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Multivariate Analysis Secondary Metabolites in Honey Samples from Terai Region of Uttar Pradesh

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ABSTRACT: The phenolic profile of the investigated samples revealed considerable variation in the concentrations of p-coumaric acid, gallic acid, caffeic acid, and pinocembrin. Among the quantified compounds, pinocembrin generally occurred at the highest concentration with the highest levels observed in Maharajganj and Pilibhit, whereas the lowest concentration was recorded in Rampur. Gallic acid was the second most abundant compound, its maximum level was observed in Balarach, followed by Pilibhit, while Rampur exhibited the lowest concentration. p-Coumaric acid showed moderate variation among the samples reaching its highest level in Bahariach and its lowest in Rampur. In contrast, caffeic acid was detected at comparatively lower concentrations, with relatively limited variation among samples. Overall, the results demonstrate distinct differences in the phenolic composition of the samples, with pinocembrin and gallic acid represented the predominant compounds, while caffeic acid contributed comparatively less to the overall phenolic profile. These variations suggested differences in the accumulation or relative abundance of individual phenolic constituents among the samples and may contribute to the differences observed in their overall phenolic and flavonoid contents. Among the locations, Bahraich exhibited the highest positive PC1 score followed by Maharajganj and Pilibhit placing these locations toward the positive phenolic gradient represented by PC1. Rampur showed the highest positive PC2 score, indicated a strong association with the positive PC2 variables, particularly quercetin, β -carotene and linalool. Conversely, Basti exhibited strongly negative scores for both PC1 and PC2, placing it toward the negative PC2 direction associated with p-coumaric acid. Shahjahanpur showed a relatively strong negative PC1 score, whereas Lakhimpur Kheri, Balrampur, Gonda and Shravasti were located close to the origin, indicated comparatively weak differentiation along the first two components.

1. INTRODUCTION

Honey is one of the oldest natural foods consumed by humans and is widely recognized for its nutritional, medicinal, and economic importance (Bonsignore et al., 2021; Martinotti et al., 2024). Although carbohydrates constitute approximately 95–99% of the dry matter of honey, it also contains proteins, amino acids, enzymes, vitamins, minerals, organic acids, and numerous bioactive phytochemicals that contribute significantly to its biological activities (Olas et al., 2020; Santos et al., 2023). Among the diverse constituents of honey, secondary metabolites are of particular scientific interest because they largely determine its biological and therapeutic potential (Mustar et al., 2022). The major classes of secondary metabolites include phenolic acids, flavonoids, terpenoids, carotenoids, coumarins, tannins, phytosterols, and volatile organic compounds (Manici et al., 2020). Phenolic acids such as gallic, caffeic, chlorogenic, ferulic, and p-coumaric acids, together with flavonoids including quercetin, kaempferol, chrysin, galangin, pinocembrin, apigenin, and luteolin, constitute the dominant

