Standardization of Callus Induction Protocol for Extraction of Secondary Metabolites from *Andrographis paniculata* (Kiratha)

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Andrographis paniculata, known as *Kiratha* or *Heen binkohomba* in Sri Lanka is an important medicinal plant with some valuable secondary metabolites. Cultivation and extraction of secondary metabolites from this plant are constricted due to limitations in conventional propagation methods. Therefore, this study aimed to standardize a callus induction protocol for this plant to extract secondary metabolites. Nodal cuttings, leaves, and seeds were tested for surface sterilization with two Clorox concentrations (10%, 15 %) and three exposure periods (5 min, 10 min, 15 min). For callus induction and multiplication, the established cultures were transferred to Murashige and Skoog (MS) medium supplemented with various combinations and concentrations of Naphthalene Acetic Acid (NAA) (1.0, 2.0 mgL^{-1}) and 2,4-Dichlorophenoxy acetic acid (2, 4 D) (0.5, 1.0, 1.5, 2.0 mgL^{-1}). All experiments were arranged according to a completely randomized design with 25 replicates. Non-contamination percentage, amount of calli formed, and appearance of callus by its colour and growth were recorded and subjected to analyse variance and Kruskal-Wallis test as required. Seeds were the most suitable explant for callus induction because of 100% non-contamination in all treatments. However, 0% of non-contamination was observed ($p \leq 0.05$) for nodes and leaves. When considering minimum resources allocation, 10% Clorox for 5 minutes exposure time period was selected as the best surface sterilization method. The highest calli formation (91.8%) was observed in MS medium supplemented with 2.0 mgL^{-1} 2, 4 D + 1.0 mgL^{-1} NAA ($p \leq 0.05$). Result was significantly different only from the treatments with 1.0 mgL^{-1} 2, 4 D + 1.0 mgL^{-1} NAA and 1.5 mgL^{-1} 2, 4 D + 2.0 mgL^{-1} NAA. Excellent callus with non-brownish colour (83%) and callus with excellent growth (67%) were observed in the same treatment. Secondary metabolites will be extracted and compared with mother plants in future studies.

Keywords: Callus, Induction, Extraction, *Andrographis paniculata*