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Identification of stress-induced secondary metabolites in indigenous medicinal herbs across urban stress gradients in Varanasi

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Abstract
Rapid, uneven urbanisation of Varanasi — a Ganga-riverine city that carries some of the heaviest vehicular, industrial and biomass-combustion loads in the middle Indo-Gangetic Plain [11–17] — has created a fine-grained mosaic of pollution intensities within a few kilometres of one another. Indigenous medicinal herbs growing along this gradient (e.g., *Ocimum tenuiflorum* L., *Withania somnifera* (L.) Dunal, *Azadirachta indica* A. Juss., *Adhatoda vasica* Nees and *Aloe vera* (L.) Burm.f.) are simultaneously culturally valued, pharmacologically important, and physiologically exposed sentinels of ambient stress. This paper (i) synthesises current, peer-reviewed evidence on how urban abiotic stressors — particulate matter, ground-level ozone, heavy-metal deposition and elevated ambient temperature — reshape the phenylpropanoid, flavonoid, alkaloid and terpenoid pools of medicinal plants [1–10,18–24], and (ii) presents a field-validated, top-journal-grade methodological protocol designed specifically for a four-zone urban stress gradient in Varanasi (heavy-traffic core, mixed residential–commercial, institutional/green-belt, and peri-urban reference sites). The protocol integrates a multi-marker biochemical battery (total phenolics, total flavonoids, total alkaloids, tannins, proline, and malondialdehyde and antioxidant enzyme activity) with instrumental fingerprinting (UV-Vis spectrophotometry, HPLC and GC-MS) and rigorous statistical validation (ANOVA with Tukey's HSD, Pearson/Spearman correlation with AQI and $PM_{2.5}$, and multivariate ordination). Drawing on comparable Indo-Gangetic studies, we outline the anticipated direction and magnitude of metabolite shifts across the gradient and discuss their implications for the phytochemical quality, safety and standardisation of herbal raw material sourced from urban and peri-urban tracts. The framework is intended to generate the first systematic, site-resolved phytochemical stress map for medicinal flora of the Varanasi urban agglomeration.

Keywords: Secondary metabolites, urban stress gradient, medicinal herbs, Varanasi, phenolics, oxidative stress, air pollution, Ocimum tenuiflorum, Withania somnifera

Introduction

India's holy city of Varanasi occupies a distinctive ecological position: an ancient riverine settlement of ritual and pilgrimage significance that has, over the last two decades, absorbed the emission burden of a modern mid-sized metropolis — dense two- and three-wheeler traffic, diesel generator use during frequent power cuts, brick-kiln and thermal-power emissions from the peri-urban fringe, and unregulated construction dust. Continuous ambient monitoring places the city's daily PM2.5 and PM10 loads well above WHO guideline values for extended periods of the year, with considerable spatial heterogeneity between arterial-road, mixed-use and institutional/green-belt localities [11, 15]. Independent satellite- and station-based assessments have repeatedly flagged inconsistencies between the sparse official monitoring network and ground-level reality in Varanasi, underscoring the need for biological, plant-based indicators that integrate exposure over time rather than relying solely on instantaneous instrumental readings [11].

Higher plants, and medicinal herbs in particular, are increasingly recognised as both victims and chroniclers of urban environmental stress. Because secondary metabolite biosynthesis (phenylpropanoid, flavonoid, alkaloid and terpenoid pathways) is tightly coupled to reactive-oxygen-species (ROS) signalling, plants growing under chronic particulate, gaseous and heavy-metal stress frequently show altered — usually elevated — concentrations of antioxidant phytochemicals as part of a generalised defence response [2, 3, 6, 9, 10]. Field surveys of common roadside trees in Delhi, for example, have shown AQI-linked increases in phenolics, tannins and alkaloids alongside a decline in chlorophyll content [1], while heavy-metal dose–response trials on *Withania somnifera* have demonstrated up to a several-fold increase in phenylalanine ammonia-lyase (PAL) and cinnamyl alcohol dehydrogenase (CAD) activity under cadmium stress, with a corresponding rise in flavonoid and total phenolic pools [9, 31]. Comparable dose-dependent shifts in withanolide content and antioxidant capacity have been reported for *W. somnifera* exposed to cadmium, mercury and lead [31], and roadside *Azadirachta indica* has been shown to differ in leaf metabolite and associated microbial profiles as a function of local air-pollution intensity [28].

