



Research Paper

Phytochemical Profiling, Seasonal Variation, and Chemometric Characterization of Essential Oils from Selected Aromatic Plants of Sidhi District

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Abstract

This study aimed to analyze the phytochemical composition of essential oils of six aromatic plants (*Ocimum canum*, *Syzygium nervosum*, *Vitex negundo*, *Murraya koenigii*, *Annona squamosa* and *Cyperus scariosus*) of Sidhi district, Madhya Pradesh, India and their seasonal variations along with chemometric relationships. Physicochemical evaluations, GC-MS, GC-FID, Principal Component Analysis (PCA), and Hierarchical Cluster Analysis (HCA) were used for essential oils hydrodistilled and physiochemically evaluated. The range in essential oil yield was significantly different among species ranging from 4.3 to 13.8 mL kg⁻¹, highest yield was obtained from *Murraya koenigii*. Analysis of seasonal production showed that the highest production was in the summer, and the lowest in the monsoon season. The GC-MS and GC-FID analyses revealed various volatile compounds of which linalool, eugenol, eucalyptol, β -caryophyllene, germacrene and patchoulol were significant marker compounds. The phytochemical class distribution revealed that *Ocimum canum* and *Murraya koenigii* were the higher monoterpene rich plants, *Cyperus scariosus* was the highest sesquiterpene rich plant, and *Syzygium nervosum* was the richest in phenolics. The chemometric analyses were able to clearly differentiate the species based on their chemical composition, and accounted for 77.55% of the total variance in PCA. The results are useful for baseline data on chemotaxonomic classification, quality assessment, conservation and utilization of aromatic plant resources of the Vindhyar region in industries.

1. Introduction

Medicinal and aromatic plants represent one of the most important natural resources because of the great diversity of biologically active secondary metabolites. These plants have been used for centuries as ingredients in traditional systems of medicine, food preservation, perfumery, cosmetics and pharmaceutical formulations and their characteristic volatile constituents and wide spectrum of biological activities render them important. Among the plant derived products essential oils (EOs) are the concentrated mixtures of volatile phytochemicals that have been receiving increasing scientific and commercial interest. The main components of these complex mixtures are monoterpenes, sesquiterpenes, phenylpropanoids, and oxygenated derivatives such as alcohols, aldehydes, esters, ketones and others which contribute to their scent, ecological roles, and industrial uses (Burt, 2004; Edris et al. 2007; Bakkali et al. 2008; Dhifi et al. 2016; Pandey et al. 2017). The qualitative and quantitative profile of essential oils is very different depending on the plant species and

can be influenced by the genetic background, geographical origin, climatic conditions, soil characteristics, altitude, developmental stage of plants, harvesting season and extraction methodology (Sharifi-Rad et al. 2017; Nazzaro et al. 2013). This can lead to the presence of different chemotypes in the same species and calls for an extensive phytochemical characterization of the species as quality control, authentication, chemotaxonomical and commercial standardization.

The development of analytical instrumentation has greatly facilitated the characterization of volatile phytochemicals. As a highly sensitive technique with a well-established library of mass spectra, gas chromatography-mass spectrometry (GC-MS) is regarded as the "gold standard" of methods for the qualitative identification of volatile constituents (Adams, 2007). GC-FID is another form of quantitative analysis which complements GC-MS because it can provide a reliable estimation of the relative abundance of individual compounds. In addition, the international organizations have recommended the use of standardized chromatographic profiling methods which ensures the

reproducibility and quality assessment of essential oils (ISO, 1998; European Pharmacopoeia Commission, 2023; AOAC, 2023).

The environmental and seasonal conditions also have a significant influence over the biosynthesis of essential oils. Direct changes in temperature, rainfall, solar radiation, atmospheric humidity, soil moisture and photoperiod affect terpene metabolic pathways; as a result, essential oil yield and composition are affected. Seasonal factors have been shown to be one of the major factors affecting essential oil quality and productivity in previous studies (Figueiredo et al., 2008).

