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Comparative profiling of phenolics, tocopherols and fatty acids in seed spices

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Abstract
Seed spices such as coriander, cumin, fennel, nigella, ajwain, and fenugreek hold great significance in Indian cuisine and traditional medicine. They are highly valued for their bioactive compounds, including phenolics, tocopherols, and fatty acids. This study aimed to profile these compounds across selected genotypes using chromatographic techniques. The plant materials consisted of seeds from two genotypes each of coriander, fennel, nigella, ajwain, and fenugreek, as well as one genotype of cumin, all procured from ICAR-NRCSS in Ajmer. Phenolics were extracted using 80% methanol and analyzed through Ion Chromatography. Tocopherols were profiled using a Polar Advantage II C-18 column, and fatty acids were quantified as fatty acid methyl esters (FAMEs) using Gas Chromatography. The results indicated that chlorogenic acid was the dominant phenolic compound in coriander, fennel, cumin, and ajwain, while fenugreek and nigella were particularly rich in caffeic acid. Coriander and fenugreek showed the highest tocopherol content, and both fenugreek and nigella had high levels of polyunsaturated fatty acids. In conclusion, coriander and fennel exhibited significant nutraceutical potential due to their resveratrol and tocopherol content, while fenugreek and nigella were rich in antioxidants and anti-inflammatory compounds. These findings provide valuable insights for developing functional foods and value-added extracts derived from seed spices.

Keywords: Phenolics, Tocopherols, Fatty acids, Nutraceutical

Introduction
Seed spices such as coriander, cumin, fennel, nigella, ajwain, and fenugreek play significant roles in both traditional medicine and modern culinary practices. This is due to their complex phytochemical compositions, which include phenolic acids, tocopherols, flavonoids, and essential oils. While their contribution to macronutrients in culinary doses is minimal, their bioactive properties provide notable antioxidant, antimicrobial, and anti-inflammatory

benefits (Mahatma *et al.*, 2022; Saxena *et al.*, 2023). Phenolic acids-such as chlorogenic, caffeic, and ferulic acids—are produced through the shikimate and phenylpropanoid pathways. They play vital roles in plant defence and human health (Shahidi & Ambigaipalan, 2015). Growing concerns about the health risks and toxicity of synthetic phenolic antioxidants have led to a significant shift towards natural antioxidants. These powerful compounds are found in a wide array of plant, spice, and herb extracts. They include a diverse group of molecules like vitamins, tocopherols, ascorbic acids, carotenoids, phenolic acids, flavonoids, tannins, stilbenes, lignans, terpenes, anthocyanins, alkaloids, essential oil components, and phospholipids, among many others. The Apiaceae family stands out as an exceptionally rich source of these natural antioxidants, with phenolic compounds—particularly phenolic acids and flavonoids—being especially abundant (Thiviya *et al.*, 2021). Apiaceae family seed oils are unique in their exceptionally high concentration of petroselinic acid, a positional isomer of oleic acid (Hajib *et al.*, 2023). The lipid soluble vitamin E encompasses a family of eight compounds: the α-, β-, γ-, and δ-tocopherols, and their corresponding α-, β-, γ-, and δ-tocotrienols (Galli *et al.*, 2017). The synthesis of these vital compounds is confined to photosynthetic organisms such as plants, algae, and cyanobacteria, as well as certain fungi, corals, sponges, and tunicates. Nature's primary reservoirs for tocopherols and tocotrienols are the oily components of nuts and oil seeds (Szewczyk *et al.*, 2021). The predominance of tocotrienols over tocopherols in seed oils of the Apiaceae family is unique since it is very rare to find tocotrienols in nature. Most known plant oils contain the γ-tocopherol and α-tocopherols with the highest concentration, while tocotrienols are not present or are found only in minor amounts (Siger and G´ orna´ s, 2023). Tocopherols, protect membrane lipids from peroxidation, while polyunsaturated fatty acids like linoleic and linolenic acids help in regulating inflammatory signalling in humans (Ahmad *et al.*, 2013). Indeed, several compounds in coriander oil, including petroselinic acid and tocochromanols (tocopherols and tocotrienols), have been recognized for their beneficial effects on skin and hair vitality, as well as their ability to reduce skin sensitivity (Kern *et al.*, 2022). Considering the above information, present study aimed to evaluate the concentrations of key bioactive compounds in various seed spices. This comprehensive study meticulously compares the phenolic acids, tocopherol isomers, and fatty acids present in coriander, fennel, nigella, ajwain, cumin, and fenugreek.

