Assessment of Antioxidant Activity by Hydrogen Peroxide Assay for Combined Herbal Formulations of *Cinnamomum verum, Curcuma longa, and Ocimum tenuiflorum* Within the Non-Toxic Range as Assessed by Brine Shrimp Lethality Assay

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Medicinal plants have been utilized globally for centuries to treat infectious and non-infectious diseases, and their therapeutic potential has been validated by scientific research. *Cinnamomum verum* bark, *Curcuma longa* rhizome, and *Ocimum tenuiflorum* leaves are well documented for their individual antioxidant activity, particularly their ability to scavenge reactive oxygen species such as hydrogen peroxide (H_2O_2), an immediate inducer of oxidative stress and cellular damage. The study is novel in evaluating the synergistic antioxidant capacity of a combined aqueous and ethanolic extract of these three herbs using the hydrogen peroxide scavenging assay, in a non-toxic concentration range established by the brine shrimp lethality test. The study was conducted under a controlled in vitro laboratory setting using conveniently sampled plant materials known for their individual antioxidant properties. Authenticated plant materials were air-dried, powdered, and macerated separately in ethanol and distilled water (1:5 w/v). Aqueous and ethanolic extracts were mixed and evaporated to obtain a crude extract. Herb powders were mixed in a 1:1:1 ratio to provide a combined extract, and one gram of it was dissolved in 10 mL of distilled water. Fifteen two-fold serial dilutions (5×10^4 to 3.013 µg/mL) were prepared and tested for toxicity using the brine shrimp lethality test. The non-toxic concentrations were used for antioxidant assays. Reaction mixtures containing phosphate buffer, H_2O_2, and the combined extract or ascorbic acid (standard) were incubated at room temperature for 10 minutes, and absorbance was measured at 300 nm. GraphPad Prism 10.4.1 analysis indicated dose-dependent hydrogen peroxide scavenging. The extract exhibited maximum scavenging activity of 10% at 3125 µg/mL, compared to 14% for the standard. Mean values were 4.004% (extract) and 4.606% (standard) with standard deviations of 3.715% and 4.033%, respectively. The LC_{50} value of the brine shrimp assay was 3354 µg/mL, which indicates moderate toxicity. Despite expectations of synergy, the combined extract showed reduced efficacy compared to individual herbs, possibly due to antagonistic interactions, suboptimal ratios, or interference with bioavailability, highlighting that synergy in herbal mixtures is not guaranteed. Careful assessment of herb–herb interactions is crucial in formulating effective polyherbal antioxidants.

Keywords: Brine shrimp lethality assay, toxicity, *Cinnamomum verum*, *Curcuma longa*, *Ocimum tenuiflorum*, hydrogen peroxide scavenging assay