India is one of the richest centres of plant biodiversity having thousands of Medicinal and Aromatic Species located in different agro-climatic zones. Vindhyan region of Madhya Pradesh is very rich in flora diversity and there exists a vast collection of aromatic medicinal plants, traditionally utilized by the local and tribal communities for various purposes such as medicinal, cultural and domestic use in Sidhi district of Madhya Pradesh. However, systematic phytochemical study of some of the native aromatic plants of this region is still very less. The available reports are limited to the plant species or studies on a specific phytochemical, while limited comparative studies are found for aromatic plants growing at different environmental conditions. As a result, there is a lack of valuable information on phytochemical diversity, seasonal variation and chemotaxonomic relationships. The selected aromatic plant *Ocimum canum*, *Syzygium nervosum*, *Vitex negundo*, *Murraya koenigii*, *Annona squamosa* and *Cyperus scariosus* are botanically different and known to have medicinal, aromatic and economic values. Essential oils of these contain different types of volatile compounds, including monoterpenes, sesquiterpenes, phenolic derivatives and oxygenated terpenoids, that are mainly responsible for the important aroma and industrial applications. But, their phytochemical characterization, in comparison to others of the Sidhi district is not yet comprehensively reported by standardized analytical methods.

The present study provides a comprehensive evaluation of essential oils from selected aromatic plants of the Sidhi district, a relatively underexplored region of Central India, by integrating essential oil yield, physicochemical properties, qualitative and quantitative phytochemical profiling, seasonal variation, and chemometric analyses using GC–MS, GC–FID, principal component analysis (PCA), and hierarchical cluster analysis (HCA). The resulting phytochemical database offers valuable insights into chemotaxonomic discrimination, quality assessment, and the scientific documentation of regional aromatic biodiversity, while serving as a baseline for the conservation and sustainable industrial utilization of aromatic plant resources from the Vindhyan region of Madhya Pradesh.

2. Materials and Methods

2.1 Study Area

The study was conducted on aromatic plant species

collected from various locations of Sidhi district, Madhya Pradesh, India, situated in the Vindhyan plateau region between approximately 23°47'–24°42' N latitude and 81°18'–82°48' E longitude. The district experiences a tropical climate with distinct summer, monsoon, and winter seasons. Owing to its diverse vegetation, ecological heterogeneity, and varied soil and climatic conditions, the region provides suitable habitats for numerous medicinal and aromatic plants, making it an ideal area for phytochemical investigations.

2.2 Collection and Identification of Plant Material

Fresh *Ocimum canum*, *Syzygium nervosum*, *Vitex negundo*, *Murraya koenigii*, *Annona squamosa* and *Cyperus scariosus* plant materials were picked up from various localities of Sidhi district at the proper stage of growth (Figure 1). All the samples collected were cleaned well to eliminate dust and impurities for analysis. Qualified taxonomists of the Department of Botany, Government Girls P.G. College, Rewa, Madhya Pradesh authenticated and botanically identified the plants. Voucher specimens were deposited in the departmental herbarium.

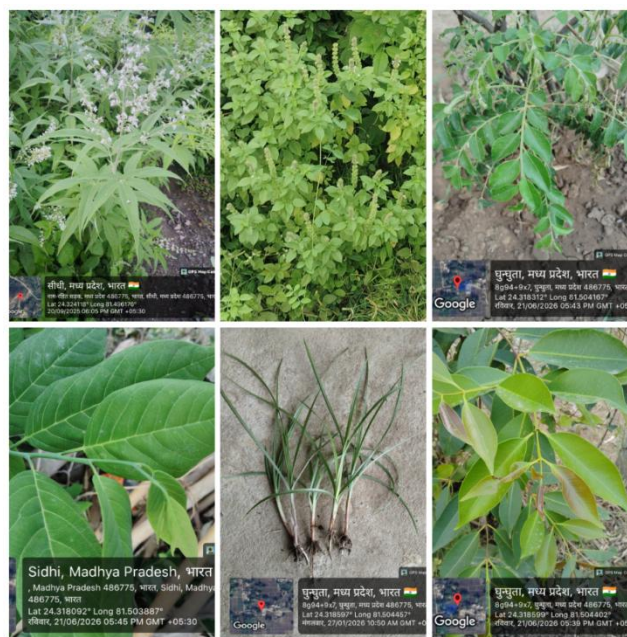


Figure 1: Representative aromatic plant species collected from Sidhi district, Madhya Pradesh, India. (A) *Vitex negundo* L., (B) *Ocimum canum* Sims, (C) *Murraya koenigii* (L.) Spreng., (D) *Annona squamosa* L., (E) *Cyperus scariosus* R.Br., and (F) *Syzygium nervosum* A.Cunn. ex DC.

