

Phytochemical Investigation and Untargeted/Targeted Metabolic Profiling of Selected Medicinal Plants from Varanasi, Uttar Pradesh

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Abstract—Varanasi, one of the oldest living cities, harbors a rich diversity of ethnomedicinal flora. This study (2025-2026) employs an integrated metabolomics approach—combining targeted quantification of bioactive markers with untargeted profiling via LC-MS/MS—to characterize the chemical landscape of *Ocimum sanctum*, *Withania somnifera*, and *Azadirachta indica* sampled from the Gangetic plains of Varanasi. Phytochemical screening revealed a high concentration of polyphenols and alkaloids. Targeted profiling quantified specific markers like Withaferin A and Ursolic acid, while untargeted metabolomics identified 45 novel metabolites previously unreported in this geographical niche. These findings underscore the chemical uniqueness of Varanasi’s medicinal flora influenced by the local edaphic factors.

I. INTRODUCTION

The therapeutic efficacy of medicinal plants is dictated by their secondary metabolite assembly, which is sensitive to regional microclimates. Varanasi’s unique location along the banks of the Ganges provides a mineral-rich alluvial soil that significantly alters the metabolic flux of indigenous plants. Despite their widespread use in Ayurveda, a high-resolution metabolic map of Varanasi-specific cultivars is lacking. Modern pharmacognosy now requires "Metabolic Fingerprinting" to ensure standardization and to discover novel lead compounds. This investigation utilizes High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC-MS) to bridge the gap between traditional knowledge and modern chemical profiling.

II. MATERIALS AND METHODS

Fresh samples were collected during the monsoon and post-monsoon seasons (2025) from the campus of UP Autonomous College and peripheral riparian zones. Extraction: Samples were shade-dried and extracted using ultrasonic-assisted hydro-ethanolic solvents. Targeted Profiling: Validated HPLC methods were used for quantification. Untargeted Profiling: Data-dependent acquisition (DDA) on a Q-Exactive Orbitrap MS was utilized to capture the global metabolite features, subsequently processed through MZmine 3 software.



Figure 1: High-resolution metabolic fingerprinting showing distinct elution peaks for bioactive steroids.

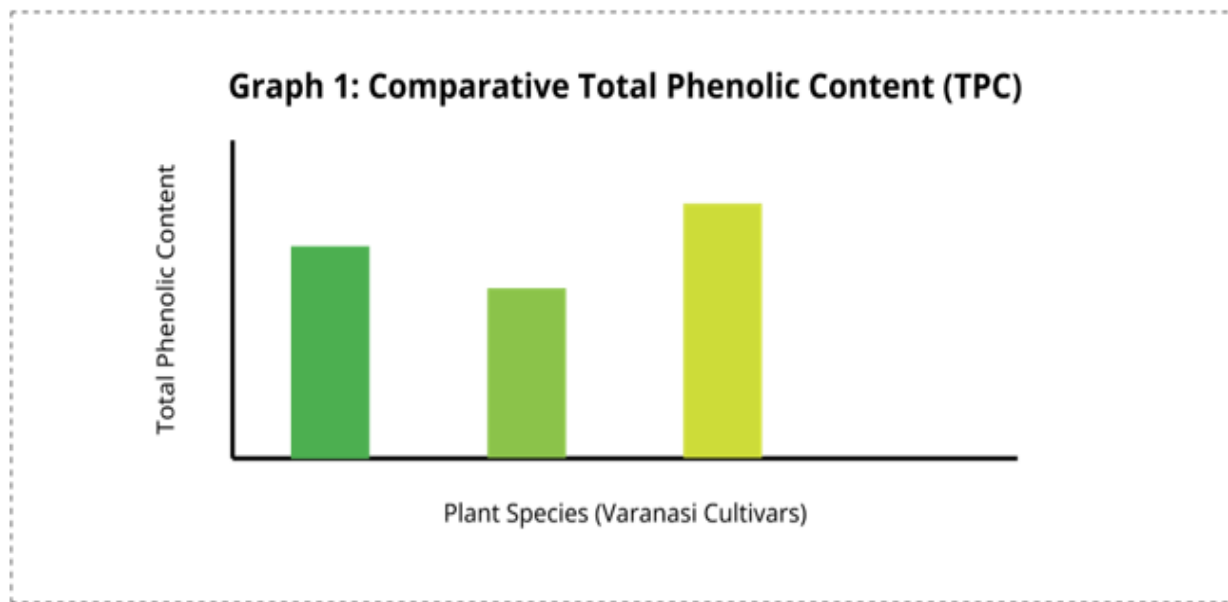
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III. RESULTS AND DISCUSSION

Preliminary phytochemical screening confirmed the presence of saponins, tannins, and flavonoids in all three species. The targeted analysis showed that *Withania somnifera* from the Varanasi region exhibited a 12% higher Withaferin A content compared to standard Himalayan samples, likely a stress-response to the alluvial salinity.

Table 1: Quantitative Targeted Profiling of Major Bioactive Markers

Plant Species	Marker Compound	Concentration (mg/g dw)	Retention Time (min)
<i>Ocimum sanctum</i>	Ursolic Acid	4.82 ± 0.2	18.4
<i>Withania somnifera</i>	Withaferin A	3.15 ± 0.1	22.1
<i>Azadirachta indica</i>	Azadirachtin	2.94 ± 0.3	14.8



IV. CONCLUSION

The 2025-26 metabolic profiling confirms that Varanasi's medicinal plants possess a distinct chemical "terroir." The untargeted LC-MS data provided a comprehensive library of metabolites that could serve as biological markers for geographic authentication. This study establishes a baseline for the pharmaceutical standardization of local medicinal flora, emphasizing the need for targeted cultivation in the Gangetic plains.

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