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## A Comparative Analysis of *Piper longum* and *Piper sarmentosum* Utilizing Thin-Layer Chromatography and Gas Chromatography-Mass Spectrometry in Exploring the Potential in Sri Lankan Traditional Medicine

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### Abstract

*Piper longum* (Thippili) is a promising medicinal plant utilized in traditional medicine in Sri Lanka. *Piper sarmentosum* (*Gas thippili*) is an underutilized plant species that is gaining popularity as a substitute for *P. longum* due to its ease of cultivation and high abundance. No previous studies have been conducted comparing both species' phytochemical profiles in Sri Lanka. This study aimed to compare the phytochemical composition of different parts of two species using chromatographic analysis. Plant materials grown under the same environmental conditions were collected from the Bandaranayake Memorial Ayurvedic Research Institute, Nawinna. Fruits, stems, roots, and leaves were collected from both species and were subjected to sequential extraction with hexane, dichloromethane, ethyl acetate, and methanol. The extracts were then subjected to thin-layer chromatography (TLC) development under optimized mobile phases. Volatile compounds in the fruits and leaves were isolated as concrete and essential oils, respectively, and analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). Based on the results of TLC, the toluene: ethyl acetate (93:7) system gave better separation for non-polar compounds extracted from hexane. Toluene: ethyl acetate (70:30) and toluene: ethyl acetate: formic acid (70:25:5) systems showed a better separation for moderately polar compounds extracted into dichloromethane and ethyl acetate, respectively. The system of ethyl acetate: methanol: water (75:15:10) was utilized to better separate polar compounds extracted into methanol. According to the TLC fingerprints obtained, both similarities and differences were observed in different parts of the two species. GC-MS analysis of fruit concrete from *P. longum* and *P. sarmentosum* revealed notable differences in the volatile constituent profiles of the two species. *P. longum* fruit concrete was found to contain primarily aliphatic hydrocarbons, including Hexadecane, Heneicosane, and Eicosane. In contrast, *P. sarmentosum* fruit concrete contained a mixture of aliphatic hydrocarbons (Dodecane, Heptadecane, Heptacosane, Nonadecane) and phenylpropanoids (Myristicin). The essential oil extracted from *P. longum* leaves contained higher amounts of  $\beta$ -Pinene,  $\alpha$ -Pinene,  $\beta$ -Myrcene, and  $\beta$ -Caryophyllene than the essential oil extracted from *P. sarmentosum* leaves, which contained Myristicin as the major compound (52.74%), followed by trans-Caryophyllene and Germacrene D. Therefore, based on the TLC fingerprints, it can be concluded that *P. longum* and *P. sarmentosum* have both similarities and differences in the phytochemical composition while differences in the volatile compounds of fruits and leaves suggests that the two species may have different therapeutic effects, and thus, could be effectively utilized in the herbal medicine of Sri Lanka.

**Keywords:** *Piper sarmentosum*, *Piper longum*, Sequential extraction, TLC, GC-MS