

Formulation of an Antioxidant Nutraceutical through the Optimization of Mixing Ratio of Antioxidant-Rich Underutilized Fruit Based on Experimental Mixture Design

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Sri Lanka, a tropical nation, boasts a diverse array of fruit varieties. Beyond commonly consumed fruits, several underutilized fruit are traditionally used, especially in rural areas. Those little-known fruit are often incorporated into traditional indigenous medicine. The antioxidants present in these fruit can prevent and mitigate the oxidative damage caused by reactive oxygen species and non-radicals to lipids, proteins, and nucleic acids. This study optimized the mixing ratio of *Manilkara zapota* L. (Sin: Sapodilla), *Cynometra cauliflora* L. (Sin: Naminan), *Elaeocarpus serratus* L. (Sin: Veralu), and *Flacourtia indica* L. (Sin: Ugurassa) dried fruit powders to select best combination for the formulation of nutraceutical containing high total phenolic content (TPC), total flavonoid content (TFC) and high antioxidant activities using a Design of Experiment-mixture design. Methanolic extracts of nineteen fruit powder blends were tested for TPC, TFC, Ferric Reducing Antioxidant Power (FRAP), and DPPH. Results showed significant differences ($P < 0.05$) between blends. The reference blend, where each component was 0.25 of the totals. Reducing *E. serratus* can improve Total Phenolic Content. Reducing *M. zapota* and increasing *C. cauliflora* can improve DPPH. Reducing *C. cauliflora* and increasing *M. zapota* and *F. indica* can improve FRAP. However, the FRAP value increased with a higher *Flacourtia indica* L. proportion and a lower Nami Nam proportion. The optimal mixing ratio was 53.20% *Flacourtia indica* L., 18.28% *Cynometra cauliflora* L., 17.52% *Manilkara zapota* L. and 11% *Elaeocarpus serratus* L. At this ratio, the predicted response values of Total Phenolic Content, Total Flavonoid Content, DPPH (IC₅₀), and FRAP activity, were 19.66 mg GAE/g dry extract, 7.83 mgQE/g, 15.17 mgTE/g, 23.28 mgTE/g respectively. The mixture design approach proved to be an effective method for optimizing a bioactive formulation and can be successfully applied to other food mixture systems.

Keywords: *underutilized fruits, antioxidant properties, experimental mixture design*