

UNIVERSITI TEKNOLOGI MARA

**ELICITATION AND
ENHANCEMENT OF PHENOLICS
AND FLAVONOIDS PRODUCTION
IN *Clinacanthus nutans* CULTURES**

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ABSTRACT

Clinacanthus nutans (*C. nutans*) plant possesses with antioxidant properties that are attributed to the presence of phenolics and flavonoids compound. However, the compounds concentration in plants is sometimes quite low, varies according to development stage and limited by environmental constraints. Elicitation technique have been recommended as an efficient method of large producing plant beneficiary compounds in *in vitro* plant at short period of time. Thus, the aim of this study was to increase the phenolic and flavonoid content of *C. nutans* by eliciting the *in vitro* cultured plant cell and tissue. The research method started with axenic culture establishment by surface sterilization, followed by the optimization of culture medium for production of differentiated plant tissue. For callus and cell suspension, the culture medium and condition had been optimized. A comparison on bioactive compound and antioxidant activities between regenerated shoots, roots, callus and cell were done. In the elicitation step, *in vitro* cultured and intact plant were treated with wood vinegar (WV), methyl jasmonate (MJ), and yeast (Y). Chalcone synthase enzyme activities and quercetin quantification were measured from all treated samples. The correlation between all parameters was also conducted to highlight the interactions between them. Findings showed that mercuric chloride at 0.2% gave the highest percentage of axenic explants and viability. For adventitious shoot formation, MS medium supplemented with 2.0 mg/L Benzyl-aminopurine (BAP) and 1% WV produced the highest number of shoots (10.60) with the longest shoot length (2.51 cm) on week 8. The MS medium supplemented with 0.5 mg/L Naphthalene acetic acid (NAA) was the best medium for indirect adventitious root formation from embryogenic calli (68.33% and 4.31 roots) on week 6. For callus induction, binodal explants on MS medium enriched with 0.5 mg/L NAA showed good callus proliferation (93.11 %) with the highest callus fresh weight (4.87 g/L) on week 6. For cell suspension culture, 0.5 mg/L NAA and 1.5 mg/L BAP gave the significant highest increment cell number (20.44×10^4 NOC/mL) in the exponential phase with the highest production of cell biomass. A comparison between plant tissue, callus and cell culture shows that the callus exhibits the significant lowest number of bioactive compounds and antioxidant activities. Thus, to maximize the production of the beneficiary compounds, callus not been selected in the elicitation procedure. In the elicitation step, WV treated samples especially adventitious root produced the highest production of bioactive compounds, followed by MJ and Y elicited. The phenolic and flavonoid compounds of 50 ppm WV treated adventitious root cultures were successfully enhanced to 53.29 and 41.74-folds higher than control, respectively. The CHS highest enzymatic activities (270.73 nkat/mg protein) and quercetin compounds (48.64 mg/g DW), were also attained in WV dose treatment towards adventitious root. Major acetic acid constituents in WV possibly stimulated plant defence response and increase the enzymatic activities in compounds production which in turn contributed to the increase in quercetin amount and antioxidant activities. Study concluded that the sterilization step, culture medium and condition significantly influenced the initiation of *in vitro* cultured shoot, root, callus and cell suspension culture. Then, selection of appropriate culture sample, elicitor types, concentration and exposure period successfully increase the bioactive constituents resulting in elevated antioxidant activity of *C. nutans in vitro* culture.

Keywords: *Clinacanthus nutans*, phenolics, flavonoids, antioxidant, elicitation

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CHAPTER ONE

INTRODUCTION

1.1 Research Background

People living in rural parts of developing nations sometimes have no other choice than to rely on medicinal plants as their only source of healthcare. Because of a lack of access to modern medical facilities, over 80 % of people living in Asia, Africa, and the Middle East continue to rely on traditional medicines, such as herbal cures, to meet their main healthcare requirements. Despite their reliance on contemporary medicine, emerging countries such as Canada, France, Germany, and Italy have populations that use traditional medicine at rates ranging from 70% to 90% of the total (Ekor, 2013). For the treatment of natural remedies, research into novel and better therapeutic substances derived from plants is now continuing (Alam *et al.*, 2021).

In this study, *Clinacanthus nutans* (*C. nutans*) was investigated as a possible therapeutic herb. The plant is native to Asia and in Malaysia is known as Sabah Snake Grass. *Clinacanthus nutans* plant extract has traditionally been used to treat a wide range of ailments, including bacterial infections, animal bites, and viral infections (Tuntiwachwuttikul *et al.*, 2004; Haida & Hakiman, 2019). The plant is also often used to treat cancer, herpes simplex virus, varicella-zoster virus lesions, skin rashes, and renal issues (Yusof *et al.*, 2018). The presence of phenols, flavonoids, glycosides, terpenoids, steroids, saponins, and carbohydrates was detected in this study's preliminary examination of the phytochemical composition of *C. nutans* leaf extracts.

Because of their diverse bioactivities and interesting prospective uses in the pharmaceutical industry, many researchers are interested in investigating plant secondary metabolites, particularly phenolics and flavonoids. Both phenolics and flavonoids have several biological effects, including reducing low-density lipoprotein levels in the blood, inhibiting platelet aggregation, increasing free radical scavenging, and restricting cell proliferation (Woodman & Chan, 2004; Lutz *et al.*, 2019). Previous research discovered that *C. nutans* contains a high concentration of phenolics, and the synergistic activities of these phenolic compounds have been linked to the treatment and prevention of oxidative stress-related disorders. Flavonoids linked to anti-cancer properties, such as quercetin, catechin, kaempferol, and luteolin, have previously been