phytochemical groups in most natural honeys (Sunkesula and Reddy, 2021). These metabolites possess remarkable antioxidant capacity by scavenging reactive oxygen species, chelating transition metals, and regulating oxidative stress pathways (Vuolo et al., 2019). In addition, they exhibit antimicrobial, antiviral, anti-inflammatory, anticancer, antidiabetic, and neuroprotective activities, making honey an important functional food with significant pharmaceutical potential (Wilczyńska & Žak, 2024). Honey secondary metabolites are minor non-sugar bioactive compounds transferred from plant nectar, pollen, or generated by the bee microbiome (Bonsignore et al., 2024). They primarily include phenolic compounds, flavonoids, and terpenoids that give honey its color, distinct flavor, antioxidant capacity, and medicinal properties (Lazaridis et al., 2024). **Phenolic acids** are recognized for a wide range of benefits for human health, such as antidiabetic, anti-inflammatory, anticancer and neuroprotective (Wu et al., 2023). In addition to the radioprotective potential, **Flavonoids**, when consumed regularly in the diet, can contribute to the prevention of certain diseases, thanks to antioxidant, anti-inflammatory, anti-allergic, antimicrobial, antiviral and anticarcinogenic, anti-hepatotoxic, anti-ulcerogenic and anti-platelet properties, among others. **Research on the bioactive compounds** that are contained in honey has shown their positive effects on human health in various aspects, such as anti-inflammatory, antimicrobial, anti-tumor, anti-aging and anti-diabetic (Albu et al., 2021; Palma-Morales et al., 2023). **Protective effects** on the cardiovascular, nervous, respiratory, cardiac and gastrointestinal systems are also added (Tlak et al., 2025). **The bioavailability of phytochemical components** and the way in which they are absorbed and metabolized determine the effects of honey on human health (Ferrassi et al., 2026). **The antioxidant action of honey** has gathered considerable attention from researchers who have analyzed in depth various types of honey, observing significant differences in relation to the different phenolic profiles (Nidhi et al., 2025). **Oxidative stress** forms free radicals and influences, among other things, the aging of cells and skin (Nicewicz et al., 2021). The enzyme inhibitory activity attributed to the phenolic compounds contained in honey may offer an answer as a clinical nutraceutical tool (Yimer, 2024). The Terai region of Uttar Pradesh forms an ecologically unique belt along the Indo-Nepal border and includes the districts of Lakhimpur Kheri, Pilibhit, Bahraich, Shravasti, Balrampur, Gonda, Maharajganj, Basti, Shahjahanpur, and Rampur. This region is characterized by tropical moist deciduous forests, extensive grasslands, wetlands, riverine ecosystems, and fertile alluvial plains that support exceptional floral diversity.

2. MATERIALS AND METHODS

2.1 Study Material

An extensive field survey was conducted to collect honey samples from natural forests and adjoining areas of the Terai regions of Uttar Pradesh. A total of ten honey samples were collected from different forest areas of the Terai region. Specifically, honey samples were collected from the districts of Lakhimpur Kheri, Pilibhit, Bahraich, Shravasti, Balrampur, Gonda, Maharajganj, Basti, Shahjahanpur and Rampur for palynological analysis to study the pollen grains preferred by honey bees in these areas

2.2 Sample Collection

Honey samples were obtained from naturally occurring bee hives across the selected districts. Collection was carried out during two distinct periods: winter (November–February) and spring (March–May). Honey was extracted by squeezing under the supervision of trained personnel from forest department and local people. Samples were filtered through cloth to remove extraneous materials such as beeswax, scum and surface debris. Each sample was labelled with the date and site of collection and stored in glass containers at room temperature in the dark.

2.3 Extraction Procedure

The present study aimed to assess the major secondary metabolites reported in natural honey from comparable floral ecosystems was undertaken to provide an integrated understanding of the phytochemical composition and potential health benefits associated with Terai honey. The details of the estimated phytochemicals (AOAC, 2023) had reflected below for completeness.

2.4 Analytical Methods

Phenolic acids	Gallic acid, Caffeic acid, Ferulic acid, p-Coumaric acid, Chlorogenic acid, Syringic acid, Vanillic acid
Flavonoids	Quercetin, Kaempferol, Chrysin, Pinocembrin, Galangin, Apigenin, Luteolin, Myricetin
Terpenoids	Linalool, Geraniol, Nerol, Thymol
Volatile compounds	Methyl syringate, Benzaldehyde, Phenylacetaldehyde
Carotenoids	β -Carotene, Lutein
Phytosterols	β -Sitosterol, Campesterol
Coumarins	Umbelliferone, Scopoletin
Tannins	Hydrolysable tannins, Condensed tannins
Saponins	Triterpenoid saponins
Alkaloids	Trace pyrrolizidine alkaloids (occasionally detected)

