

UNIVERSITI TEKNOLOGI MARA

**THE EFFECTS OF PLANT GROWTH
REGULATORS ON
ACCUMULATION OF
PINOCEMBRIN AND CARDAMONIN
OF ADVENTITIOUS ROOT
SUSPENSION CULTURES OF
Boesenbergia rotunda (L.) MANSF.**

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MSc

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Boesenbergia rotunda is an important medicinal plant. The rhizome requires a long period of time to produce in a large scale for this plant. *In vitro* culture is the best method as a continuous source of supply of disease free planting material for commercial utilization because it offers an opportunity to exploit the cell, tissue, organ or entire organism by growing them *in vitro*. Secondary metabolites of *B. rotunda* possess many biological and pharmacological activities such as antioxidant, antibacterial, antitumor, anti-inflammatory and anti-cancer activities. Pinocembrin has shown cytotoxicity against certain cancer cell lines whereas cardamonin was reported to have strong inhibition of *HIV-1 protease* activity. There were a few studies on secondary metabolites of *B. rotunda* in cell culture and callus culture. However, this plant has low content of secondary metabolites in cell suspension cultures and callus culture. This study was conducted to establish the protocol for adventitious root induction from shoot meristem of *B. rotunda* and to determine the effects of PGRs on the biomass and accumulation of pinocembrin and cardamonin in adventitious root suspension cultures of *B. rotunda*. Surface sterilization with 60% clorox for 30 minutes, followed by 20% clorox for 15 minutes was the best treatment to establish the aseptic explants with mean value of 4.667 ± 0.724 . For the adventitious root induction, half strength of Murashige and Skoog (MS) medium was supplemented with various concentrations of auxins; 1-naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA), and 2,4-dichlorophenoxyacetic acid (2,4-D) at a range of 0.5 mg/L-3.0 mg/L either alone or in combination of cytokinins; kinetin (Kn) and thidiazuron (TDZ) at a range of 0.1 to 0.5 mg/L. The most effective treatment for adventitious root induction was supplemented with 0.5 mg/L NAA which produced the highest number and length of roots with a mean value of 7.667 ± 2.082 and 1.733 ± 0.416 respectively. The most effective combination of auxin and cytokinin for adventitious root induction was 0.5 mg/L NAA+ 0.1 mg/L Kn that produced the highest number and length of roots with a mean value of 3.400 ± 2.408 and 1.330 ± 1.021 respectively. The best treatment for the biomass production of adventitious root of *B. rotunda* was 0.5 mg/L NAA + 0.1 mg/L TDZ with a mean value of 3.684 ± 1.233 g fresh weight and also produced the highest number of shoots with a mean value of 3.167 ± 1.169 . Histological examinations of adventitious root of *B. rotunda* revealed the presence of important root anatomic structures including epidermis, endodermis, pericycle, phloem, cortex, late metaxylem and protoxylem pole. HPLC analysis showed that both pinocembrin and cardamonin were present in the extract of adventitious root suspension cultures of *B. rotunda*. The highest compound contents were 13.09 ± 3.92 mg/g for pinocembrin whereas 16.95 ± 4.01 mg/g for cardamonin when the adventitious root were respectively cultured into 0.5 mg/L NAA + 0.3 mg/L TDZ and 0.5 mg/L NAA + 0.1 mg/L TDZ. The result shows that the PGRs (NAA and TDZ) is a good approach for enhancing the secondary metabolite content in adventitious root cultures of *B. rotunda*. Further research using bioreactor, elicitors, application of hairy root culture, histological analysis at cellular level and pharmacological properties should be done as *B. rotunda* is an important aromatic and medicinal plant.

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