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Increasement of Eleutherosides and Antioxidant Activity in *Eleutherococcus senticosus* Adventitious Root by Jasmonic acid

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Abstract : This study was carried out to investigate the impacts of jasmonic acid (JA) on adventitious root culture of *Eleutherococcus senticosus*. Adventitious root of *E. senticosus* were treated with jasmonic acid (JA) and cultured for 30 days. JA inhibited the root growth but increased eleutherosides accumulation, total phenolic contents and antioxidant activity. Among various concentrations of JA, 1.0 mg/L JA increased the total phenolic contents in *E. senticosus* adventitious root to 39.81 μg/g, about 2.6 times higher than that of the control. Consequently, high accumulation of total phenolic contents led to increase the antioxidant activity to 82.41%. The antioxidant activity of control was 37.89% at 2500 μg/mL. A linear correlation (R^2 = 0.9937, 0.9648 and 0.9883) was also shown between antioxidant activity (at 1250, 1875, and 2500 μg/mL) and total phenolic contents of adventitious root of *E. senticosus*.

Key words : Eleutherococcus senticosus, *adventitious root, jasmomic acid, eleutherosides, total phenolic content, antioxidant activity*

Introduction

Eleutherococcus senticosus is a useful medicinal plant and its root have been used to promote robustness and as a remedy for many ailments including diabetes, hypertension and tumor (Guo *et al.*, 2006). It contains eleutheroside A, B E, F, flavonoids and polysaccharides (Feng *et al.*, 2005). Among these compounds, eleutheroside B and -E were acknowledged as major active components of the *E. senticosus*. Eleutheroside B has an anti-fatigue function and eleutheroside E has antistress effect (Sandberg, 1973).

Generally, adventitious root cultures have been used to produce secondary metabolites as alternatives of field cultivation with advantages of fast growth and stable metabolite production. However, secondary metabolites and biological activity from adventitious roots are usually lower than those in cultivated plants and this is the problem to solve in root culture.

Elicitation is seen as an effective strategy to enhance the production of secondary metabolites and the biological activities. Therefore, elicitor induced accumulation of secondary metabolites has received much attention and jasmonic acid has been confirmed as an effective elicitor for induction of secondary metabolites in plant cell cultures (Aerts *et al.*, 1994). Jasmonic acid (3-oxo-2-(2'-cis-pentenyl)-cyclopentene-1-acetate, JA) and its derivatives are a group of native plant growth regulators (Eng *et al.*, 1998). These compounds are widely distributed in higher plants, showing phytohormone action and playing important roles as inhibitors of plant growth. JA induces phytoalexin-related enzymes and therefore applied to induce the production of phytoalexins (Chong *et al.*, 2005).

JA and its methyl ester, methyl jasmonate (MeJA) have been employed successfully to induce a number of secondary metabolites in cultures, such as saponin in *Panax ginseng*, rosmarinic acid in *Coleus blumei* and taxane in *Taxus chinensis* (Yu *et al.*, 2002). However, the study on the effect of JA in the adventitious root culture of *A. senticosus* was not performed.

In this study, the effect of JA on adventitious root growth and eleutherosides production of *E. senticosus* have been examined. We also measured the contents of total phenolic contents and antioxidant activity of *E. senticosus* adventitious root treated with JA. Further, we also evaluated the correlations between antioxidant activity and total penolic content of *E. senticosus* adventitious root.

Materials and Methods

1. Materials

E. senticosus adventitious roots induced from the callus were proliferated on modified MS medium supplemented with 3 mg/L naphthaleneacetic acid and 60 g/L sucrose.

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Bulb type air-lift bioreactor cultures were established containing 1/3 MS medium supplemented with 3 mg/L naphthaleneacetic acid and 60 g/L sucrose. Bioreactor cultures were maintained at 22°C in continuous dark. Various amount of JA (0.3, 0.5 and 1.0 mg/L) was added to the bioreactor cultures after 30 days of incubation. Root growth, eleutherosides content, total phenolic content and antioxidant activity were measured after 30 days of addition JA.

2. Extraction

Dried *E. senticosus* adventitious roots were finely ground and extracted with ethanol (EtOH) at 60°C for 30 min and then evaporated to give the crude extract.

3. Determination of Eleutherosides contents using HPLC

Eleutherosides were quantified by HPLC equipped with a ODS RP-18 column according to Patric *et al.* (1998) and using a photodiode array detector. Eleutherosides were separated using a flow rate of 0.6 mL/min with water and acetonitrile as the mobile phase with a gradient of 10% acetonitrile for 0-5 min, 20% acetonitrile for 20 min, 40% acetonitrile for 15 min and equilibration with 5% acetonitrile for 5 min.

4. Total phenolic contents

Total phenolic contents were measured according to the method of Cheung *et al.* (2003). Each sample (1 mL) was mixed with Folin and Ciocalteu's phenol reagent (1 mL, Sigma). After 3 min, 1 mL of saturated Na_2CO_3 was added to the mixture and it was made up to 10 mL by adding distilled water. After the reaction was kept in the dark for 90 min, absorbance was taken at 725 nm. A calibration curve was constructed with different concentrations of gallic acid (Wako pure chemical Industries) (0.01-0.1 mM) as a standard. Total phenolic contents were expressed as gallic acid equivalents (mg GAE/g extract).