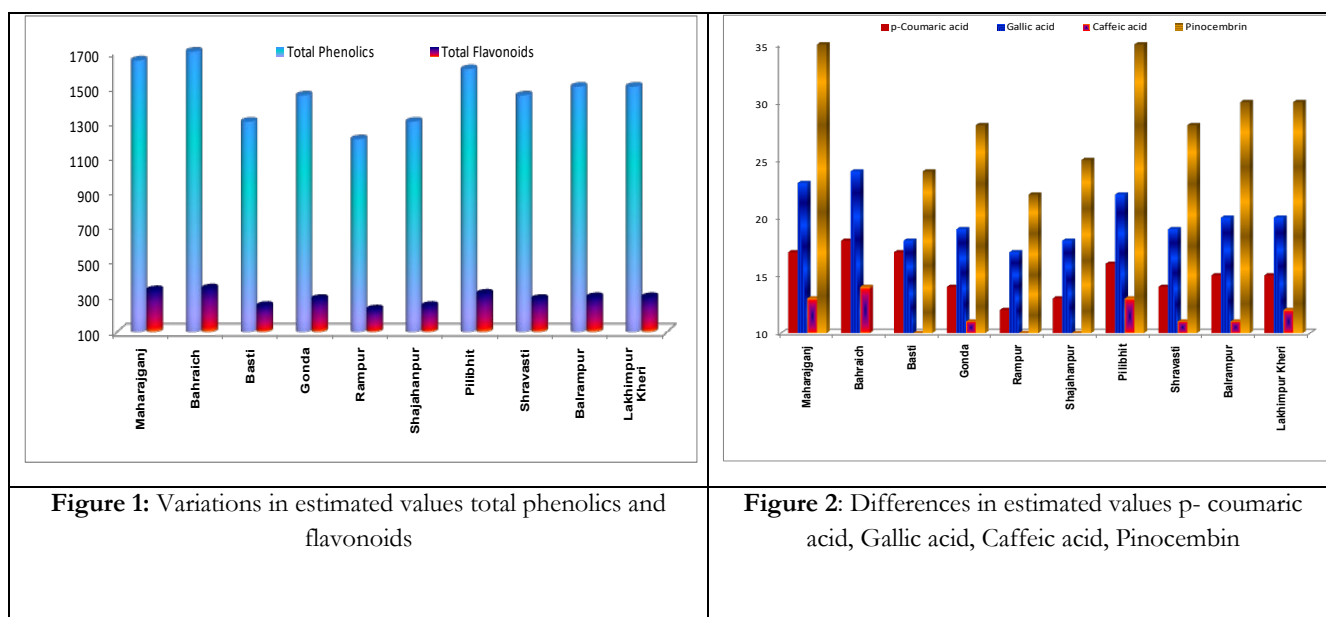
2.5 Statistical Analysis

Analysis of variance (ANOVA) test was used in order to find the significant differences ($p \leq 0.05$) among the honey samples. In addition, the data were analyzed using principal components analysis (PCA) and multivariate hierarchical cluster analysis (HCA) to classify and discriminate among the honey samples based on general physicochemical parameters etc. All the statistical analyses were performed using JMP version 9.3 and R studio.

3. RESULTS AND DISCUSSION

3.1 Preliminary Analysis

The bar chart in figure 1 had compared total phenolic content (TPC) and total flavonoid content (TFC) among ten samples of the study. Total phenolics showed substantially higher values than total flavonoids across all samples as reported by Bhalchandra and Joshi, 2021. Baharaich had the highest total phenolic content, approximately 1,750, followed by Maharajganj (~1,700) and Pilibhit (~1,650). Rampur showed the lowest total phenolic content, approximately 1,250, while Basti was around 1,350. Total flavonoids had ranged approximately from 260 to 380. Baharaich and Maharajganj had expressed the highest flavonoid contents (~370–380), whereas Rampur had the lowest (~260). Overall, the samples showed considerable variation in phenolic and flavonoid contents, however the consistently phenolics were much higher than flavonoids.



