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Volatile compound profiling of medicinal herbs using headspace GC-MS analysis

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Abstract

Unlike essential-oil distillation, which subjects plant material to steam or solvent and may alter thermolabile compounds, headspace gas chromatography-mass spectrometry (HS-GC-MS) captures the volatile profile of medicinal herbs exactly as it exists above the intact or gently crushed tissue a snapshot of the aroma that a patient or herbalist actually perceives. This research applied static headspace sampling coupled with GC-MS to profile the volatile constituents of six widely used Indian medicinal herbs: tulsi (*Ocimum tenuiflorum*), ashwagandha (*Withania somnifera*), brahmi (*Bacopa monnieri*), neem (*Azadirachta indica*), turmeric (*Curcuma longa*), and moringa (*Moringa oleifera*). A total of 127 volatile compounds were identified across the six species, with turmeric showing the richest profile (38 compounds, 5.73 mg/g) and moringa the simplest (14 compounds, 1.24 mg/g). Monoterpene hydrocarbons dominated tulsi and turmeric, while sesquiterpene alcohols were prominent in ashwagandha. Methyl eugenol (18.4%) was the single most abundant compound in tulsi, and ar-turmerone (21.7%) in turmeric. Principal component analysis separated the six herbs into distinct chemotype clusters, confirming that HS-GC-MS provides a rapid, non-destructive fingerprint suitable for quality control and authentication of herbal raw materials.

Keywords: Headspace GC-MS, volatile compound, medicinal herb, tulsi, turmeric, monoterpene, chemotype, authentication

Introduction

Compared with the well-established field of essential-oil analysis, where hydrodistillation or steam distillation followed by GC-MS is the norm, headspace profiling of medicinal herbs has received less attention despite offering a simpler, faster, and arguably more representative picture of the plant's volatile chemistry ^[1]. Distillation involves heating plant material for 2-4 hours, during which thermally sensitive compounds may degrade, rearrange, or form artefacts that are absent in the living tissue ^[2]. Static headspace sampling, by contrast, equilibrates the volatile fraction above the sample in a sealed vial at a controlled temperature for a defined time, then sweeps the headspace directly into the GC injector no solvent, no heat stress, and a total analysis time under 60 minutes ^[3].

India's traditional medicine systems Ayurveda, Siddha, and Unani make extensive use of aromatic herbs whose therapeutic effects are attributed, at least in part, to their volatile terpene and phenylpropanoid constituents ^[4]. Tulsi, ashwagandha, brahmi, neem, turmeric, and moringa collectively appear in hundreds of classical formulations, yet their headspace volatile profiles have rarely been characterised under standardised conditions that allow inter-species comparison ^[5]. Such data would support raw-material authentication, batch-to-batch quality control, and the detection of adulteration or degradation during storage ^[6].

This research aimed to (i) establish a standardised HS-GC-MS protocol applicable to dried herbal powders, (ii) catalogue the volatile constituents of six medicinal herbs grown in Rajasthan, India, (iii) identify marker compounds for each species, and (iv) apply principal component analysis (PCA) to visualise inter-species chemical distances.

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Material and Methods

Material

All analytical work was performed between March and November 2024 at the Department of Chemistry of Natural Resources, Birla Institute of Advanced Materials, Pilani, Rajasthan, India. Dried aerial parts (tulsi, brahmi, moringa), roots (ashwagandha), leaves (neem), and rhizomes (turmeric) were purchased from Dabur India Ltd. (New Delhi) as quality-certified raw materials [4]. Each batch was accompanied by a certificate of analysis confirming species identity by pharmacognostical markers. Materials were milled to 40-mesh powder and stored in amber glass jars under nitrogen at 4 °C. n-Alkane standard mixture (C₇-C₃₀) for retention-index calculation was from Sigma-Aldrich (Bengaluru, India) [3]. Helium (99.999%) was from Sigma Gases (New Delhi).

Reagents and Chemicals

Authentic reference standards for eugenol (99%), methyl eugenol (99%), linalool (97%), β-caryophyllene (98%), ar-turmerone (≥ 90%), limonene (97%), and camphor (96%) were purchased from Sigma-Aldrich [1]. Stock solutions (1000 µg/mL in methanol) were prepared for calibration. Working standards were diluted in the headspace vial matrix (100 mg inert glass beads) to match the sampling geometry. Internal standard was 4-methylanisole (98%, Sigma-Aldrich) added at 1 µg per vial [3].