2.3 Extraction of Essential Oils

Fresh plant materials were subjected to hydrodistillation (clevenger type apparatus) according to the standard procedure recommended by the European Pharmacopoeia and the AOAC. Approximately three hours of distillation were performed for each sample. The oils collected were separated from the aqueous phase, dried over anhydrous sodium sulfate to remove any moisture, and stored in airtight, amber-colored vials at 4°C until analyzed. The percentage volume of the essential oil

obtained from each unit weight of the fresh plant material was taken as the essential oil yield.

2.4 Physicochemical Characterization

The essential oils extracted were tested for various physicochemical parameters such as color, odor, density, refractive index, optical rotation and yield of oil. The density was determined using a calibrated pycnometer, the refractive index was determined with an Abbe refractometer and the optical rotation was measured with a digital polarimeter under controlled laboratory conditions. The analysis was carried out three times and the results were reported as mean $\pm$ SD.

2.5 GC–MS Analysis

The essential oils were subjected to qualitative phytochemical profiling with the help of Gas Chromatography–Mass Spectrometry (GC–MS). The volatile constituents were separated on a fused silica capillary column in helium carrier gas (Shah et al. 2020). Mass spectra were obtained under e.i. (70eV). The identification of compounds was done by matching the retention indices and mass spectra to the NIST Mass Spectral Library and literature. The identification of compounds was based on the relative abundance measured using the areas of the peaks of the chromatograms.

2.6 GC–FID Quantitative Analysis

Flame Ionization Detector (FID) was used for quantitative analysis of the major volatile constituents, using Gas Chromatography. The conditions employed in chromatographic were the same as those used in the GC–MS analysis for consistency. The relative percentage of each compound in the phytochemical composition of each plant species investigated was determined by peak area normalization of individual compounds, thus allowing comparative assessment of phytochemical composition among the various investigated plant species.

2.7 Seasonal Evaluation of Essential Oil Yield

The plant samples were taken in summer, monsoon and winter season to investigate the seasonal influences on the production of essential oil. To reduce the analytical variation, essential oils were extracted from seasonal samples using the same experimental conditions. Oil yields were compared and evaluated for each season to understand the effects of environmental and climatic factors on essential oil productivity.

2.8 Chemometric Analysis

Multivariate statistical methods were used to analyse the phytochemical relationships between the species studied. Data dimensionality reduction and identification of the major variables that contribute to the differentiation among the species were carried out using Principal Component Analysis (PCA). The analysis of the

species in terms of their chemical composition and the understanding of chemotaxonomic relationships were performed using Hierarchical Cluster Analysis (HCA) with Euclidean distance and Ward's method.

2.9 Statistical Analysis

All experiments were conducted in triplicate, and data were expressed as mean $\pm$ standard deviation. Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Differences among plant species and seasonal samples were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Statistical significance was considered at $p < 0.05$. Principal Component Analysis and Hierarchical Cluster Analysis were carried out using OriginPro 2024 software (OriginLab Corporation, Northampton, MA, USA) to examine phytochemical similarities and variations among the selected aromatic plant species".

3. Results

3.1 Essential Oil Yield and Physicochemical Characteristics

Significant variations were observed in the physicochemical properties and essential oil yield among the six aromatic plant species (Table 1 & Figure 2). Essential oil yield ranged from 4.3 to 13.8 mL kg⁻¹, indicating substantial interspecific differences in oil productivity. *Murraya koenigii* recorded the highest oil yield (13.8 $\pm$ 0.5 mL kg⁻¹), followed by *Ocimum canum* (12.5 $\pm$ 0.4 mL kg⁻¹), whereas *Annona squamosa* exhibited the lowest yield (4.3 $\pm$ 0.2 mL kg⁻¹). The densities ranged from 0.89 to 0.96 g mL⁻¹ and refractive indices from 1.472 to 1.505. There were also differences in the optical rotation, which were caused by differences in the volatile chemical composition of the species.

