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Phytochemical, physicochemical, toxicological, morphological and genetic variations of two *Piper longum* L.populations grown in Sri Lanka

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Piper longum is a therapeutically important medicinal plant belonging to the family Piperaceae. It is widely used as an ingredient in traditional and Ayurveda systems of medicine for the treatment of many ailments. Since Sri Lanka has two *P. longum* populations, there is confusion when identifying the authentic raw materials. Therefore, the present study was undertaken to differentiate the two *P. longum* populations available in Sri Lanka, by comparing phytochemical, physicochemical, toxicological, and genetic variations using previously published protocols. Results demonstrated that all tested phytochemical groups of saponins, alkaloids, tannins, flavonoids and steroid glycosides were present in both populations. However, physicochemical parameters of total ash, water soluble ash, moisture content and extractable matter were different as 7.02 ± 0.00 and 7.15 ± 0.00, 2.87 ± 0.00 and 2.89 ± 0.00, 16.84 ± 0.00 and 17.82 ± 0.00, 10.85 and 26.88, for the population 1 and population 2, respectively. Results of the brine shrimp toxicity assay demonstrated the significantly higher toxicity in population 1. Although, some of the morphological parameters were monomorphic for both populations, some characters such as growth habit, leaf colour, leaf width, leaf length, leaf surface colour, petiole length and intermodal space were different from each other. The amplification and sequencing success was 100% for *rbcL* and *matK* loci, and showed higher interspecific divergence but not intraspecific divergence within the populations. Single nucleotide polymorphisms (SNPs) were found in three positions in each locus namely 5, 7, 56 and 221, 449, 549 for *rbcL* and *matK*loci respectively. Even though there are some phytochemical and morphological similarities in both populations, they could be clearly separated by physicochemical, toxicological, and genetic parameters. The finding of the current study could be effectively used for the differentiation of two *Piper longum* populations.

Keywords: *Piper longum*, Piperaceae, morphology, genetic variance

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