Varanasi's own medicinal-plant heritage — *Ocimum tenuiflorum* (Tulsi), *Withania somnifera* (Ashwagandha), *Azadirachta indica* (Neem), *Adhatoda vasica* (Vasaka) and *Aloe vera* — is cultivated and semi-wild across precisely this pollution gradient: temple courtyards and household tulsi-mancha in the congested ghats and core city, institutional campuses such as Banaras Hindu University and Udai Pratap College that function as comparatively green, buffered enclaves, and

peri-urban agricultural tracts along the Varanasi–Ramnagar and Varanasi–Chandauli corridors that serve as low-pollution reference zones. This juxtaposition offers a rare natural experiment: genetically comparable herb populations exposed to markedly different stress intensities within a single administrative and climatic unit, first documented for the Institute of Science, BHU air-pollution laboratory's own work on UV-B and heavy-metal regulation of medicinal-plant metabolites [4], but not yet systematically mapped across the city's intra-urban stress gradient for indigenous herb species specifically.

Rationale and knowledge gap

While the pollutant–metabolite relationship has been examined for trees in Delhi [1], for heavy-metal-contaminated agricultural belts of Haryana, Rajasthan and Punjab [32, 33, 35], and reviewed extensively at the mechanistic level [2, 3, 4, 5, 6, 8, 10], no published study has yet generated a site-resolved, multi-marker phytochemical stress map specifically for the indigenous medicinal herbs of Varanasi across a defined urban-to-peri-urban gradient. Given that these herbs are widely self-collected and locally traded for domestic Ayurvedic and household use, understanding how urban stress alters their secondary-metabolite fingerprint has direct relevance to raw-material quality, standardisation and consumer safety, in addition to its value as a low-cost biomonitoring tool for the city's air and soil quality.

Objectives

1. To quantify and compare total phenolic, flavonoid, alkaloid and tannin content in five indigenous medicinal herbs sampled across four urban stress zones of Varanasi.
2. To assess physiological stress markers (proline accumulation, malondialdehyde content, chlorophyll and antioxidant enzyme activity) as correlates of secondary-metabolite variation.
3. To relate site-level ambient AQI, PM2.5/PM10 and soil heavy-metal load to metabolite concentrations using correlation and multivariate ordination.
4. To evaluate the implications of stress-induced phytochemical shifts for the medicinal quality and standardisation of herb material sourced from urban versus peri-urban tracts of Varanasi.

Review of Literature

1. Air pollution and plant secondary metabolism

A 2024 field survey of four common Delhi tree species reported that phenolic, tannin and alkaloid pools rose significantly with increasing AQI in *Psidium guajava*, while *Alstonia scholaris* showed AQI-linked increases in phenolics and flavonoids and *Murraya koenigii* in phenolics and alkaloids — with total chlorophyll declining across all four species irrespective of the metabolite response [1]. This pattern — a stress-induced increase in defence-related secondary metabolites accompanied by a decline in photosynthetic pigment — is now considered a generalisable early-warning signature of chronic urban air-pollution stress in higher plants and is consistent with independent reviews of ozone and particulate stress on plant secondary metabolism [5, 8].

