



## ***IN VITRO* SEED GERMINATION AND CALLUS INDUCTION OF *Catunaregam spinosa***

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### **Abstract**

*Catunaregam spinosa* is a medicinal plant with significant diversity of phytochemicals. There is insufficient evidence based on *in vitro* growth of *C. spinosa*. *In vitro* seed germination and callus induction of *C. spinosa* were studied as part of direct and indirect organogenesis. Best surface sterilization protocol was tested with different concentrations and time intervals of Carbendazim and Clorox. Seed germination protocol was optimized using mature seeds grown in Murashige and Skoog (MS) medium treated with different concentrations (1.0-4.0mg L<sup>-1</sup>) of Gibberellic Acid (GA<sub>3</sub>). Leaf discs from *in vitro* grown seedlings were cultured on MS medium supplemented with different concentration combinations (1.0-6.0 mg L<sup>-1</sup>) of 6-Benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA). Experiment was conducted in Completely Randomized Design with ten replicates. Data was statistically analysed using ANOVA and means were compared at significant level of  $p=0.05$ . Disinfecting with 0.3% (w/v) Carbendazim for 10 minutes and 10.0% Clorox for 10 minutes followed by two washings of sterile distilled water found to be the best surface sterilization technique. Highest seedling height was recorded in MS medium supplemented with 4.0 mg L<sup>-1</sup> of GA<sub>3</sub> after 16 days of incubation. Highest mean dry calli weight (0.097±0.92 g) was recorded at concentrations of 1.0 mgL<sup>-1</sup> BAP and 3.0 mg L<sup>-1</sup> NAA after 3 months of incubation under dark conditions. Visual observations highlighted morphologically different calli at low (compact and green) and high (friable and white) concentrations of NAA. Statistical data showed calli induction and growth is significantly different upon concentrations of BAP and NAA. This study describes an efficient sterilization protocol, best medium for *in vitro* seed germination and callus induction of *C. spinosa*.

**Keywords:** *Catunaregam spinosa*, *in vitro* growth, callus induction, seed germination