PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITIES OF LEAF, NODE AND *IN VITRO*-INDUCED CALLUS OF *Bougainvillea glabra* Choisy USING DIFFERENT SOLVENTS

By

NASRAT MOHAMMAD NASIM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

June 2022

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DEDICATION

This thesis is dedicated to my family
Malika Rahimi, Najla Nasrat, Mohammad Emran Nasrat and Viana Nasrat
and
My respected mentors
Dr. Mohd Hakiman Mansor
Prof. Madya Dr. Siti Zaharah Sakimin

Thank you for your endless patience, encouragements, advices,
sacrifices and motivations throughout my master degree journey

Abstract of the thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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June 2022

Chairman : **Mohd Hakiman Mansor, PhD**
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Bougainvillea, popularly known as ‘Bunga kertas’ in Malaysia, is thoroughly explored for nutritional and medicinal purposes. *Bougainvillea* has been shown to possess alkaloids, flavonoids, cardiac glycosides, saponins, and beta-cyanins, which are widely used in folk medicine to treat different illnesses. Despite its major conventional therapeutic importance, only limited attempts have been made to investigate this species' chemical and pharmacological properties in relation to its medicinal uses. Therefore, this study was conducted to determine the effect of *in vitro* induced callus under different light conditions and plant growth regulators on phytochemical and antioxidant activities using different extraction solvents. In this study, the leaves and nodes were collected and dried in an oven at a temperature of 40 °C for 48 hours until the weight remained constant. The dried materials made into fine powder using mortar and pestle, then different solvents (aqueous, ethanol, acetone, and hexane) were used for extraction purposes. Subsequently, the phytochemicals (total phenolic and total flavonoid contents), and antioxidant activities such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2’-azinol-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and iron (II) chelating activity were assayed using a spectrophotometer. For the second experiment, leaves and nodes of the plants were used for *in vitro* surface sterilization. Different concentrations of Clorox® (10, 20, 30 and 40%) and exposure time (10, 15, and 20 minutes) were applied. For the third experiment, the sterilized nodal segments were used for *in vitro* callus induction. Different concentrations and combinations of 2,4-D (2.5, 5, and 7.5 µM) and BAP (0.5, 1, and 1.5 µM) were used for callus induction purposes under dark and light conditions. Finally, the phytochemicals screening and antioxidant activities of *in vitro*-derived calluses and conventional propagated donor plants were measured as per the first experiment to find out and compare the influence of callus induction on secondary metabolite production potential. The results from the first experiment showed that leaf segment extracted with aqueous extract had a significantly superior effect than other plant parts and solvents where it achieved the highest total phenolic content (58.04 mg GAE/g DW), total flavonoid content (127.93 mg RE/g DW), DPPH free radical scavenging (10.08 mg TE/g DW), ABTS scavenging activity (1.59 mg TE/g DW), respectively. Based on the results of the callus induction experiment, the maximum days (18.25) to callus initiation recorded when nodal segment cultured on woody plant medium (WPM) supplemented with 7.5 µM 2,4-D without addition cytokinin under light condition. On the contrary, the minimum days (7) to callus initiation were recorded when nodal treated with 2.5, 5 and 7.5 µM 2,4-D + 1 and 1.5 µM BAP under dark conditions, which showed significant differences between various concentrations and combinations of auxin and cytokinin. This study showed that the dark condition is better for callus induction than light condition.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Master Sains

AKTIVITI FITOKIMIA DAN ANTIOKSIDAN DAUN, NOD DAN KALUS DARIPADA *IN VITRO Bougainvillea glabra* Choisy DALAM PELBAGAI PELARUT BERBEZA