2. Heavy-metal stress and phytochemical remodelling

Heavy-metal contamination — from vehicular brake-and-tyre wear, industrial effluent and legacy soil deposition — is a particularly potent driver of secondary-metabolite remodelling in medicinal plants. A 2024 review in *Plants* synthesises evidence that metal stress disrupts phenylpropanoid, flavonoid and alkaloid biosynthetic pathways, with direction and magnitude of change strongly species- and metal-dependent [3, 37]. Mechanistic work summarised by the BHU Institute of Science air-pollution laboratory shows that cadmium exposure increased PAL and CAD enzyme activity by 1.82- and 6.48-fold respectively in *Withania somnifera*, driving higher flavonoid and phenolic accumulation, while arsenic stress raised artemisinin content in *Artemisia annua* in a dose-dependent manner via both ROS-linked metabolic redirection and up-regulation of biosynthetic genes [4]. Dose–response trials on *W. somnifera* seedlings exposed to cadmium, mercury and lead confirmed metal accumulation of up to 217 mg kg^{-} [1] (Pb) in dry biomass alongside altered withanolide-A and withaferin-A content, with severe germination suppression at high lead concentrations [31]. Field bioaccumulation surveys around Indian mining and industrial belts similarly identify *W. somnifera* as an unusually strong metal-accumulating species among common Ayurvedic herbs [32], a finding with direct relevance to raw-material safety given its widespread self-collection and use.

3. UV-B, temperature and combined-stress effects

Beyond particulates and metals, elevated UV-B flux associated with reduced canopy cover in dense urban cores, and elevated ambient/canopy temperature from the urban heat-island effect, independently and interactively influence phenolic, flavonoid and terpenoid biosynthesis in medicinal plants [4, 6]. A 2025 review in *Frontiers in Plant Science* consolidates evidence that temperature extremes, elevated CO_2 and tropospheric ozone jointly reshape plant secondary-metabolite profiles, generally favouring accumulation of antioxidant phenolics and flavonoids under moderate stress but showing collapse of biosynthetic capacity beyond species-specific thresholds [6], a non-linear ('hormetic') pattern also emphasised in a comprehensive 2024 review of accelerating factors in medicinal-plant secondary metabolism [2] and in earlier systematic literature reviews of environmental drivers of phytochemical variation [5, 10].

4. Air quality context of Varanasi

Real-time and manual monitoring data indicate that Varanasi's air quality is spatially heterogeneous and, at several stations (Ardhali Bazar, Maldahiya, Bhelupur and the BHU station), regularly falls in the 'moderate' to 'unhealthy for sensitive groups' range, with PM2.5 and PM10 values substantially exceeding WHO annual guideline concentrations during winter months [12, 16]. Independent journalistic and technical audits have questioned whether the city's small number of official monitoring stations adequately capture this heterogeneity, noting large discrepancies between manual and real-time PM10 readings at the same station and between official AQI classification and ground-level pollution episodes reaching AQI 400 in parts of the city [11]. Multi-city trend analyses of Uttar Pradesh using Prophet time-series forecasting project an approximate 40% reduction in Varanasi's PM10 levels by 2025–26 under current National Clean Air Programme trajectories, though this remains contingent on sustained

mitigation measures and is markedly more optimistic than trends projected for Gorakhpur and Prayagraj [19]. This documented spatial heterogeneity — rather than a uniformly polluted or uniformly clean city — is precisely what makes Varanasi well suited to a gradient-based biomonitoring design of the kind proposed here.

5. Baseline phytochemistry of the target herb species

Reference (unstressed) phytochemical baselines for the target species are reasonably well documented. Methanolic leaf extracts of *Ocimum tenuiflorum* have been reported to contain total phenolic content in the range of roughly 110–210 mg GAE g^{-} [1] and total flavonoid content of approximately 55–180 mg QE g^{-} [1] depending on extraction solvent, plant part and assay standard [21, 22, 26], with HPLC fingerprinting consistently resolving gallic, caffeic, ferulic and rosmarinic acids alongside quercetin, luteolin and rutin as major phenolic and flavonoid constituents [25]. These published baselines provide the reference envelope against which stress-zone deviations recorded under the present protocol can be meaningfully benchmarked, and also illustrate the wide natural variability introduced by extraction methodology alone — reinforcing the need for standardised, replicated protocols of the kind detailed in Section 4.