The figure 2 presented the comparative levels of four phenolic compounds p-coumaric acid, gallic acid, caffeic acid, and pinocembrin among collected samples. Pinocembrin was the predominant compound in all samples, with values ranged approximately from 22 to 35 units as mentioned by Kharkamni, 2021. The highest levels were observed in Maharajganj and Pilibhit (~35), followed by Balrampur and Lakhimpur Kheri (~30). Rampur had the lowest pinocembrin concentration, at approximately 22. Gallic acid was the second most abundant compound, varied from 17 to 24 units. Bahariach showed the highest gallic acid level (~24), followed by Pilibhit (~22). Rampur exhibited the lowest level (~17), while Basti, Shahjahanpur, Shravasti, Balrampur, and Lakhimpur Kheri showed intermediate concentrations. p-Coumaric acid occurred at moderate concentrations, nearly 12–18 units. The highest value was recorded in Bahariach (~18), whereas Rampur (~12) had the lowest. Maharajganj and Basti were close to 17, while Pilibhit was approximated to 16. Caffeic acid was observed at comparatively low concentrations, around 11–14 units. Bahariach showed the highest level (~14), while Rampur, Gonda, Shravasti, and Balrampur were close to 11. The relatively narrow range indicated less variation among samples compared with pinocembrin and gallic acid.

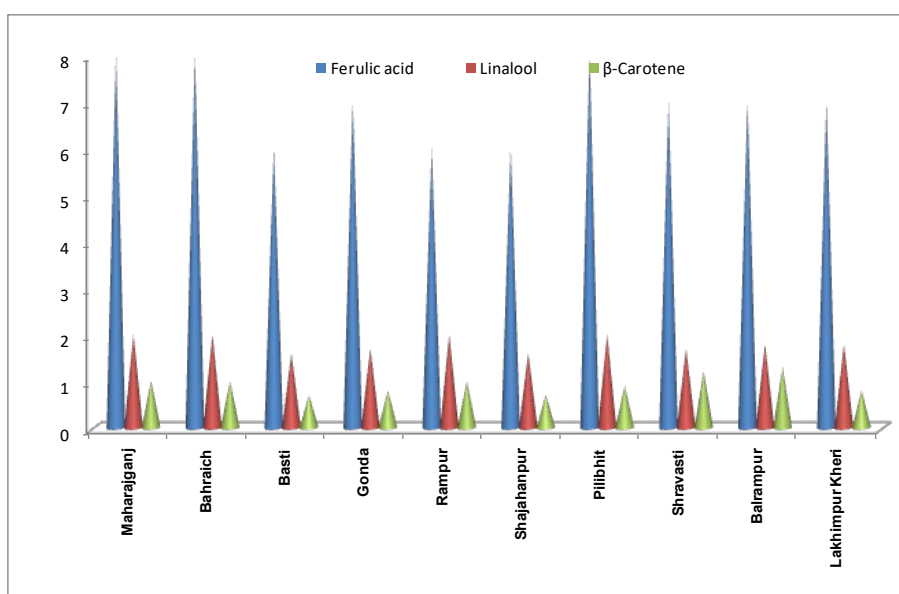


Figure 3: Range Values of Ferulic acid, Linalool and beta- carotene

The figure 3 exhibited the comparative concentrations of ferulic acid, linalool, and β -carotene across ten samples. Ferulic acid was the predominant compound in all ten samples, with concentrations ranged from 5.9 to 8.0 Shukla and Kumar, 2020). The highest levels were observed in Maharajganj and Baharaich (~8.0), followed by Pilibhit (~7.8). Gonda showed approximately 7.0, whereas Shravasti, Balrampur, and Lakhimpur Kheri were around 6.9–7.0. The lowest ferulic acid concentration was observed in Rampur (~5.9), followed closely by Basti and Shahjahanpur (~6.0). Linalool occurred at intermediate concentrations as reported by Thakur et al., 2021, varied from 1.6 to 2.1. Maharajganj, Baharaich, Gonda, and Pilibhit showed values close to 2.0–2.1. Rampur also exhibited a relatively high linalool concentration of approximately 2.0. The lowest levels were observed in Basti and Shahjahanpur (~1.6–1.7). β -Carotene was present at the lowest concentrations among the three compounds as observed by Sabrina et al., 2023, with values approximately between 0.7 and 1.4. The highest β -carotene levels were observed in Balrampur (~1.4) and Shravasti (~1.3). Maharajganj, Baharaich, Rampur, and Gonda showed intermediate values of 0.9–1.1. Basti and Shahjahanpur had lower concentrations, around 0.7–0.8.