3.2 Seasonal Variation in Essential Oil Yield

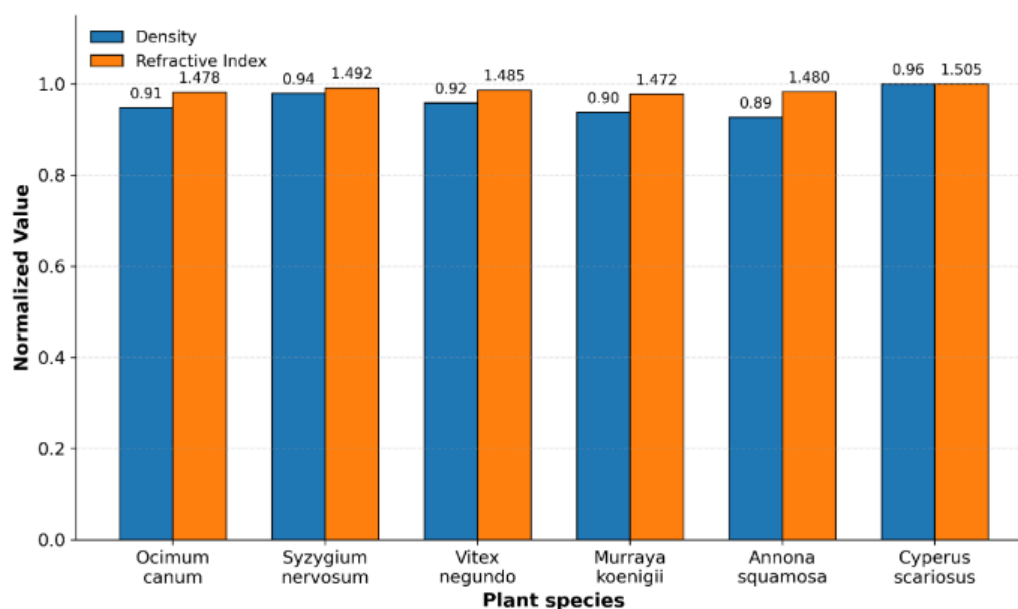
The seasonal evaluation showed that the environmental conditions have a significant effect on essential oil production (Table 2 & Figure 3). Summers were found to be the best season for producing oil for all species, followed by winter and monsoon seasons were found to be the worst. *Murraya koenigii* recorded the best productivity all year long, with a maximum production of 14.6 $\pm$ 0.5 mL kg⁻¹ in summer. On the other hand, *Annona squamosa* had the lowest seasonal production. It can be inferred from the observed trend that the augmentation of terpene biosynthesis in summer when the temperature is high and solar radiation is high, but a decrease of essential oil accumulation during the monsoon period when humidity is high and rainfall is abundant. The results highlight the need for the harvest timing to achieve maximum oil yield with uniformity of raw material quality.

3.3 GC–MS and GC–FID Phytochemical Characterization

The GC–MS analysis revealed presence of various volatile components, primarily monoterpenes, sesquiterpenes, phenolics, and oxygenated terpenoids.

Table 1: Essential oil yield and physicochemical characteristics of selected aromatic plants