5. Antioxidant activity

The antioxidant activity was measured by the DPPH method according to the procedure of Park *et al.* (2004). MeOH solution (4 mL) of samples at various concentrations were added to a solution of DPPH in MeOH (4.5×10^{-4} M, 1 mL) and the reaction mixture were shaken vigorously. After leaving the mixtures for 30 min at room temperature, the remaining parts of DPPH were determined by colorimetry (8452A Diode Array Spectrophotometer, Hewlett Packard Co., USA) at 520 nm. The mixture of 4 mL MeOH with a solution of 1 mL DPPH was used as control. The mean values were obtained from triplicate experiments.

Results and Discussion

1. The Effect of jasmonic acid on adventitious root growth

The growth of *E. senticosus* adventitious root in 10 L bioreactor treated with jasmonic acid is presented in Table 1. The roots in the untreated cultures reached 36.8 g dry wt but 1.0 mg/L JA attained only 7.0 g dry wt. We can observe that the fresh weight and dry weight of *E. senticosus* adventitious root were decreased as the jasmonic acid concentration increased.

According to Ketchum *et al.* (1999), similar results were obtained in *Taxus* which inhibited root growth by methyl jasmonate.

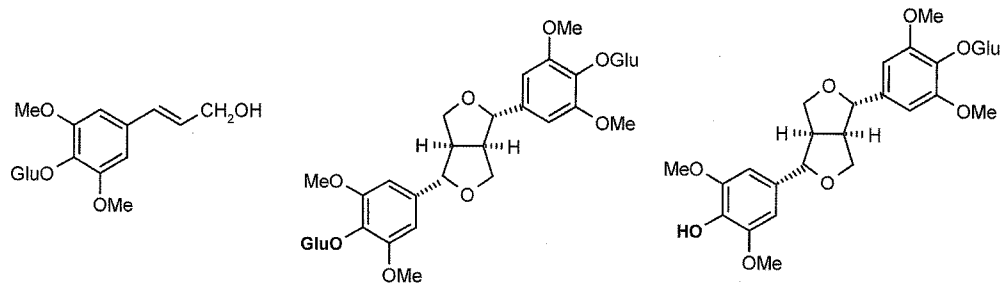
2. The Effect of jasmonic acid on Eleutherosides production

Since it is known that eleutheroside B, E, and E_1

Table 1. Effects of jasmonic acid elicitation on growth of *E. senticosus* adventitious root cultivation in 10 L bioreactors containing 1/3 MS medium

Jasmonic Acid (mg/L)[1)]	Fresh weight (FW, g)	Dry weight (DW, g)	DW/FW (%)
0	278.1	36.8	13.2
0.3	73.7	11.7	15.9
0.5	63.0	9.5	15.1
1.0	47.0	7.0	14.9

[1)]JA was added to the cultures after 30 days of culture and roots were harvested 30 days after JA treatment.



Eleutheroside B Eleutheroside E Eleutheroside E1

Figure 1. Chemical structure of eleutheroside B, E, and E_1.

Table 2. Effect of JA elicitation on eleutherosides composition of adventitious root of *E. senticosus* cultivation in 10 L bioreactors containing 1/3 MS medium

Jasmonic Acid (mg/L)[1)]	Eleutherosides (ug/g)		
	B	E	E_1
0	149.3	325.1	14.2
0.3	329.3	624.9	12.2
0.5	476.3	676.0	12.8
1.0	522.1	702.5	12.5

[1)]JA was added to the cultures after 30 days of culture and roots were harvested 30 days after JA treatment.

shown in Figure 1 were major active components of the *Acanthopanax* species, including *E. senticosus*, we measured the content of these compounds.

Production of elutheroside B, E and E_1 by the adventitious root of *E. senticosus* in 10 L bioreactor treated with jasmonic acid is presented in Table 2. Maximum elutheroside B, and E contents were 522.1, and 702.5 ug/g, respectively, with 1.0 mg/L JA. Recent studies report that stress including elicitation induced during *in vitro* culture changes primary metabolism leading to formation of different secondary metabolites such as phenolics, alkaloids and flavonoids (Shohael *et al.*, 2006). Similar results were also reported that elicit inhibited the root growth and promoted secondary metabolite production with cell cultures of *Taxus* (Yukimune *et al.*, 1996).

3. The Effect of jasmonic acid on the total phenolic contents

It is reported that total phenolic contents played an important role in the DPPH radical scavenging activity (Pyo *et al.*, 2004). Generally, the antioxidant activity increased with the increase in the total phenolic contents. The total phenolic contents of adventitious root of *E. senticosus* are presented in Figure 2.

