

Exploring HPLC-mapped variations of marker compounds in commercially cultivated *Withania somnifera* from Andhra Pradesh, India

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The study aimed to assess five marker compounds (Withanoside IV, Withanoside V, Withaferin A, Withanolide A, and Withanone) extracted from the root of the indigenous medicinal plant Ashwagandha (*Withania somnifera*) across 125 different cultivars in Andhra Pradesh, India. This is the first report of the simultaneous determination of these markers using a novel buffer-free HPLC method. Results showed dynamic variations in marker levels across different mandals and villages, likely influenced by microclimate and soil conditions. Notably, Withanoside IV was most prevalent in Alur, Aspiri, Pattikonda, and Holagunda mandals of Kurnool, followed by Withanolide A and Withaferin A. Conversely, Withaferin A exhibited dominance in the Halharvi mandal. The integration of PCA added a deeper layer of understanding, shedding light on the interrelationships between these compounds and the regional cultivation variances of Ashwagandha in Andhra Pradesh. Based on principal component analysis (PCA), the first two components accounted for over 97.24% of the variance, with eigenvalues of 0.0083 and 0.0012, respectively. The first PCA explained a significant amount of variability. Withanoside IV, Withanoside V, and Withaferin A notably contribute to the first principal component, explaining 84.92% of its variability. Withaferin A stands out as the primary contributor to the second principal component.

Keywords: Ashwagandha (*Withania somnifera*), Cultivars, HPLC, Marker compounds, Principal component analysis

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