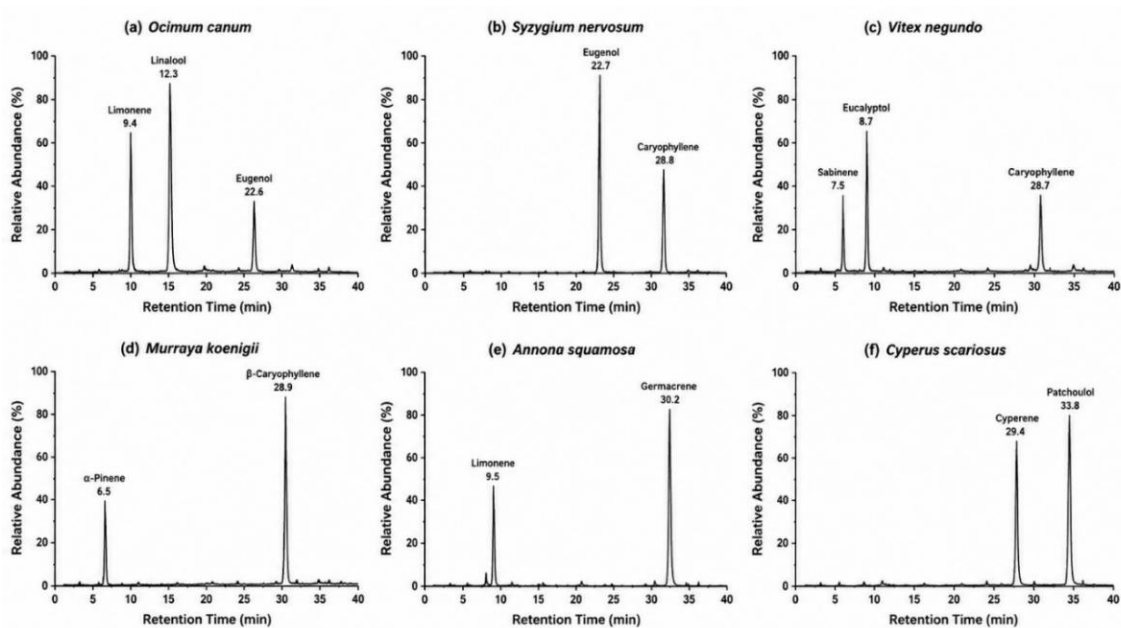
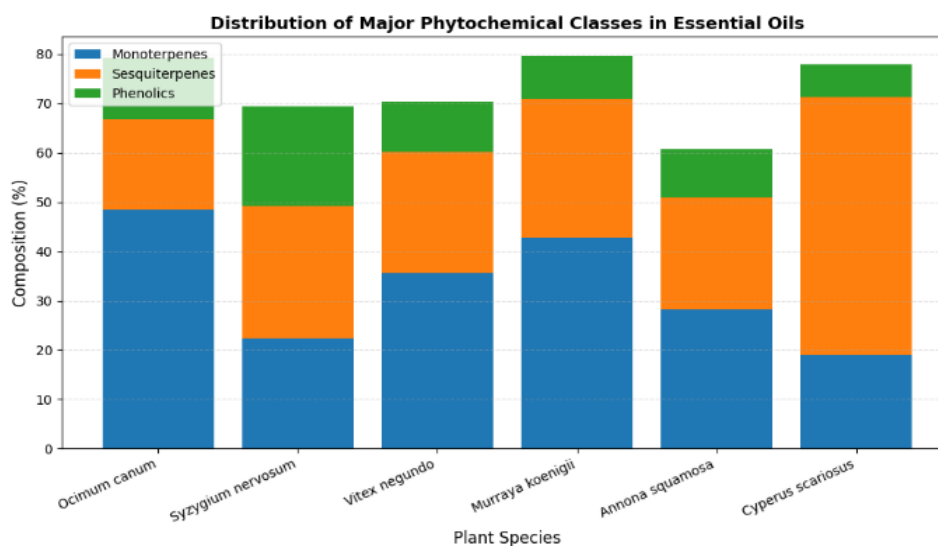
Plant species	Density (g mL ⁻¹)	Refractive index	Optical rotation (°)	Essential oil yield (mL kg ⁻¹)
<i>Ocimum canum</i>	0.91 ± 0.01	1.478 ± 0.002	+4.2 ± 0.3	12.5 ± 0.4 ^b
<i>Syzygium nervosum</i>	0.94 ± 0.01	1.492 ± 0.003	+1.8 ± 0.2	8.2 ± 0.3 ^d
<i>Vitex negundo</i>	0.92 ± 0.01	1.485 ± 0.002	+2.5 ± 0.2	9.4 ± 0.3 ^c
<i>Murraya koenigii</i>	0.90 ± 0.01	1.472 ± 0.002	+5.4 ± 0.4	13.8 ± 0.5 ^a
<i>Annona squamosa</i>	0.89 ± 0.01	1.480 ± 0.002	+0.9 ± 0.1	4.3 ± 0.2 ^e
<i>Cyperus scariosus</i>	0.96 ± 0.01	1.505 ± 0.003	-2.1 ± 0.2	7.5 ± 0.3 ^d

**Figure 2:** Physicochemical characteristics (density and refractive index) of essential oils from six aromatic plant species collected in Sidhi district.**Table 2:** Seasonal variation in essential oil yield (mL kg⁻¹)

Plant species	Summer	Monsoon	Winter
<i>Ocimum canum</i>	13.5 ± 0.5	10.2 ± 0.4	11.8 ± 0.4
<i>Syzygium nervosum</i>	9.0 ± 0.3	6.8 ± 0.2	7.9 ± 0.3
<i>Vitex negundo</i>	10.2 ± 0.4	7.5 ± 0.3	8.8 ± 0.3
<i>Murraya koenigii</i>	14.6 ± 0.5	11.0 ± 0.4	12.7 ± 0.4
<i>Annona squamosa</i>	5.2 ± 0.2	3.8 ± 0.1	4.5 ± 0.2
<i>Cyperus scariosus</i>	8.3 ± 0.3	6.9 ± 0.2	7.6 ± 0.3