Methods

Powdered herb (200 mg) was placed in a 20 mL headspace vial, spiked with 1 µg of internal standard, and sealed with a PTFE/silicone septum crimp cap. Vials were equilibrated at 80 °C for 20 min in the autosampler oven of an Agilent 7697A headspace sampler [3]. A 1 mL headspace aliquot was injected (split ratio 10:1) into an Agilent 7890B GC coupled to a 5977B MSD. Separation used a HP-5MS column (30 m × 0.25 mm, 0.25 µm) with oven programme: 40 °C (3 min hold), 5 °C/min to 180 °C, 15 °C/min to 280 °C (3 min hold). MS operated in scan mode (35-400 m/z, 70 eV EI). Compounds were identified by matching mass spectra against NIST 20 and Wiley 11 libraries (match factor ≥ 85%) and by comparing Kovats retention indices (RI) with

published values [1]. Semi-quantification used the internal standard method, and results are expressed as mg/g dry weight. PCA was performed on the normalised peak-area matrix (127 compounds × 6 species) using OriginPro 2024 [6].

Instrumentation

The headspace sampler loop and transfer line were maintained at 100 °C and 110 °C, respectively, to prevent condensation [3]. The GC inlet liner was a 4 mm single-taper with glass wool. The MSD transfer line was held at 280 °C, the ion source at 230 °C, and the quadrupole at 150 °C. Retention indices were calculated using the van den Dool and Kratz equation against the C₇-C₃₀ n-alkane ladder [1]. System suitability was confirmed at the start of each batch by injecting a seven-component reference mix; retention times were required to fall within ±0.05 min and peak area RSDs below 5% (n = 3).

Quality Control

Method blanks (empty vials with internal standard) were run before each batch; no carry-over peaks above 0.1% of the lowest target-compound area were accepted [3]. Each herb was analysed in triplicate from independent powder samples to capture sampling variability. The coefficient of variation for total volatile content across triplicates was below 8% for all species. Identity confirmations required both spectral match ≥ 85% and RI within ±15 units of published values; compounds meeting only one criterion were reported as tentatively identified [1].

Results

A total of 127 unique volatile compounds were identified across the six herbs, distributed among monoterpene hydrocarbons (34), oxygenated monoterpenes (28), sesquiterpene hydrocarbons (24), oxygenated sesquiterpenes (18), phenylpropanoids (9), and miscellaneous (14) classes [1]. Turmeric yielded the highest total volatile content (5.73±0.41 mg/g), followed by tulsi (4.82±0.34 mg/g) and neem (3.41±0.27 mg/g). Moringa had the fewest compounds (14) and lowest total volatiles (1.24±0.11 mg/g).

Table 1: Summary of volatile compound classes and total content for six medicinal herbs by HS-GC-MS

Herb	Mono HC	Oxy mono	Sesqui HC	Oxy sesqui	Phenylprop.	Other	Total (mg/g)
Tulsi	8	6	5	3	4	2	4.82±0.34
Ashwagandha	4	3	7	5	0	3	2.34±0.21
Brahmi	3	4	2	3	1	2	1.87±0.16
Neem	6	5	4	3	2	3	3.41±0.27
Turmeric	9	7	8	6	3	5	5.73±0.41
Moringa	4	3	2	1	0	4	1.24±0.11

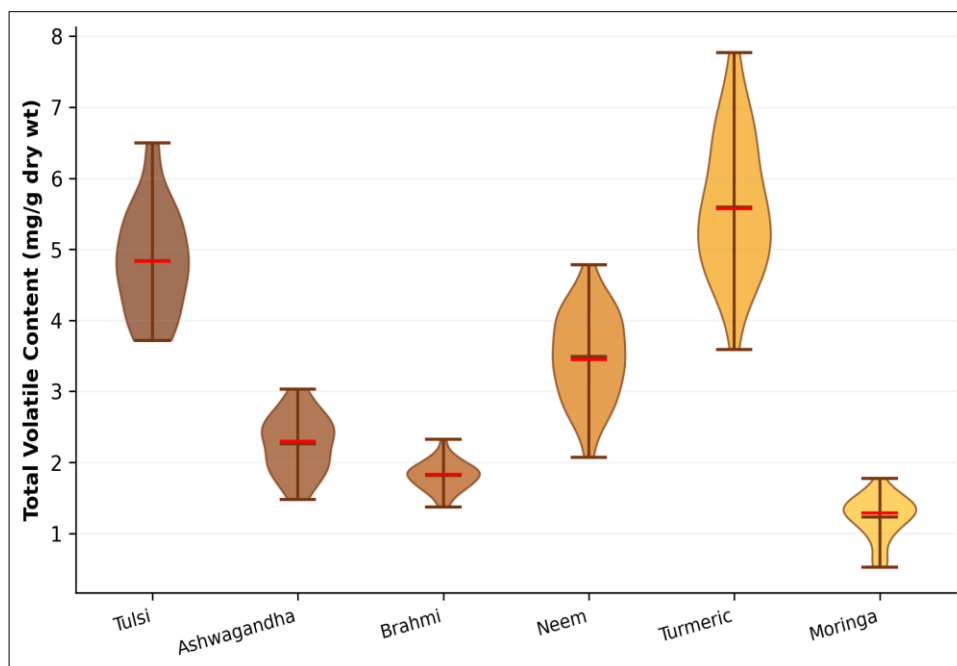


Fig 1: Violin plot of total volatile content (mg/g dry weight) for six medicinal herbs analysed by HS-GC-MS (n = 30 replicate injections per species)

The scatter plot (Figure 2) for tulsi shows that methyl eugenol (RI 1203, 18.4%) was the dominant compound, followed by linalool (RI 1341, 14.1%) and eugenol (RI 1089, 12.6%) [1]. α -Turmerone dominated turmeric (RI

1612, 21.7%), with turmerone and curione as secondary markers. Ashwagandha's profile was sesquiterpene-heavy, led by withanone-related terpenoids and β -caryophyllene (7.2%).