Biplot analysis

The first two PCs together explained 80.19% of the total variation in the phytochemical dataset. This was a strong representation of the original multivariate information. The table contained two sets of information i.e. trait loadings to observe how strongly each phytochemical contributed to PC1 and PC2. Location scores were also studied to their positions where each geographical location lied on PC1 and PC2. As PC1 represented the major pattern of variation among phytochemicals. PC2 expressed the second, independent pattern of variation. Locations with similar PC1 and PC2 scores had similar multivariate phytochemical profiles. A positive or negative score indicated the direction in which a location lied along

that component. PC1 explained 64.53% of the total variation, making it the dominant component. This meant that almost two-thirds of the observed variation among the phytochemical profiles was represented by a single underlying gradient. PC2 explains an additional 15.66%.

The strongest positive PC1 loadings were of Caffeic acid (0.4072), Gallic acid (0.4020), Ferulic acid (0.3974), Total phenolics (0.3875). These four variables were therefore the principal characteristics defined PC1. Because all four had positive and relatively similar loadings, PC1 could reasonably be described as a phenolic-acid/total-phenolics gradient. The positive association of linalool, β -carotene, quercetin and p-coumaric acid with PC1 was weaker though contributed to this component of biplot analysis. Pinocembrin had a loading of only -0.0857 . This was substantially smaller than the other loadings. Therefore, pinocembrin contributed relatively little to PC1. The strongest positive contributors of PC2 were Quercetin (0.4274), β -Carotene (0.4257), Linalool (0.4126) as these three compounds defined the positive side of PC2. The strongest negative loading was of p-Coumaric acid (-0.5868). This was the largest absolute loading in the entire PC2 column. Therefore, PC2 primarily distinguishes a pattern characterized by Quercetin + β -carotene + Linalool from a contrasting pattern characterized by p-Coumaric acid.

Table 1: Loadings of phytochemical and locations based on first two principal components

Traits	PC1	PC2	Locations	PC1	PC2
Gallic acid	0.4020	-0.1822	Lakhimpur Kheri	0.0189	-0.0031
Caffeic acid	0.4072	-0.0975	Maharajganj	0.4174	0.0725
Ferulic acid	0.3974	-0.0513	Bahraich	0.5586	-0.2575
p-Coumaric acid	0.2731	-0.5868	Basti	-0.4096	-0.6462
Quercetin	0.2964	0.4274	Gonda	-0.1175	0.0256
Pinocembrin	-0.0857	0.1881	Rampur	-0.2501	0.7069
Linalool	0.3220	0.4126	Shajahanpur	-0.3886	0.0002
β -Carotene	0.3018	0.4257	Pilibhit	0.3215	0.0969
Total Phenolics	0.3875	-0.2021	Shravasti	-0.1175	0.0256
Total (80.19 %)	64.53%	15.66%	Balrampur	-0.0331	-0.0210

Bahraich had the highest positive PC1 score among the locations. It therefore lied strongly toward the positive PC1 direction, which was mainly defined by caffeic acid, gallic acid, ferulic acid, and total phenolics. Its negative PC2 score placed it toward the negative PC2 direction, which was associated particularly with p-coumaric acid. Thus, Bahraich occupied a position characterized by a strong positive PC1 contribution and a negative PC2 contribution. Maharajganj had a strongly positive PC1 score and a slightly positive PC2 score. It was therefore primarily associated with the PC1 phenolic gradient, with a weaker contribution from the positive PC2 traits such as quercetin, β -carotene and linalool. Pilibhit also occurred in the positive PC1 and positive PC2 region. Its position suggested an association with both the phenolic-acid/total-phenolics pattern represented by PC1, and the quercetin- β -carotene-linalool pattern represented by PC2. However, its PC1 score was larger than its PC2 score, so its separation was more strongly related to PC1. Rampur was particularly important because it has the highest positive PC2 score. Its position strongly corresponded to the positive PC2 direction, which was associated with quercetin, β -carotene, and linalool. Therefore, Rampur was strongly differentiated along the second principal component. Its negative PC1 score indicated that it was positioned away from the positive PC1 phenolic gradient. Basti had strongly negative scores on both components. Its PC2 score was particularly important because it was strongly negative. Since p-coumaric acid had the strongest negative PC2 loading (-0.5868). Basti was positioned toward the p-coumaric-acid-associated direction. At the same time, its negative PC1 score places it opposite the locations strongly associated with the major positive PC1 phenolic gradient. Basti had a distinctly different multivariate position in the PCA space. Shahjahanpur had a moderately strong negative PC1 score but virtually zero PC2 score. Therefore, its major differentiation was along PC1 rather than PC2. It was