Table 3: Major phytochemicals identified and quantified in essential oils

Plant species	Major compounds identified by GC–MS	Dominant compound (% by GC–FID)
<i>Ocimum canum</i>	Limonene, Linalool, Eugenol	Linalool (28.5 ± 1.1)
<i>Syzygium nervosum</i>	Eugenol, Caryophyllene	Eugenol (18.7 ± 0.8)
<i>Vitex negundo</i>	Sabinene, Eucalyptol, Caryophyllene	Eucalyptol (20.1 ± 0.9)
<i>Murraya koenigii</i>	β -Caryophyllene, α -Pinene	β -Caryophyllene (25.6 ± 1.0)
<i>Annona squamosa</i>	Germacrene, Limonene	Germacrene (12.4 ± 0.5)
<i>Cyperus scariosus</i>	Cyperene, Patchoulol	Patchoulol (27.9 ± 1.1)

**Figure 3:** Representative GC–MS chromatograms illustrating the characteristic volatile phytochemical profiles of the six investigated aromatic plant species.**Figure 4:** Distribution of major phytochemical classes (monoterpenes, sesquiterpenes, and phenolics) in the essential oils of selected aromatic plants.

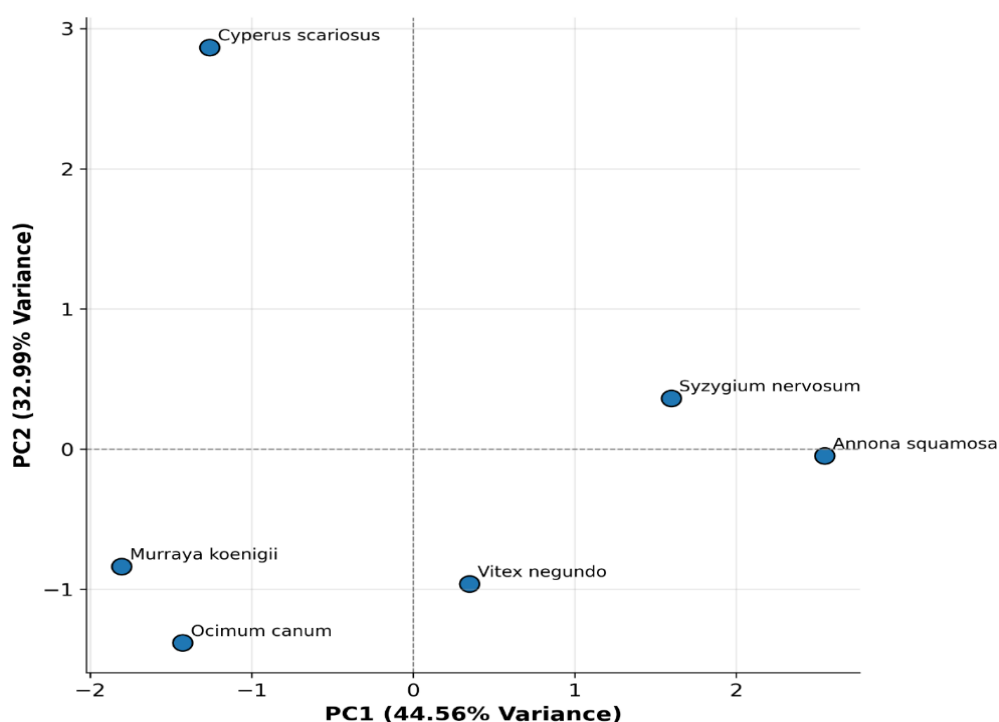


Figure 5: PCA score plot showing chemometric clustering and phytochemical differentiation among the six aromatic plant species based on essential oil composition.

Phytochemical fingerprint of each species showed a great chemical diversity of the aromatic vegetation of Sidhi district. Quantitative GC–FID analysis also showed that there were significant differences in the concentration of the major compounds (Table 3). *Ocimum canum* had high percentage of linalool (28.5%) and *Murraya koenigii* had a significant amount of β -caryophyllene (25.6%). In the case of *Syzygium nervosum* eugenol (18.7%) was predominant while *Cyperus scariosus* was rich in patchoulol (27.9%). Such species-specific marker compounds play an important role in the aroma, biological activity and value of the essential oil.

3.4 Distribution of Major Chemical Classes and Chemometric Analysis

The qualitative phytochemical classes of essential oils showed significant differences between all the species (Table 4). The highest monoterpene content was found in *Ocimum canum* while *Cyperus scariosus* was the richest source of sesquiterpenes (52.4%). *Syzygium nervosum* had the highest phenolic fraction (20.3%). The differences indicate that there are different chemotypes among the aromatic species that were examined.

