Development of cell suspension cultures of *Gyrinops walla* for agarwood resin extraction

Arosha Nirmal Buddhapriya and Wathuge Thamara Priyanthi Sandun Kumari Senarath

Department of Botany, University of Jayewardenepura, Nugegoda, Sri Lanka

Natural habitats for *Gyrinops walla* (Thymelaeaceae) are disappearing fast due to geographical instabilities and climate change. Bioactive compounds of agarwood currently extracted from plant raw materials and incorporated in cosmetics especially in perfumes. This led to consider the possibilities of using cell suspension cultures as an alternative for the production of phytochemicals present in agarwood resin. Cell suspension cultures were established by transferring white friable calli of *G. walla* into Murashige and Skoog (MS) liquid medium supplemented with 1-naphthaleneacetic acid, NAA (3.0 mg/L), 6-benzylaminopurine,BA (1.0 mg/L) and agitated on a rotary shaker at 100 rpm at 25 ± 1 °C in the dark. After eight weeks, an aliquot of homogenized liquid culture was centrifuged at 13,000 rpm and phytochemicals were extracted from the supernatant of liquid culture with dichloromethane using a separatory funnel. Thereafter, dichloromethane was evaporated using a rotary evaporator. The crude was re-dissolved in ethyl acetate and centrifuged at 5,000 rpm prior to gas chromatography-mass spectrometry analysis. The phytochemical constituents were identified using an external standard method from chromatograms obtained in GC-MS analysis. Four weeks old cell cultures appeared in brownish yellow colour due to accumulation of secondary metabolites during the incubation period. The cell aggregates were well dispersed throughout the liquid culture and globular in shape. Seven important phytochemicals were identified in chromatograms which present in agarwood constituents such as azulenone, stigmasterol and γ-sitosterol etc. Further optimization of cell cultures of *G. walla* could extend and enhance the synthesis of bioactive compounds in agarwood, can ultimately provide a continuous and reliable source of natural products.