positioned away from the positive PC1 phenolic gradient but did not show a strong directional separation along PC2. Gonda lied relatively close to the origin. This indicated that it did not have a particularly strong score on either PC1 or PC2. Interestingly, Shravasti had exactly the same PC1 and PC2 scores as Gonda. Therefore, same position in the PC1–PC2 space **was** occupied by Gonda and Shravasti values. This expressed highly similar projected phytochemical profiles with respect to the first two principal components. Balrampur was extremely close to the origin. It therefore had neither a strong positive nor negative association with either principal component. Lakhimpur Kheri was also almost exactly at the origin. Consequently, it did not show a strong differentiation along either of the two major PCA axes.

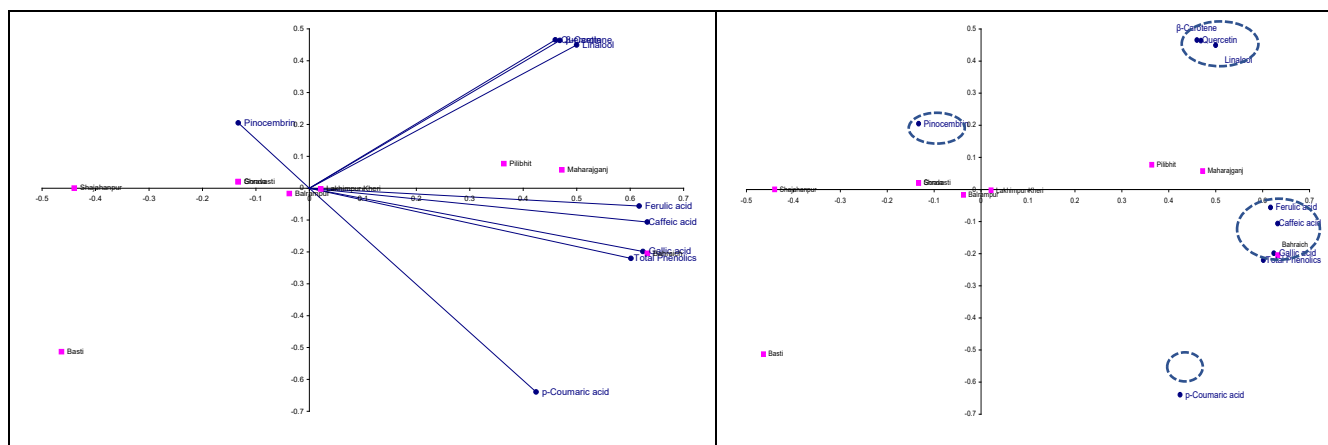


Figure 5: Biplot analysis of locations vis-à-vis secondary metabolites

Clustering of compounds and locations of the study

Principal component analysis (PCA) was performed to assess the multivariate relationships among the phytochemical traits and to discriminate the studied locations based on their overall phytochemical profiles. The first two principal components accounted for 80.19% of the total variation, PCA biplot provided an effective two-dimensional representation of the major variation present in the dataset. The biplot further demonstrates that variables with similar loading directions tend to exhibit similar patterns of variation across locations, whereas variables oriented in opposite directions represent contrasting patterns. The two dendrograms provide clustering at two levels **i.e.** compounds/traits (right side) and districts (bottom).