Relationships among chemotaxonomic groups were further emphasized by Principal Component Analysis (PCA) and by Hierarchical Cluster Analysis (HCA). The first two principal components explained 77.55% of the total variance (PC1 = 44.56%, PC2 = 32.99%). PCA grouped *Ocimum canum* with *Murraya koenigii* as they contain high amount of monoterpenes whereas *Cyperus scariosus* was grouped separately because of high

amount of sesquiterpenes. *Syzygium nervosum* was characterized as having a high phenolic content. The same clustering pattern was seen in the HCA dendrogram, which showed the reliability of the phytochemical classification.

4. Discussion

The present study revealed that the six species of aromatic plants collected from Sidhi district of Madhya Pradesh showed significant differences in the essential oil yield, their physicochemical characteristics and phytochemical composition. This interspecific variation is often attributed to genetic, ecological or climatic factors and species-specific metabolic pathways in the biosynthesis of secondary metabolites (Sangwan et al., 2001; Maffei, 2020). This difference in essential oil yield of *Murraya koenigii* and *Ocimum canum*, which showed higher essential oil yield than *Annona squamosa* may relate with the difference in the secretory structures, glandular trichome density and terpene biosynthetic efficiency. This differential productivity of the aromatic plants in oil production has been observed earlier and the role of genetic and environmental factors on essential oil accumulation has been found (Elshafie & Camele, 2017; Raut & Karuppayil, 2014).

The seasonal evaluation showed that the highest production of essential oil was in summer, intermediate in winter and the lowest in monsoon. This trend is consistent with previous research with aromatic plants, in which the higher temperature, more solar radiation and the higher photosynthetic activity favored the biosynthesis of terpenes and the accumulation of essential oils (Hussain

et al. 2010; Duong et al. 2022). However, during monsoon season, the high amount of rainfall and humidity can lead to dilution of metabolites and influence physiological activity. These results highlight the criticality of harvest time for obtaining high oil yields and quality for commercial oil production.

In the volatile constituents of the species investigated, considerable qualitative and quantitative differences were found by GC–MS and GC–FID analysis. These essential oils contained mainly monoterpenes, sesquiterpenes, oxygenated terpenoids and phenolic compounds that are well known for their biological and industrial importance (Elshafie & Camele, 2017; Maffei, 2020). The phytochemical profile was different in each species, suggesting that species-specific chemotypes exist. The total amount of oxygenated monoterpenes (linalool and limonene) in *Ocimum canum* is in line with previous report on *Ocimum* species in which the oxygenated monoterpenes group is the dominant bioactive group (Sharifi-Rad et al. 2021). Linalool is known for its antimicrobial, antioxidant, sedative and anti-inflammatory properties and limonene for antioxidant and therapeutic properties. This essential oil of *O. canum* is rich in these compounds and has a significant pharmaceutical and nutraceutical potential.

In the same way, *Murraya koenigii* was found to possess high concentration of β -caryophyllene and α -pinene which are known for their anti-inflammatory, antioxidant, neuroprotective, antimicrobial and antioxidant and bronchodilatory properties, respectively (Raut & Karuppaiyil, 2014). The terpenes found in these bioactive molecules indicate the traditional medicinal significance of *M. koenigii* and its application in obtaining functional natural products.

Cyperus scariosus, on the contrary, showed a sesquiterpene rich chemical profile, where patchoulol and cyperene are the predominant compounds. The oils rich in sesquiterpenes have a longer lasting fragrance and are more chemically stable than those rich in monoterpenes, and as such are very sought after for perfumery and cosmetic uses. Patchoulol is also found to be a major compound that possesses antioxidant, antimicrobial, insecticidal and anti-inflammatory activity in previous studies (Srivastava et al. 2014). Thus, the present results further support the commercial value of *C. scariosus* essential oil.

An interesting finding is that *S. nervosum* had a very high concentration of eugenol. Eugenol is one of the most powerful naturally occurring phenolic antioxidants which works by scavenging free radicals and blocking the formation of lipid peroxides (Elshafie & Camele, 2017; Raut & Karuppaiyil, 2014). This correlation of eugenol-rich essential oils and potent antioxidant activity has been found in a number of aromatic species. Thus, *S. nervosum* could be a source of natural antioxidant substances for pharmaceutical, cosmetic and food preservation.