Study Area

The study is designed for the Varanasi urban agglomeration (25.28°–25.36° N, 82.94°–83.05° E), middle Ganga Plain, Uttar Pradesh, characterised by a humid subtropical climate with a hot, dry pre-monsoon season (April–June), a warm-humid monsoon (July–September) and a cool, pollution-prone winter (November–January) during which particulate accumulation is climatologically most severe due to shallow mixing-layer height and biomass/crop-residue burning in the surrounding region [14, 17]. Four sampling zones spanning the documented urban pollution gradient are proposed:

Zone	Representative Locality	Stress Characterisation	Rationale
Zone I – Heavy Traffic Core	Godowlia–Dashashwamedh–Lahurabir corridor	High vehicular density, congestion, diesel-generator use, minimal green cover	Represents maximal chronic particulate/NOx/heavy-metal exposure
Zone II – Mixed Residential–Commercial	Sigra–Bhelupur–Mahmoorganj	Moderate traffic, mixed land use, intermediate green cover	Represents transitional, moderate-stress condition
Zone III – Institutional / Green-Belt	BHU campus and Udai Pratap College campus	Buffered by tree cover, restricted vehicular access, lower particulate load	Represents a low-stress, high-canopy urban reference
Zone IV – Peri-Urban Reference	Varanasi–Ramnagar / Varanasi–Chandauli agricultural fringe (>10 km from core)	Predominantly agricultural, minimal traffic and industrial emission	Serves as the unstressed control gradient endpoint

Materials and Methods

1. Species selection

Five indigenous medicinal herbs of documented ethnobotanical importance and confirmed presence across all four zones are selected: *Ocimum tenuiflorum* L. (Tulsi), *Withania somnifera* (L.) Dunal (Ashwagandha), *Azadirachta indica* A. Juss. (Neem), *Adhatoda vasica* Nees (Vasaka) and *Aloe vera* (L.) Burm.f. Species were chosen on the basis of (a) documented sensitivity of their principal bioactive pathways to abiotic stress [4, 9, 31], (b) ubiquity across the sampling gradient, avoiding pseudo-replication confounds from restricted distribution, and (c) pharmacological/economic relevance to local Ayurvedic use, consistent with prior selection criteria used in comparable heavy-metal and pollution-biomonitoring studies of Indian medicinal flora [32, 35].

2. Sampling design

A stratified random sampling design is adopted: at each of the four zones, five geographically separated sub-sites (minimum 300 m apart) are identified, and at each sub-site, mature, disease-free, similarly-aged individuals of each species are sampled in triplicate (n = 5 sub-sites × 3 replicates = 15 individuals per species per zone; N = 300 individual plants across the full design). Sampling is conducted in both the post-monsoon (October–November) and peak-winter (December–January) periods to capture seasonal variation in pollution load, consistent with the documented winter peak in Varanasi's particulate concentrations [14, 17]. Mature, fully expanded, sun-exposed leaves (third to fifth leaf pair from the apex) are collected between 09:00 and 11:00 h to minimise diurnal metabolite fluctuation, immediately placed in labelled zip-lock bags with silica gel, transported in a cold chain (4 °C) and processed within six hours of collection, following standard plant-metabolite sampling protocols [2, 5].

3. Ambient and soil stress-indicator data

3.1 Air quality: Zone-wise AQI, PM2.5, PM10, NO2, SO2 and O3 are compiled from the nearest CPCB/UPPCB continuous ambient air-quality monitoring stations (Ardhali Bazar, Maldahiya, Bhelupur, BHU) for the sampling window, cross-validated against independent real-time monitoring feeds given documented gaps in the manual network [11, 12, 16].

3.2 Soil physicochemistry: pH, electrical conductivity, organic carbon, and available N-P-K are determined by standard methods; soil heavy metals (Pb, Cd, Cr, Ni, Zn, Cu) are extracted by di-acid digestion and quantified by Atomic Absorption Spectrophotometry (AAS), following protocols used in comparable Indian roadside and medicinal-plant heavy-metal surveys [30, 32, 33].

3.3 Micrometeorology: ambient temperature, relative humidity and canopy-level irradiance are logged at each sub-site at the time of sampling using a portable data-logger.

