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TABLE OF CONTENTS

Putu Gede Martha Setiawan, Woro Anindito Sri Tunjung, Luthfi Nurhidayat
Effect of diazepam on kidney function and histological structure of white rat's kidney **1**

Asep Sadili
Trees structure and composition on logged over forest at Kopiyo Meratus, Hulu Sungai Tengah District, South Kalimantan **7**

Saikhu Akhmad Husen, Dwi Winarni, Firas Khaleyla, Septian Hary Kalqutny
Activity test of various mangosteen (*Garcinia mangostana*) pericarp extract fractions to decrease fasting blood cholesterol levels and lipid peroxidation activity in diabetic mice **13**

Sarno Sarno, Moh. Rasyid Ridho
The growth of seedlings of rhizophoraceae in the nursery utilizing fresh water **18**

Djajadi Djajadi, Sulis Nur Hidayati, Roni Syaputra
Effect of Organic Matter and Si Liquid Fertilizer on Growth and Yield of Sugar cane **22**

Sutini Sutini, Susilowati Susilowati, M. Rasjad Indra, Djoko Agus Purwanto
Growth and accumulation of flavan-3-ol in *Camellia sinensis* through callus culture and suspension culture method **27**

Angga Yudaputra, Rizmoon N. Zulkarnaen, Irvan F. Wanda, Joko R. Witono
Flowering of *Areca catechu* in Bogor Botanic Gardens **32**

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Original Article

Growth and accumulation of flavan-3-ol in *Camellia sinensis* through callus culture and suspension culture method

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Abstract

This study was aimed to assess flavan-3-ol biomass in *C. sinensis* through callus cultures and suspension cultures derived from leaf explants. Callus initiation of both cultures were using Murashige and Skoog medium were enriched with plant growth regulators Naphthalene Acet ic Acid 3.0 mg/L and kinetin 2.0 mg/L. The procedures in this study were: (1) callus initiation by cutting the leaves of *C. sinensis* shoots then planted on Murashige and Skoog medium that were enriched with plant growth regulators, (2) sub callus culture on fresh medium that enriched with the same growth regulators, (3) suspension culture initiation of liquid callus, (4) growth examination of callus and suspension cultures in week 12, (5) e xamination of qualitative-quantitative content of flavan-3-olin suspension cultures at week 4. The results show that suspension cultures contain biomass flavan-3- ol that increase in the same manner of the increase of callus age and weight

Key words: biomassa flavan-3-ol, *C. sinensis*, Murashige and Skoog, naphthalene acetic acid, kinetin.

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Introduction

Flavan-3-ol is one of the secondary metabolites contained in *C.* sinensis, which can be utilized in various fields including medical industry. This compound is classified into phenol group which can performs anti-oxidant activity (Giri et al., 2012; Vinha et al., 2013; El-kassas 2014; Khalaf et al., 2008; Makanjuola et al., 2015). In agricultural industry, flavan-3-ol monomer in the form of catechins can be used as allelochemical (Inderjit, 2008; Castells E. 2008). It also *trimethyl xanthina* is utilized in food industries to supports food and beverage becoming functional (Sutini et al., 2011; 2014; 2016; Ferruzzi, 2012; Astri, 2009; Ivan, 2005).

Flavan-3-ol can be harvested from *C. sinensis* plant in disturbed farming area after 3-5 years of plantation with special treatments (Ryo, 2009; Asri, 2010). *In vitro* culture method has been used widely for various purpose such as secondary metabolites production, obtaining identic clone of *C. sinensis*, obtaining Jatropha that is resistant to drought, and obtaining sugar cane with abundant yield (Sumaryono et al., 2005; Sandal et al., 2005; Mochamad et al., 2012; PTPN. 2014). Callus cultures form was crumb and varies, so it should be kept in optimum condition that corresponds to research purposes. Production of flavan-3-ol through *in vitro* culture (callus cultures and suspension) has several advantages, namely less time consuming compare to field production, more efficient (laboratory scale results that can provide industrial needs), and to free from climatic change effect. Therefore, this study was purposed to evaluate the biomass of flavan-3-ol in callus cultures and suspension cultures derived from leaf explant of *C. sinensis*.

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Methods

Initiation of Callus

Initiation of callus was done by cutting *C. sinensis* leaves then planted on Murashige and Skoog medium (MS) enriched with plant growth regulators Naphthalene Acetic Acid (NAA) and kinetin (Aljabari et al., 2014; Farzana et al., 2011; Nikolaeva et al., 2009; Orihara and Furuya, 1990). *C. sinensis* leaves were washed with running water for 30 minutes, and then dipped in mix solution of fungicide-bactericide 3% and 5% of calcium hypochlorite. The leaves then were rinsed with distilled water and soaked in ascorbic acid 3% for 15 minutes in culture tubes. Sterilization was done by soaking the leaves in 5% of sodium chlorine solution for 30 minutes then rinsed three times with sterile distilled water. Leaves were cut in sterile area for about 1 cm with forceps. Leave cuts were initiated in solid medium MS enriched with NAA 3 mg/L kinetin and 2 mg/L at laminar air flow cabinet. At last, they were stored in temperature 20-25 °C (Sutini et al., 2008, 2012).

Callus Subculture

Calluses were cut into 2-4 pieces sub-callus using forceps and needles. The sub-calluses were subsequently transferred to the fresh medium that has similar composition as initiation medium. Sub-calluses were stored at the same temperature as previous step or 20-25°C (Sutini et al., 2008, 2012).

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