Clustering of phytochemicals

The dendrogram on the right side grouped the eight measured variables according to their similarity across the districts. First cluster was comprised of Gallic acid, caffeic acid and p-coumaric acid. Gallic acid and caffeic acid were closely associated. p-Coumaric acid subsequently joins this group. This indicated that the spatial variation of these three phenolic acids across the districts was relatively similar. In other words, districts that tended to have similar levels of gallic acid also tended to show similar patterns for caffeic acid and p-coumaric acid. This is biologically plausible because all three were phenolic compounds associated with phenylpropanoid/phenolic metabolic pathways, although the clustering itself is based on the measured data rather than their chemical classification. Second cluster was consisted of Ferulic acid, linalool and β-carotene. Within this group, linalool and β-carotene appear to form a relatively close pair, which subsequently joined with ferulic acid. An important point was that ferulic acid did not cluster with the other phenolic acids in this dataset, despite being chemically classified as a phenolic acid. This suggested that its geographical variation among the districts was more similar to that of linalool and β-carotene than to gallic, caffeic or p-coumaric acid. The clustering reflected patterns of variation among districts, rather than simply grouping compounds according to their chemical classes.

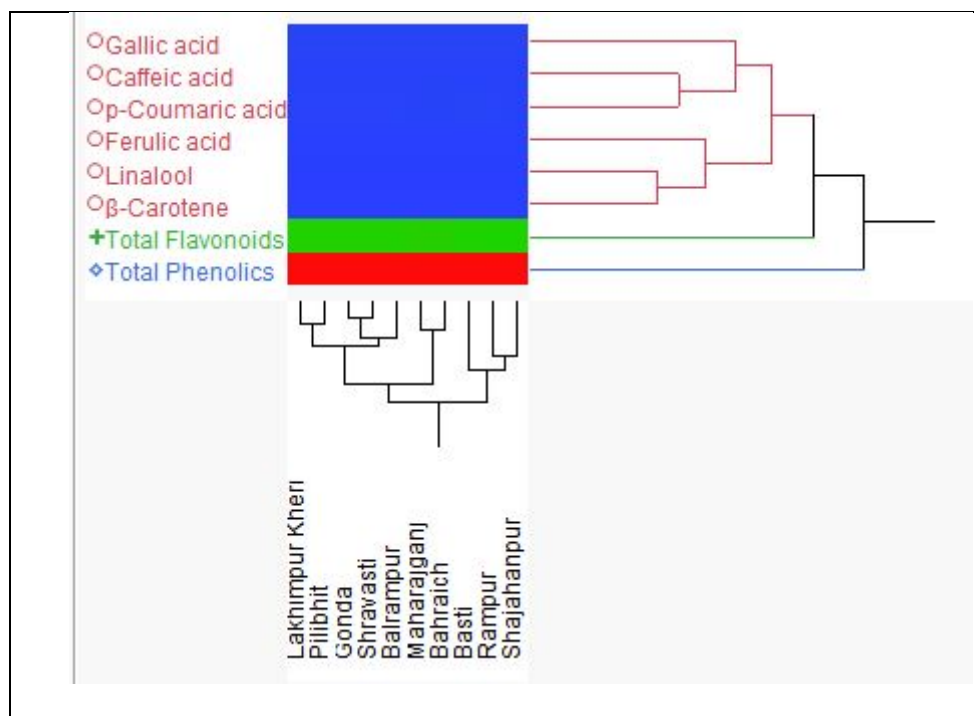


Figure 4: Multivariate hierarchical clustering of phytochemicals and locations

The two aggregate parameters were separated from the six individual compounds. Total flavonoids form a distinct branch in the dendrogram. This pointed out that the spatial pattern of total flavonoid content across the districts differs substantially from the patterns of the individual compounds. Total phenolics were located on a separate, relatively distant branch. The comparatively long branch associated with total phenolics indicated that its distribution among districts was relatively distinct from the other variables included in the analysis. This distinction was important because total phenolic content is an aggregate measure and did not necessarily follow the distribution of any single phenolic compound.