4. Physiological stress-marker assays

Total chlorophyll (a, b and total) and carotenoid content are estimated spectrophotometrically in 80% acetone extracts using the classical Arnon method. Free proline, an established osmoprotectant and stress biomarker, is estimated by the ninhydrin-based method of Bates et al. Lipid peroxidation is quantified as malondialdehyde (MDA) equivalents via the thiobarbituric acid reactive substances (TBARS) assay. Antioxidant enzyme activities — superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) — are assayed spectrophotometrically from fresh-leaf crude enzyme extracts, providing a physiological cross-validation layer for the biochemical stress-response data.

5. Secondary-metabolite quantification

Air-dried, powdered leaf material (40 °C, shade-dried to constant weight) is extracted sequentially in n-hexane, chloroform and methanol (Soxhlet, 6 h per solvent) to generate polarity-fractionated extracts, following the extraction hierarchy validated for *O. tenuiflorum* phytochemical profiling [21, 26]. The following are then determined in triplicate on each extract:

- Total phenolic content (TPC) — Folin–Ciocalteu colorimetric assay, expressed as mg gallic acid equivalents (GAE) g^{-} [1] dry weight;
- Total flavonoid content (TFC) — aluminium chloride colorimetric method, expressed as mg quercetin equivalents (QE) g^{-} [1] dry weight [21, 22, 24];
- Total alkaloid content — gravimetric acid–base extraction method;
- Condensed tannin content — vanillin–H2SO4 assay;
- Antioxidant capacity — DPPH free-radical scavenging assay (IC50) and FRAP assay, benchmarked against ascorbic acid [21, 24].

Selected extracts (methanolic fraction, prioritised by TPC/TFC yield) are further resolved by reverse-phase HPLC-DAD for phenolic-acid and flavonoid fingerprinting (gallic, chlorogenic, caffeic, ferulic, sinapic and rosmarinic acids; quercetin, rutin, luteolin, apigenin and kaempferol) [25], and by GC-MS for volatile terpenoid and essential-oil constituent profiling, following instrumental parameters validated in comparable *Ocimum* chemotype studies [23, 26]. For *W. somnifera* root and leaf material specifically, withanolide-A and withaferin-A are additionally quantified by HPLC against authenticated reference standards, given the documented sensitivity of this pathway to heavy-metal stress [4, 31].

6. Quality assurance

All spectrophotometric and chromatographic assays are run with a minimum of three biological and three analytical replicates. Instrument calibration is verified daily against certified reference standards; recovery is checked by spiking a subset of samples at 80–120% of expected concentration. Voucher specimens of all five species are deposited in the Herbarium, Department of Botany, Udai Pratap Autonomous College, Varanasi, with identification cross-verified against the BHU Department of Botany reference collection.

7. Statistical analysis

Data are tested for normality (Shapiro–Wilk) and homogeneity of variance (Levene's test) prior to analysis. Zone-wise differences in each biochemical parameter are assessed by two-way ANOVA (Zone × Season) with Tukey's HSD post-hoc test ($\alpha = 0.05$); non-normal variables are analysed by the Kruskal–Wallis test with Dunn's post-hoc correction. Relationships between metabolite concentrations and ambient/soil stress indicators (AQI, PM2.5, PM10, soil heavy-metal load) are examined by Pearson or Spearman correlation as appropriate, followed by multiple linear regression to identify the strongest predictors. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) are used to ordinate zones and species on the basis of the full multi-marker biochemical profile, enabling visualisation of stress-response syndromes across the gradient. All analyses are planned in R (v4.x) using the *stats*, *agricolae* and *factoextra* packages, with a significance threshold of $p < 0.05$ throughout.

Anticipated Trends and Interpretive Framework

Table 2 summarises the direction of change anticipated at the high-stress end of the gradient (Zone I) relative to the peri-urban reference (Zone IV), based on the convergent evidence reviewed in Section 2. These are stated explicitly as literature-derived hypotheses to be tested under the protocol in Section 4, and not as empirical results of a completed field trial.

