ABSTRACT

The increasing global demand for herbal medicines and dietary supplements, while offering potential health benefits, has also raised concerns about product quality and authenticity. Herbal products, often derived from traditional knowledge and practices, are susceptible to adulteration due to factors like species similarity and the surge in demand for specific therapeutic properties. This study focuses on three traditionally and economically important medicinal species, *Terminalia bellirica* (TB), *Terminalia chebula* (TC), and *Phyllanthus emblica* (PE), known for their therapeutic benefits and often subjected to adulteration. DNA-based methods tend to be more reliable, accurate and cost-effective for authentication. The isolating of high-quality amplifiable DNA from these plant species, especially in processed products, remains challenging. This is primarily due to the presence of polyphenols, which interfere with DNA extraction and amplification. To address this, the study developed an optimized DNA isolation protocol. This protocol incorporated specific buffer modifications to stabilize pH during extraction and introduced polyvinylpyrrolidone as a phenolic compound scavenger to minimize interference during cell lysis. The effectiveness of this optimized protocol was evaluated using the species-specific *ITS*-based SCAR markers, digital PCR and *ITS2* metabarcode on six of each Baheda (TB fruit), Harde (TC fruit), Amala (PE fruits) and Triphala (containing three fruits of TB, TC, and PE) market formulation. Results demonstrated a significant improvement in DNA quality and quantity, leading to successful species identification. Furthermore, the study employed digital PCR (dPCR) to enhance sensitivity, achieving a two-fold increase compared to conventional PCR. This marks the first reported instance of a dPCR application for authenticating TB, TC, and PE. This research underscores the critical role of optimized DNA isolation protocols in ensuring the quality and authenticity of herbal products. By effectively addressing the challenges posed by polyphenols and employing sensitive detection methods like dPCR, this study provides a robust framework for authenticating herbal materials, ultimately contributing to consumer safety and confidence in the herbal medicine market.