

EVALUATION OF TOXICITY OF AQUEOUS EXTRACT OF *HEMIDESMUS INDICUS* R. BR. (*IRAMUSU*) USING BRINE SHRIMP ASSAY

H.D.T. Madhuranga^{1*}, G.K.B.C.M. Gunasena¹, W.A.S.H. Dharmasena¹, T.G.K.U. Thambiliyagoda¹, M.C.M. Riham¹, D.N.A.W. Samarakoon¹ and H.M.L.P.B. Herath²

¹Department of Biomedical Science, Faculty of Health Science, KIU, Battaramulla, Sri Lanka.

²Department of Chemistry, Faculty of Science, University of Colombo, Colombo 03, Sri Lanka.

*tmadhuranga@kiu.ac.lk

Hemidesmus indicus R. Br. [*Iramusu*], belongs to the family Apocynaceae and is used in various ayurvedic preparations in Sri Lanka. Even though the medicinal value of the plant has been extensively studied, the toxicity of the plant remains unknown. Therefore, it is vital to identify the toxicity for dosage determination of formulations. This study aimed to identify the minimum toxic concentration of the crude extract of *H. indicus* using a brine shrimp assay. Using the maceration technique, the air-dried plant material was powdered and extracted into distilled water in a 1:3 ratio. The dilution series was prepared with two-fold dilution, 1g of bark aqueous extract powder dissolved in 4 ml of distilled water, starting from 1g mL⁻¹ to 2 × 10⁻³ g mL⁻¹ concentration and observed the ability to kill a cultured larva (nauplii). Ten larvae were exposed to plant extract for 24 hours, the number of live larvae was observed using the naked eye, and the mortality percentage and LC₅₀ were calculated using GraphPad Prism 9.5.1 software according to a log concentration and percentage mortality graph and compared with Meyer's or Clarkson's toxicity scale (if LC₅₀ < 1000 µg/ml considered as toxic, if LC₅₀ > 1000µg/ml considered as non-toxic). The study was triplicated. The highest mortality percentage (100%) was observed among 63×10⁻³ g mL⁻¹ - 1g mL⁻¹. The resulting LC₅₀ was = 0.0482 g mL⁻¹ (48,200 µg/mL) for the aqueous extract. The results confirmed that the plant extract has not shown toxic properties according to Meyer's or Clarkson's toxicity scale. In conclusion, the concentration range from 1g mL⁻¹ to 2 × 10⁻³ g mL⁻¹ has not shown any significant toxicological activities with regards to the toxicity scale; the minimum toxic concentration of *H. indicus* in the brine shrimp assay is 0.0482 g mL⁻¹ (48,200 µg/mL). Therefore, it is recommended to determine further by an activity-guided fractionation to identify less toxic, active compounds present in the plant extract. However, "*Iramusu*" is a safe Ayurvedic preparation according to the results of the toxicity assay conducted (according to Meyer's or Clarkson's initial threshold set < > 1000 ug/mL).

Financial assistance from the KIU undergraduate research fund is acknowledged.

Keywords: Brine shrimp assay, *Hemidesmus indicus*, Medicinal properties, Toxicity