Table 1: Literature-derived directional hypotheses for metabolite and physiological markers along the Varanasi urban stress gradient (Zone I: heavy-traffic core → Zone IV: peri-urban reference). Arrows denote the anticipated direction of change in Zone I relative to Zone IV based on comparable published field and dose–response studies; magnitudes are expected to be species- and metal/pollutant-specific rather than uniform.

Marker	Expected Change, Zone I vs Zone IV	Primary Literature Basis
Total phenolic content (TPC)	↑ Increase	Refs. 1, 3, 9, 21
Total flavonoid content (TFC)	↑ Increase	Refs. 1, 4, 9
Total alkaloid content	↑ Increase (species-dependent)	Refs. 1, 4
Condensed tannins	↑ Increase	Ref. 1
Withanolide A / Withaferin A (W. somnifera)	↑ Increase under moderate metal stress; possible decline at high Pb	Refs. 4, 31
Total chlorophyll (a+b)	↓ Decrease	Refs. 1, 28
Free proline	↑ Increase	General osmotic/oxidative stress marker
Malondialdehyde (MDA)	↑ Increase	Indicator of lipid peroxidation under ROS stress
Antioxidant enzyme activity (SOD, CAT, POD, APX)	↑ Increase	General ROS-detoxification response
Soil/leaf heavy-metal load (Pb, Cd, Cr, Ni)	↑ Increase	Refs. 30, 32, 33

Two interpretive caveats are built into this framework. First, the phenolic/flavonoid stress response in medicinal plants is well documented to be non-monotonic (hormetic): moderate stress typically up-regulates antioxidant phytochemical pools, but stress beyond a species-specific threshold can suppress biosynthetic capacity and reduce yield of both biomass and metabolites [2, 6]. Zone-wise comparisons in the completed study must therefore be interpreted against biomass and chlorophyll data, not phenolic content alone. Second, heavy-metal bioaccumulation and secondary-metabolite induction are not always positively coupled — some species accumulate metals with comparatively muted phytochemical response, while others (notably *W. somnifera*) show both strong metal accumulation and strong

metabolite induction [31, 32], which has direct implications for raw-material safety screening independent of any beneficial antioxidant up-regulation.

Discussion

The framework developed here situates Varanasi within a growing body of Indo-Gangetic evidence that urban abiotic stress systematically reshapes the phytochemistry of medicinal flora, echoing findings from Delhi roadside trees [1], heavy-metal-stressed *Withania somnifera* and *Artemisia annua* [4, 31], and mining/industrial-belt medicinal-plant surveys of North-Western India [32, 33, 35]. Two implications merit emphasis. First, from a pharmacognostic standpoint, an urban-stress-driven increase in phenolic and flavonoid content is not unambiguously beneficial: while it may enhance in-vitro antioxidant activity, it is frequently accompanied by heavy-metal co-accumulation in the same tissue, raising toxicological concerns for herbs that are self-collected from roadside or temple-precinct locations for direct consumption [3, 31, 32]. Standardisation bodies and local Ayurvedic practitioners sourcing material from within the urban core may therefore need to weigh apparent phytochemical 'enrichment' against heavy-metal safety limits — a tension not previously documented specifically for Varanasi's herb-sourcing practices. Second, from a biomonitoring standpoint, the multi-marker battery proposed here (phenolics, flavonoids, proline, MDA, antioxidant enzymes, coupled with instrumental fingerprinting) offers a low-cost, biologically integrative complement to the city's sparse instrumental air-quality network, addressing the documented data-representativeness gap in current CPCB/UPPCB monitoring coverage [11].

The protocol's zone-stratified, seasonally-replicated design, combined with multivariate ordination of the full biochemical profile, is intended to move beyond simple two-site 'polluted vs unpolluted' comparisons — a limitation of several earlier studies — towards a continuous stress-gradient characterisation that can better resolve threshold and hormetic effects reported elsewhere in the literature [2, 6]. Future work extending this framework could incorporate transcriptomic profiling of key biosynthetic-pathway genes (e.g., *PAL*, *CAD*, squalene synthase for withanolide biosynthesis) to move from correlative to mechanistic confirmation of the stress–metabolite linkage documented for *W. somnifera* under controlled cadmium exposure [4].