Dendrogram for clustering of districts

The dendrogram below the heatmap groups the 10 districts according to similarity in their overall chemical profiles. Districts joined by short branches were more similar to one another in their measured phytochemical profiles, whereas districts connected only through longer branches were more dissimilar. The bottom dendrogram showed several relatively close groupings, followed by progressively larger clusters. Thus, the districts did not all exhibit an identical phytochemical pattern. The dendrogram indicated that some districts had relatively similar combinations of phenolic acids, linalool, β -carotene, total flavonoids, and total phenolics. These smaller clusters were subsequently merged into larger groups as the clustering distance increased.

The locations could broadly be understood according to their PCA positions. The strongest positive PC1 scores were of Bahraich, Maharajganj, and Pilibhit. These locations were positioned toward the phenolic-acid/total-phenolics gradient. The strongest negative PC1 scores were of Basti, Shahjahanpur and Rampur. These locations were positioned on the opposite side of the major PC1 gradient. Rampur was especially prominent because of its very high PC2 score. It was associated with the direction defined by Quercetin + β -carotene + Linalool. Basti had the strongest negative PC2 score, followed by Bahraich. This placed these locations toward the negative PC2 direction, which was strongly associated with p-coumaric acid. Lakhimpur Kheri, Balrampur, Gonda and Shravasti were relatively close to the origin and therefore were not strongly differentiated by either PC1 or PC2.

The loading values also provide information about relationships among traits. Gallic acid, caffeic acid and ferulic acid. Their very similar PC1 loadings indicated that their similar contribution to PC1 and also had relatively small negative PC2 loadings. Therefore, their major variation was captured by PC1 rather than PC2. Quercetin, β -carotene and linalool had remarkably similar PC2 loadings. This suggested a very similar contribution to PC2 and agrees with their close positioning in the PCA

biplot. p-Coumaric acid had PC2 loading of **-0.5868** was particularly notable. It was substantially larger in absolute magnitude than the PC2 loadings of the other traits, making p-coumaric acid a major variable responsible for the negative direction of PC2.

4. CONCLUSION

The comparative analysis revealed substantial variation in the concentrations of ferulic acid, linalool, and β -carotene among the ten samples. The highest concentrations of Ferulic acid were observed in Maharajganj and Balarach, followed by Pilibhit, whereas Rampur exhibited the lowest concentration. Relatively higher concentrations of Linalool observed in Maharajganj, Balarach, Rampur, Gonda, and Pilibhit. The highest levels β -Carotene was detected at Balrampur and Shravasti. The phenolic profile of the investigated samples revealed considerable variation in the concentrations of p-coumaric acid, gallic acid, caffeic acid, and pinocembrin. Pinocembrin highest levels observed in Maharajganj and Pilibhit, whereas the lowest concentration was recorded in Rampur. Gallic acid had expressed maximum level Balarach, followed by Pilibhit, while Rampur exhibited the lowest concentration. p-Coumaric acid showed highest level in Bahariach and its lowest in Rampur. In contrast, caffeic acid was detected at limited variation among samples.

Principal component analysis revealed substantial multivariate variation among the studied locations as first principal component was characterized predominantly by positive loadings for caffeic acid, gallic acid, ferulic acid and total phenolics . PC2 was strongly and positively associated with quercetin, β -carotene and linalool, while p-coumaric acid showed the strongest negative loading. Among the locations, Bahraich exhibited the highest positive PC1 score, followed by Maharajganj and Pilibhit. Rampur showed the highest positive PC2 score, indicated a strong association with quercetin, β -carotene and linalool. Conversely, Basti exhibited strongly negative scores for both PC1 and PC2, placed it toward the negative PC2 direction associated with p-coumaric acid. Shahjahanpur showed a relatively strong negative PC1 score, whereas Lakhimpur Kheri, Balrampur, Gonda and Shravasti were located close to the origin.

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AUTHOR CONTRIBUTIONS

Sunita Verma: Conceptualization, Investigation.

Shilpa Pandey: Methodology, Laboratory Analysis.

Alka Rani: Data Curation, Formal Analysis.

Sunita Erma: Supervision, Writing—Review & Editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Data will be available on request

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