Total phenolic contents of adventitious root of *E. senticosus* treated with jasmonic acid (0.3, 0.5, and 1.0 mg/L) were 16.38, 29.39 and 39.81 ug/g, respectively. From the results obtained from Figure 2, an increase in total phenolic content was observed as the jasmonic acid concentration increase. The results revealed that 1.0 mg/L jasmonic acid treatment was most efficient in the production of total phenolics.

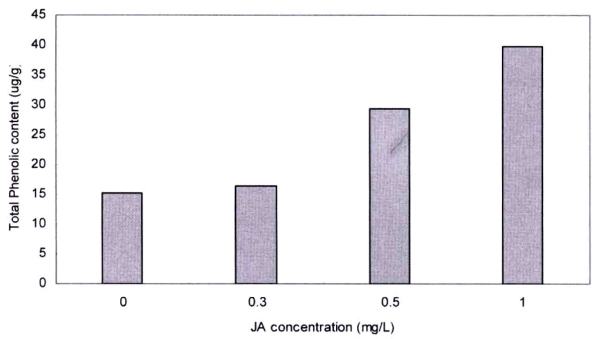


Figure 2. Effect of JA elicitation on the total phenolic contents of adventitious root of *E. senticosus* cultivation in 10 L bioreactors containing 1/3 MS medium. JA was added to the cultures after 30 days of culture and roots were harvested 30 days after JA treatment.

4. The Effect of jasmonic acid on antioxidant activity

Free radicals are chemical fragments that cause oxidation and antioxidants act as free radical scavengers. The free radical scavenging activities of adventitious root of *A. senticosus* are shown in Figure 3.

The antioxidant activities increased as the jasmonic acid concentration and dose concentration increased, and the free-radical scavenging activities of adventitious root of *E. senticosus* treated with 0.3, 0.5, and 1.0 mg/L of jasmonic acid were 43.07%, 68.04% and 82.41% at 2500 ug/mL, respectively. The antioxidant activity of adventitious root of *E. senticosus* appeared to be concentration dependent.

According to Lim *et al.*, (2005), in the cultivation of *Panax ginseng* adventitious root, methyl jasmonate led to increase the antioxidant activity expressed to the DPPH scavenging activity. Similar results were also reported by Ali *et al.*, (2005) in tissue cultured root of mountain *Panax ginseng* subjected to methyl jasmonate.

5. Correlation of antioxidant activity with total phenolic content

It is reported that total phenolic contents played an important role in the DPPH radical scavenging activity (Pyu *et al.*, 2004). The scavenging activity of adventitious root of *E. senticosus* treated with 0.3, 0.5, and 1.0 mg/L of jasmonic acid on DPPH radicals increased with increasing total phenolic contents. A linear correlation (R^2 = 0.9937, 0.9648 and 0.9883) was shown between antioxidant activity (at 1250, 1875, and 2500 μg/mL) and total phenolic contents of adventitious root of *E. senticosus*.

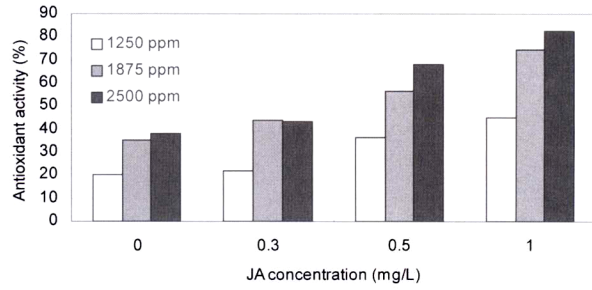


Figure 3. Effect of JA elicitation on antioxidant activity of adventitious root of *E. senticosus* cultivation in 10 L bioreactors containing 1/3 MS medium. JA was added to the cultures after 30 days of culture and roots were harvested 30 days after JA treatment.

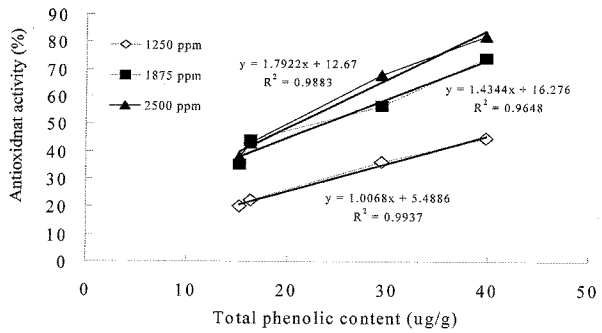


Figure 4. The correlation between antioxidant activity and total phenolic content of adventitious root of *E. senticosus* cultivation in 10 L bioreactors containing 1/3 MS medium.

Conclusion

This study showed that jasmonic acid elicitation could increase eletheroside B, E, and E_1 production and antioxidant activity but with a reduction in biomass in *E. senticosus* adventitious root cultures. Total phenolic contents of the adventitious root of *E. senticosus* increased as jasmonic acid concentration increase. This results obtained from our study suggest that jasmonic acid might have increased the enzyme activities for the synthesis of eleutherosides. Therefore, further studies will be required to investigate related enzymes involved in the enzymatic biosynthesis of eleutherosides and other phenolic compounds related with antioxidant activity.

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