

Establishment of Axenic Explants and Callus Induction of *Vernonia amygdalina* Del.

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ABSTRACT

Vernonia amygdalina is a plant with great medicinal potentials due to the phytochemicals it possesses. The establishment of axenic culture and callus induction are important as the primary steps to acquire callus and cell suspension cultures which are prospective to be used as alternatives in the production of phytochemicals. The effect of different regimes of sterilization unveiled that explants treated with mercuric chloride showed significant difference in the mean number of axenic explants, but observations of serious necrosis in all explants that later lead to death were recorded. Whilst, the explants treated with Clorox[®] were not significantly difference in the mean number of axenic explants but the explants obtained were healthy and the growth was sustained. For callus induction, there was no significant difference in the callus induction frequency (CIF) among different concentrations of 2,4-D or picloram. In conclusions, the explants were recommended to be surface sterilized with 15% of Clorox[®] for 15 minutes as it was more economical and less time consuming. Whereas, MS media supplemented with 0.5 mg/L 2,4-D or 0.5 mg/L picloram would be a better choice for callus induction due to observation of root formation at lower concentrations of 2,4-D.

Keywords: *Vernonia amygdalina*, axenic, callus induction, surface sterilization, plant growth regulator, phytochemical

INTRODUCTION

Bitter leaf or scientifically known as *Vernonia amygdalina* Del. is a small tree or shrub that belongs to the family Asteraceae (Fomum, 2004; Ofori *et al.*, 2013). *Vernonia* genus has a total of 109 species being reported to have medicinal properties. The most commonly used species is *V. amygdalina* (Toyang & Verpoorte, 2013). The leaf of *V. amygdalina* has been used as traditional medicine to treat fever, malaria, dysentery, hepatitis, diarrhea as well as cough. Also, the leaves are used to treat headache, stomachache, scabies, malaria and gastrointestinal disorders (Fomum, 2004). Secondary metabolites or phytochemicals possess in *V. amygdalina* are the one that is responsible for pharmacologic activities such as hypoglycaemic, hypolipidemic, antimalarial, antioxidant and anti-inflammatory activities (Audu *et al.*, 2012). The major bioactive compounds of *V. amygdalina* are sesquiterpene lactones and stigmastane-type steroid glucoside compounds (Yeap *et al.*, 2010).

However, bitter leaf with great medicinal potentials is grown for personal needs rather than for commercial purposes. The processed

leaves of *V. amygdalina* are sold at high price as compared to raw commodity in Cameroon. Conventional propagation of *V. amygdalina* is commonly by rooting of stem cuttings where mature stems are selected while seeds are also used (Fomum, 2004). Propagation is plausible by using seeds collected from dry flower heads but stem cuttings are used in general due to faster growth (Ofori *et al.*, 2013). In spite of conventional propagation, studies shown that *in vitro* micropropagation played a key role in clonal propagation of elite genotypes of *V. amygdalina* for the production of secondary metabolites that are valuable by pharmaceutical means (Khalafalla *et al.*, 2007).

Plant cell culture is promising potential alternative sources to produce secondary metabolites at commercial scale (Rao & Ravishankar, 2002). There are reports on the production of phytochemicals in both suspension and callus cultures. Some secondary metabolites are produced in higher concentrations in cultured cells as compared to the whole plants of the same species (Linden, 2002). On top of that, plant cell culture makes great strides for the production of pharmaceuticals and chemicals as the synthesis