TRITERPENES PRODUCTION OF *LAGERSTROEMIA SPECIOSA* (L.) Pers. TISSUE CULTURE

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INTRODUCTION

Lagerstroemia speciosa (L.) Pers is a tree in family Lythraceae and distributes in tropical countries. It has been extensively studied for hypoglycemic activity. The leaves contain corosolic acid which has very good hypoglycemic activity and now used as dietary supplement for reducing blood glucose level. It can be developed to new drug in the future after more clinical studies. So, the production of corosolic acid from this plant is interested to many researchers. In this study we induced callus and cell suspension culture from the leaves of *L. speciosa* and determination of corosolic acid production from callus and cell suspension culture.

MATERIALS AND METHODS

Plant materials and explants preparation. The leaves of *L. speciosa* were surface sterilized by rinsing with 70% ethanol for 10 s, and immerging in 1.2% NaOCl containing 0.1% of Tween 20 for 20 min, and then 3 times rinsing with sterile distilled water and cut into small size. The explants were put on solid media. In the preliminary study, four media were used for the callus induction. The four medium included
(1) MS medium supplemented with 2 mg/l of 2,4-D and 1 mg/l of kinetin (Kongduang *et al.*, 2008), (2) WPM medium supplemented with 2 mg/l of 2,4-D and 1 mg/l of kinetin, (3) MS medium supplemented with 0.1 mg/l IBA and 2 mg/l BA, (4) B5 medium supplemented with 0.1 mg/l IBA and 2 mg/l BA. Explants were cultured at 25°C under 16-h photoperiod of fluorescent light and subcultured every four weeks. The medium, in which the callus could grow well, has been chosen as a medium for cell suspension culture at 250 rpm in darkness and subculture every four weeks.
Sample extraction. The 2-month old dried callus and cell suspension were ground into powder and extracted with methanol by sonication at room temperature. The methanol crude extract was then used for analysis by thin layer chromatography. A 10 mg crude extracts was dissolved in methanol with a final volume of 5 ml and filtered through 0.2-μm membrane filter [2].
Thin layer chromatography (TLC) analysis. TLC plate silica gel GF_{254} was used as stationary phase. Three solvent systems include chloroform and acetone (4:1), chloroform and methanol (9:1) and ethyl acetate and methylene chloride (8:2) were used for separation. After running on each solvent system, TLC plate was dried and sprayed by anisaldehyde-sulfuric acid spraying reagent before heated at 105°C for detection of triterpenoid compounds.
High performance liquid chromatography (HPLC) analysis. An Agilent 1100 series equipped with UV detector and autosampler was used. Separation was performed using C18 column (Vertisep Ges, 5 μm, 4.6 x 250 mm). Isocratic elution was carried out with acetonitrile and 0.1% phosphoric acid in water (80:20, v/v) at a flow rate of 1 ml/min, detection wavelength was at 210 nm [2].

RESULTS

Callus induction. The calli were induced after 5 days. The MS medium supplemented with 2 mg/l 2,4-D and 1 mg/l kinetin gave the fastest callus induction and the highest growth. The character of callus was white-green and dense. The callus was stable growth even after several subcultures. Calli in other 3 media were also green but the growth of calli in these media decreased after first subculture. Finally, the callus turned brown and died.

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Cell suspension culture. The cell suspension culture was generated from 2-month callus and cultured in MS liquid media supplemented with 2 mg/l 2,4-D and 1 mg/l kinetin. The cells had brown-green color with high growth. The cell suspension was stable growth after several subcultures.

TLC analysis. The crude extract was directly subjected on TLC plates and developed in three different solvent systems chloroform and acetone (4:1), ethyl acetate and methylene chloride (8:2) and chloroform and methanol (9:1). Corosolic acid was used as authentic standard with R_f of 0.18, 0.36 and 0.47 respectively. Analysis of triterpenes from cell suspension and callus culture on TLC showed several separate spots. The spots at the same R_f as that of corosolic acid could be seen in both callus and cell suspension culture in all 3 solvent systems..

Determination of triterpene from callus and cell suspension culture

Dry callus and cell suspension (300 mg each) were extracted and analyzed for corosolic acid content by HPLC. HPLC chromatograms of callus and cell suspension methanol extracts showed peaks of compound at 9.50 and 9.51 min, respectively. The spiking technique was introduced to confirm the compounds. Corosolic acid was added to methanol extract of callus and cell suspension and was analyzed by HPLC. The only peak at 9.50 and 9.51 min were increased. This indicated that compound at 9.50 and 9.51 min were corosolic acid from callus and cell suspension, respectively. HPLC analysis revealed the production of corosolic acis in cell suspension culture was 2.65 times higher than natural leaves while production of corosolic acid from callus culture was 0.44 times of natural leaves.

DISCUSSION

Due to the highest corosolic acid accumulation was found in *L. speciosa* leaves [1]. The leaves were then used as explants for induction of callus. The callus grew well in MS medium supplemented with 2 mg/l 2,4-D and 1 mg/l kinetin even after several subcultures. Callus was transferred to liquid MS medium, with same hormone concentrations to the induction medium, for production of cell suspension. Corosolic acid production from cell suspension culture was higher than in callus and intact leaves, showing the potential of cell suspension culture to be an alternative source for corosolic acid production.

CONCLUSION

L. speciosa callus and cell suspension culture were successfully induced from natural leaves. The induction medium for callus and cell suspension was MS medium supplemented with 2 mg/l 2,4-D and 1 mg/l kinetin. The corosolic acid accumulation in cell suspension culture was 2.65 times higher than natural leaves, while that of callus was 0.44 times lower than natural leaves.

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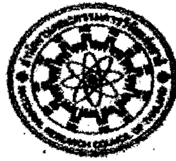

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