Oleh

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Bougainvillea, dikenali sebagai Bunga kertas di Malaysia, diterokai secara menyeluruh bagi tujuan pemakanan dan perubatan. *Bougainvillea* telah terbukti mempunyai alkaloid, flavonoid, glikosida, saponin dan beta-sianin, yang digunakan secara meluas dalam perubatan tradisional untuk merawat pelbagai penyakit. Walaupun terdapat kepentingan terapeutik yang banyak, hanya sedikit kajian telah dijalankan untuk mengetahui sifat kimia dan farmakologi spesies ini berhubung dengan kegunaan perubatannya. Oleh itu, kajian ini dijalankan untuk menentukan kesan induksi kalus secara *in vitro* di bawah keadaan cahaya yang berbeza dan pengawalatur tumbesaran tumbuhan terhadap aktiviti fitokimia dan antioksidan menggunakan pelarut yang berbeza. Dalam kajian ini, daun dan nod diambil dan dikeringkan di dalam ketuhar pada suhu 40 °C selama 48 jam atau sehingga tiada perubahan berat. Sampel kering dijadikan serbuk halus menggunakan mortar dan alu, kemudian pelarut yang berbeza (akuas, etanol, aseton, dan heksana) digunakan untuk pengekstrakan. Selepas itu, fitokimia (jumlah kandungan fenolik dan flavonoid), dan aktiviti antioksidan seperti 2,2-difenil-1-picrilhidrazil (DPPH), asid 2,2'-azinol-bis (3-etilbenzothiazolin-6-sulfonik (ABTS) dan aktiviti *chelation* besi (II) telah diukur menggunakan spektrofotometer. Pada eksperimen kedua, daun dan nod tumbuhan disterilkan permukaan secara *in vitro*. Kepekatan Clorox® yang berbeza (10, 20, 30 dan 40%) dan tempoh masa (10, 15 dan 20 minit) digunakan. Pada eksperimen ketiga, segmen nod yang steril digunakan untuk induksi kalus *in vitro*. Kepekatan dan kobinasi berbeza 2,4-D (2.5, 5 dan 7.5 µM) dan BAP (0.5, 1 dan 1.5 µM) telah digunakan untuk tujuan induksi kalus dalam keadaan gelap dan terang. Akhir sekali, penyaringan fitokimia dan aktiviti antioksidan bagi kalus daripada *in vitro* dan tumbuhan penderma yang dibiakkan secara konvensional diukur sebagai eksperimen pertama untuk mengetahui dan membandingkan pengaruh induksi kalus terhadap potensi pengeluaran metabolit sekunder. Hasil eksperimen pertama menunjukkan bahawa segmen daun yang diekstrak dengan pelarut akueus mempunyai kesan unggul yang ketara berbanding bahagian tumbuhan dan pelarut lain di mana masing-masing mencapai jumlah kandungan fenolik tertinggi (58.04 mg GAE/g DW), jumlah kandungan flavonoid (127.93 mg RE/g DW), DPPH penghapusan radikal bebas

(10.08 mg TE/g DW), ABTS aktiviti penipisan (1.59 mg TE/g DW). Hasil dari eksperimen induksi kalus, hari maksimum (18.25) bagi penghasilan kalus pertama diperhatikan apabila segmen nod dikultur dalam medium tumbuhan berkayu (WPM) ditambah dengan 7.5 µM 2,4-D tanpa penambahan sitokinin dalam keadaan terang. Sebaliknya, hari minimum (7) bagi penghasilan kalus pertama diperhatikan apabila nod dirawat dengan 2.5, 5 dan 7.5 µM 2,4-D + 1 dan 1.5 µM BAP dalam keadaan gelap, menunjukkan perbezaan ketara antara pelbagai kepekatan dan kombinasi auksin dan sitokinin dalam keadaan cahaya yang berbeza.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
UPM	Universiti Putra Malaysia
BAP	6-benzylaminopurine
2,4-D	2,4-dichlorophenoxyacetic acid
TPC	Total phenolic content
TFC	Total flavonoid Content
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2’-azinol-bis (3-ethylbenzothiazoline-6-sulfonic aci**d)**

CHAPTER 1

INTRODUCTION

1.1 Background of the study

The genus of *Bougainvillea* belongs to the family Nyctaginaceae, which is one of the utmost valuable ornamental and medicinal plants native from South America (Brazil, Peru, and northern Argentina). It is commonly used for many industries such as horticulture, medicinal, cosmetic, agriculture, and environmental in tropical and subtropical regions due to broad stability in the various agro-climate areas. The name derives from the French navigator Louis Antoine de Bougainville, who was the first to state this plant in Brazil in 1786 (Naito et al., 2020; Kumar et al., 2020).