Also, there were significant chemotypic differences

between species in the comparative distribution of phytochemical classes. *Ocimum canum* and *Murraya koenigii* had high percentage of monoterpenes while *Cyperus scariosus* had maximum amount of sesquiterpenes. However, *Syzygium nervosum* showed a high phenolic level profile. This is the case of chemotypic differentiation of aromatic plants from different geographical areas which is known to be an important determinant of biological activity and industrial uses (Ferreira, 2022). Oils with high contents of monoterpenes tend to be used for pharmaceutical or antimicrobial purposes, while sesquiterpene-rich oils have been used in perfumery because of their fragrance stability and chemical stability.

Additional evidence of phytochemical differentiation was obtained through a chemometric analysis. PCA accounted for 77.55% of the total variance and was able to make clear separation of species based on the dominant chemical classes. *Ocimum canum* and *Murraya koenigii* grouped together due to the presence of more monoterpene and *Cyperus scariosus* grouped separately due to the presence of more sesquiterpene. *Syzygium nervosum* was characterized by the higher phenolic fraction. The clustering pattern which was achieved by HCA was in close agreement with the PCA results indicating the reliability of phytochemical classification. PCA and HCA have been successfully used in chemotaxonomic studies, metabolomic studies and authentication of medicinal and aromatic plants (Ward, 1963; Massart, 1997; Jolliffe et al. 2016; Lebanov et al. 2021; Abraham et al. 2023).

The environment also seems to be an important factor in determining phytochemical composition. Sidhi district has various ecological and climatic conditions, which could be responsible for chemotypic variability. The production of terpenes and essential oils has been shown to be significantly affected by the temperature, rainfall, solar radiation, soil properties and water availability in previous studies (Figueiredo et al. 2008; Sangwan et al. 2001). The present study further indicates the same relationship due to the seasonal variations found, which highlights the importance of ecology for determining the quality of essential oil.

In general, the identified phytochemical profiles show significant prospects for use in pharmaceutical industries, nutraceuticals, cosmetics and fragrance industries. Major constituents like linalool, limonene, eugenol, β -caryophyllene, α -pinene, patchoulol and eucalyptol are widely reported for their antimicrobial, antioxidant, anti-inflammatory and therapeutic properties (Raut & Karuppaiyil, 2014; Elshafie & Camele, 2017; Sharifi-Rad et al. 2021). Thus, the extensive phytochemical database obtained during the present study serves as a source of basic information for chemotaxonomic classification, quality control, authentication, conservation and possible future commercial exploitation of aromatic plant resources of Vindhyan region of Madhya Pradesh.

5. Conclusion

The current study showed that there were substantial differences in essential oil yield, physical and chemical characteristics, and phytochemical composition of the six aromatic plant species gathered from the Sidhi district in Madhya Pradesh. *Murraya koenigii* and *Ocimum canum* gave the maximum essential oil yields and *Annona squamosa* was least productive. The effect of the environment on the biosynthesis of the terpenes was clearly observed in the seasonal assessment where the highest yield of EO was obtained in summer and the lowest in the monsoon season. Volatile components were detected by GC–MS and GC–FID, which revealed a variety of compounds, of which linalool, β -caryophyllene, eugenol, eucalyptol, germacrene and patchoulol were marker compounds of the respective species. Significant differences in the levels of various major chemical classes (monoterpenes, sesquiterpenes and phenolics) were observed, confirming the presence of distinct species-specific chemotypes.

The PCA and HCA chemometric analysis discriminated against the species investigated on the basis of their phytochemical composition and provided a clear chemotaxonomy relationship. *Ocimum canum* and *Murraya koenigii* were clustered together as monoterpene rich species, *Cyperus scariosus* were clustered together as sesquiterpene rich species and *Syzygium nervosum* was distinguished by its high phenolic profile. In summary, the study has created a database of the phytochemical constituents of the aromatic flora of Vindhyan region and demonstrated they have immense potential in pharmaceuticals, nutraceuticals, cosmetics, perfumery and food sectors. The results also highlight the need for the optimization and sustainable use of the aromatic plant resources to improve the quality and use them commercially.

6. Statements and Declarations

Ethical Approval: The study involved collection of plant materials only and did not involve human participants or experimental animals. Plant collection was conducted in accordance with applicable institutional and regional regulations.

Conflict of Interest: The authors declare that there are no competing financial interests or personal relationships that could have influenced the work reported in this manuscript.

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