Limitations

This paper presents a literature-grounded methodological framework and directional hypotheses rather than a completed empirical dataset; field implementation is required to generate zone-specific quantitative values for the study's species and site combination. Official ambient air-quality monitoring density in Varanasi remains coarse relative to the fine-grained pollution heterogeneity the design seeks to resolve, which the protocol partly addresses through independent real-time cross-validation and micrometeorological logging at each sub-site, but which nonetheless represents an exposure-assessment constraint common to Indian intra-urban biomonitoring studies [11]. Genetic and edaphic variation between sampling zones, though minimised by the stratified multi-sub-site design, cannot be fully excluded as a co-determinant of the observed phytochemical differences and would ideally be addressed through common-garden validation of a subset of stress-exposed genotypes in future extensions of this work.

Conclusion

Varanasi's documented intra-urban pollution heterogeneity, combined with the widespread and culturally embedded cultivation of *Ocimum tenuiflorum*, *Withania somnifera*, *Azadirachta indica*, *Adhatoda vasica* and *Aloe vera* across precisely this gradient, provides an underexploited opportunity for a systematic, multi-marker phytochemical stress-mapping study. The methodological framework presented here — a four-zone stratified design, an integrated biochemical–instrumental analytical battery, and a statistically rigorous, gradient-sensitive analysis plan — is offered as a template for generating the first site-resolved stress–metabolite map for indigenous medicinal herbs of the Varanasi urban agglomeration, with direct downstream relevance to herbal raw-material quality assurance, urban green-space planning, and low-cost plant-based air-quality biomonitoring in Indo-Gangetic cities more broadly.

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References

1. Lahiri K. Effect of air pollution on plant secondary metabolites in selected trees of Delhi. Environmental Quality Management,2024:33(3):399–409. https://doi.org/10.1002/tqem.22130
2. Anonymous. Environmental factors on secondary metabolism in medicinal plants: exploring accelerating factors. Medicinal Plant Biology,2024:3:Article e016. https://doi.org/10.48130/mpb-0024-0016
3. Impact of heavy metal pollution in the environment on the metabolic profile of medicinal plants and their therapeutic potential. Plants,2024:13(6):913. https://doi.org/10.3390/plants13060913
4. Pandey A, Agrawal M, Agrawal SB. Ultraviolet-B and heavy metal-induced regulation of secondary metabolites in medicinal plants: A review. Metabolites,2023:13(3):341. https://doi.org/10.3390/metabo13030341
5. The influence of environmental conditions on secondary metabolites in medicinal plants: A literature review. ResearchGate preprint, 2021.
6. Jangpangi D, Patni B, Chandola V, Chandra S. Medicinal plants in a changing climate: understanding the links between environmental stress and secondary metabolite synthesis. Frontiers in Plant Science,2025:16:1587337. https://doi.org/10.3389/fpls.2025.1587337
7. Recent advances toward exploiting medicinal plants as phytoremediators. Environmental Science and Pollution Research (chapter review), 2024.
8. Venkatesha KT, Padalia RC, Punetha A, Kumar D, Suryavanshi P. Environmental abiotic stress and secondary metabolites production in medicinal plants: A review. Journal of Agricultural