Medicinal plants or herbs are enriched in various phytochemicals, which have been shown to have various biological effects and should be researched further (Saleem et al., 2020). One of the medicinal plants that has currently expanded much attention because of its therapeutic constituents is *Bougainvillea glabra* Choisy, locally known as 'Bunga kertas' in Malaysia. Since 1970, the chemical components of the *Bougainvillea* genus have been extensively studied. Phytochemical studies were carried out to classify various bioactive compounds from the extracts of different polarity solvents from buds, leaves, or bracts with or without flowers, bark stems, and roots of the species (Abarca-Vargas and Petricevich, 2018). The type of solvents used in the extraction technique has a big impact on the success of determining biologically active compounds from plant material. Extraction is essential in phytochemical isolation for detecting pharmacologically active constituents in plant parts (Thomas et al., 2020). Selecting a suitable solvent system for extraction is crucial for standardization of pharmaceutical products. It isolates the essential compounds in medicinal plants while excluding the unwanted matrix (Kumarasamy and Selvi, 2020). *Bougainvillea* is mainly grown in tropical and subtropical climates, and there is a lack of raw material in temperate weather to treat the diseases. In such places, this plant is propagated in the greenhouse to protect from cool weather and can produce raw material, but by this method, raw material production is low. A massive amount of raw materials is needed to extract the high amount of secondary metabolites. This problem can be overcome by producing the secondary metabolites in the laboratory by plant tissue culture technique (Haida et al., 2020). Plant tissue culture has arisen as an approaching instrument and forms the foundation of plant biotechnology. Plant tissue culture is conducted by excising any part of the plant and culturing it onto a nutrition medium that has been created artificially in culture vessels under aseptic conditions and maintained under controlled conditions (Colombo et al., 2018).

As far as we know, there are not enough studies on the influence of white light and dark on callus induction and phytochemical/antioxidant activity in *B. glabra.*

Hence, we studied the effect of light quality on morphological and biochemical components of *in vitro* grown node-derived callus cultures of *B. glabra*. This research will help understand the effect of light on the production of commercially essential secondary metabolites and their optimization in the *in vitro* cultures of *B. glabra* Choisy.

1.2 Statement of the problem

B. glabra is vegetatively propagated by stem cuttings. However, in the traditional propagation method, the production of secondary metabolites is known to be unsuitable due to external factors in the environment such as climate, plant pests and diseases, and fertilizer application. Therefore, the plant tissue culture technique is the most suitable technique for the consistent production of secondary metabolites under a controlled environment. Protocol for micro propagation of many woody plants is different, because each species could be influenced by different factors from which plant growth regulators, physical conditions, growth parameters and growing media are the most important ones. Although, micro propagation protocol for *B. glabra* has been carried out before, but no protocol can be found on callogenesis of *B. glabra* Choisy under different light regime. So, there is a need to develop a micro propagation protocol for callogenesis of *B. glabra* not only as a mass of biomass but also as somatic embryogenesis for future work.

1.3.1 Objectives of the study

The objectives of this research were;

1. To determine the suitable extraction solvent on phytochemical and antioxidant activities of leaf and node in *Bougainvillea glabra* Choisy.
2. To compare the effective concentration of Clorox® and exposure time for explant surface sterilization of *Bougainvillea*.
3. To determine the optimum concentration of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzyl amino purine (BAP) on callus induction of *Bougainvillea* under light conditions.
4. To compare the phytochemical and antioxidant activities of *in vitro*-induced callus and conventionally propagated nodal segment.

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