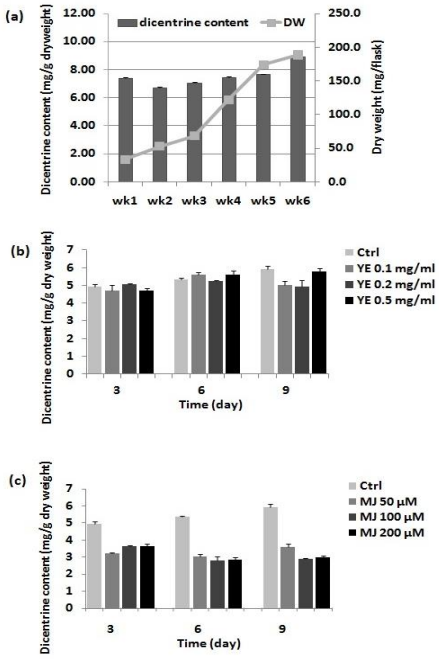

Figure 2 Hairy root cultures of *S. suberosa*; (a) growth curve (b) effect of yeast extract and (c) methyl jasmonate on dicentrine production at day 3, 6 and 9. The data are presented as the mean ± SD.

Conclusion

To obtain the high dicentrine production from *S. suberosa in vitro* cultures, hairy root cultures of *S. suberosa* was more suitable than cell suspension cultures. And elicitation with yeast extract only increase dicentrine in cell suspensions of *S. suberosa* on day 9 while methyl jasmonate suppressed dicentrine production in both cell suspensions and hairy root cultures of *S. suberosa*.

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