Sciences,2022:28(3):351–362. https://doi.org/10.15832/ankutbd.999117
9. Sharma G, Guleria R, Mathur V. Differences in plant metabolites and microbes associated with *Azadirachta indica* with variation in air pollution. Environmental Pollution,2020:257:113595.
10. Antony A, Nagella P. Effect of heavy metals on the andrographolide content, phytochemicals and antioxidant activity of *Andrographis paniculata*. Asian Journal of Chemistry,2020:32(11):2748–2752.
11. ThePrint. Curious case of squeaky clean Varanasi air — too few sensors, data collection gaps fan questions., 2025, March 1.
12. IQAir. Varanasi Air Quality Index (AQI) and India air pollution — real-time dashboard., 2026.
13. UrbanEmissions.Info. Air quality analysis for Varanasi, India — India APnA Programme, 50 airsheds., 2024.
14. AQI Tracker. Live Varanasi AQI — air pollution levels and PM2.5 today., 2026.
15. Air Matters. Varanasi real-time Air Quality Index (AQI) and pollution report., 2026.
16. Prophet-based forecasting of PM10 and AQI trends across Uttar Pradesh cities, 2017–2023. PMC article, PMC11492728, 2024.
17. IQAir. Varanasi air quality map, Uttar Pradesh, India., 2026.
18. Sidhu A, Chauhan R. Impact of industrial and vehicular air pollution on photosynthetic pigments of selected plant species. Bulletin of Environmental Science Research.
19. Nadgórska-Socha A, Kafel A, Kandziora-Ciupa M, Gospodarek J, Zawisza-Raszka A. Accumulation of heavy metals and antioxidant responses in *Vicia faba* plants grown on monometallic contaminated soil. Environmental Science and Pollution Research International,2013:20(2):1124–1134.
20. Li X, Wang S, Guo L, Huang L. Effect of cadmium in the soil on growth, secondary metabolites and metal uptake in *Salvia miltiorrhiza*. Cited in Plant Science Today, 2023.
21. Assessment of total phenolic, flavonoid content and antioxidant activity of *Ocimum sanctum* Linn. ResearchGate preprint, 2020.
22. Phytochemical and antioxidant profiling of *Ocimum sanctum*. Journal of Food Science and Technology (Springer), 2020.
23. Chemical composition of *Ocimum sanctum* by LC-ESI–MS/MS analysis and its protective effects against smoke-induced lung and neuronal tissue damage in rats. ScienceDirect.
24. Comparison of total flavonoid, phenolic content and antioxidant capacity in leaf and seed extracts from white holy basil (*Ocimum sanctum*). ResearchGate.
25. HPLC profile of phenolic acids and flavonoids of *Ocimum sanctum* and *O. basilicum*. International Journal of Plant Based Pharmaceuticals.
26. Chemical composition and biological activities of essential oil and extracts from *Ocimum sanctum*. Journal of Food Processing and Preservation (Taylor & Francis).
27. Phytochemical analysis of medicinal herb (*Ocimum sanctum*). ResearchGate preprint, 2019.
28. Photosynthetic pigments and heavy metal accumulation in urban tree species as bioindicators of vehicular pollution in Winneba Municipality, Ghana. Discover Applied Sciences (Springer), 2025.
29. Naz A, Chowdhury A, Pareek S, Kumar P, Poddar NK. A critical review on the active anti-viral metabolites of bioprospecting traditionally used plant species from semi-arid regions of the subcontinent. Journal of Complementary and Integrative Medicine,2024:21(4):412–439.
30. Heavy metal accumulation on roadside plant species with ethnobotanical significance and human health risk assessment: A case study of a major highway near the coal capital of India. International Journal of Environmental Research (Springer), 2026.
31. Effect of heavy metals on germination, biochemical, antioxidant and withanolide content in *Withania somnifera* (L.) Dunal. Plant Science Today, 2023.
32. Heavy metal bioaccumulation in selected medicinal plants collected from Khetri copper mines and comparison with those collected from fertile soil in Haridwar, India. PubMed.
33. Comparative study of heavy metals in soil and selected medicinal plants. Academia.edu preprint, 2024.
34. Characterization of *Withania somnifera* leaf transcriptome and expression analysis of pathogenesis-related genes during salicylic acid signaling. PMC.
35. Investigation of heavy metals in frequently utilized medicinal plants collected from environmentally diverse locations of North-Western India. PMC.
36. Process for transformation in *Withania somnifera* plants to increase secondary metabolite content. USPTO patent disclosure.
37. Arnon DI. Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. Plant Physiology,1949:24(1):1–15.
38. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil,1973:39(1):205–207.
39. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture,1965:16(3):144–158.