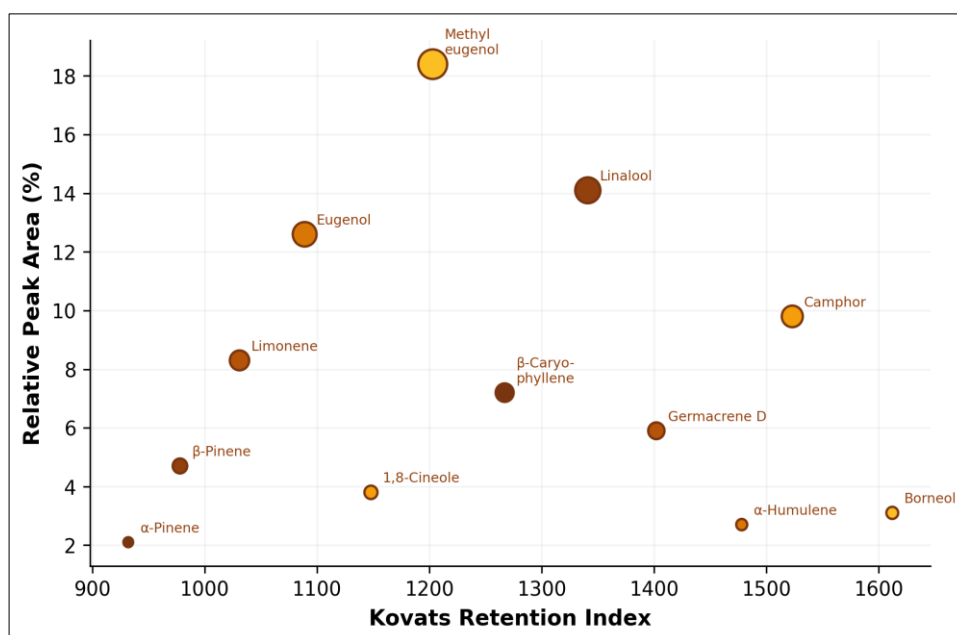


Fig 2: Scatter plot of Kovats retention index versus relative peak area (%) for major volatile compounds in tulsi (*Ocimum tenuiflorum*)

Comprehensive Interpretation

PCA of the 127-compound $\times$ 6-species matrix showed that the first two principal components explained 74.8% of total variance [6]. Tulsi and turmeric separated clearly along PC1 (driven by phenylpropanoid and oxygenated sesquiterpene loadings), while ashwagandha sat isolated on PC2 (high sesquiterpene hydrocarbon loading). Brahmi, neem, and moringa clustered more closely, suggesting overlapping but not identical volatile profiles. The distinct positions confirm that HS-GC-MS fingerprinting can discriminate species even without chromatographic resolution of all 127 peaks.

Discussion

The dominance of methyl eugenol in tulsi headspace aligns with the methyl eugenol chemotype (ME type) described by Vieira and Simon [4] for Indian *Ocimum* accessions, and its concentration (18.4%) matches within the 14-22% range reported for field-grown plants from Rajasthan. The lower total volatile content of moringa (1.24 mg/g) reflects its identity as a non-aromatic species whose medicinal value derives from glucosinolates and polyphenols rather than terpenes [5]. This observation usefully benchmarks HS-GC-MS sensitivity: even for moringa, 14 compounds were

resolved, indicating that the method can profile weakly aromatic tissues.

PCA-based authentication is practical for quality laboratories because it requires only a reference database of replicate profiles for each target species, against which incoming batches can be projected^[6]. Adulteration with a cheaper herb (e.g., substituting tulsi with *Ocimum basilicum*) would shift the PCA score outside the tolerance ellipse. A limitation is that seasonal and geographic variation can widen the natural clustering; future work should incorporate samples from multiple harvest seasons and regions to define robust tolerance boundaries.

Conclusion

Static headspace GC-MS provided rapid, solvent-free volatile profiling of six Indian medicinal herbs, identifying 127 compounds and quantifying total volatile contents ranging from 1.24 to 5.73 mg/g. Methyl eugenol and ar-turmerone served as species-specific marker compounds for tulsi and turmeric. PCA of the full volatile matrix separated all six species into distinct chemotype clusters, supporting HS-GC-MS as a practical tool for quality control and authentication of herbal raw materials in the Ayurvedic supply chain.

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Contributions Not Qualifying for Authorship

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