Materials and Methods

Plant Material

Seeds of two genotypes each of coriander (ACr-1, ACr-2), fennel (AF-1, AF-2), nigella (AN-1, AN-20), ajwain (AA-1, AA-2), and fenugreek (AFg-1, AFg-2), and one genotype of cumin (GC-4) were procured from ICAR-NRCSS, Ajmer.

Phenolic Extraction and Analysis

To extract phenolic substances, one gram of powdered seed spices was thoroughly mixed with 80% HPLC-grade methanol in screw-cap glass tubes. This mixture was then stored under refrigeration at 4 °C for 48 hours. Following this initial soaking, the seed material was further processed by homogenization using a mortar and pestle. The resulting extract was centrifuged at 10,000 rpm for 10 minutes. This extraction and centrifugation cycle was repeated four times for each sample. The supernatant from each centrifugation step was carefully collected in a volumetric flask and brought to a final volume of 25 mL with 80% methanol. To concentrate the phenolic compounds, the supernatant was evaporated to dryness in a vacuum concentrator at 50 °C. The dried residue was subsequently redissolved in 1 mL of the mobile phase solution. For analysis, a 25 µL aliquot of the sample was filtered using a nylon filter (25 mm × 0.45 µm) via a syringe before being injected into the Ion Chromatograph (Khatediya *et al.*, 2018).

Tocopherol Profiling

Tocopherols were quantified from seed oils and analyzed using Ion Chromatograph (Dionex, ICS 3000) on a 5 µm Polar Advantage II C-18 column (4.6 × 150 mm) at 25 °C. To prepare the sample, 100 µL of oil was dissolved in 5 mL of absolute methanol. Absolute methanol served as the mobile phase, with a gradient flow rate. Initially, the flow rate was 1.0 mL min-1, which was then reduced to 0.4 mLmin-1for up to 9.0 minutes. For the final 1 minute, the flow rate was adjusted to 0.6 mL min-1. Tocopherols were detected at 295 nm using a UV detector. Individual tocopherol peaks (specifically α-Tocopherol, β+γ-Tocopherol, and δ-Tocopherol) were identified by comparing their retention times to those in a standard mixture obtained from Sigma Aldrich, USA. Peak integration was performed using Chromaleon software.

Fatty Acid Methyl Esters (FAMEs) Analysis

For the fatty acid profiling, the fatty acids were converted into fatty acid methyl esters (FAMEs) through transesterification, and these FAMEs were prepared following a modified protocol described by Mahatma *et al.*, 2016. For this, 100 µl of oil was combined with 5 ml of n-hexane in a 10 ml screw-cap test tube. This mixture was gently agitated by intermittent vortexing and left at room temperature for 1 hour. Subsequently, 3 ml of freshly prepared sodium methoxide solution (80 mg NaOH in 100 ml methanol) was added to the same tube and incubated at room temperature for 30 minutes. To this, 3 ml of 0.8% aqueous sodium chloride was added, and the mixture was thoroughly shaken. After a 5-minute separation period, the upper hexane layer, containing the methyl esters, was carefully decanted into a separate centrifuge tube pre-filled with 100 mg of anhydrous sodium sulfate. This hexane layer, now enriched with methyl esters, was then ready for Gas Chromatograph (GC) analysis. FAMEs were analyzed using an Agilent gas chromatograph, (Agilent make 7890A). One l of FAME was injected into injection port with a split ratio of 1:50. FAMEs were separated on HP5 capillary column (30 m x 320 µm x 0.25 µm) with a flow of 0.5 ml carrier gas. Oven temperature was set at 170 °C and then gradually increased to 205 °C at the rate of 5 °C/min then to 211 °C at the rate 1 °C/min. and finally to 255 °C at the rate 10 °C/min and detected using flame ionization detector. Detector temperature was kept at 280°C. Fatty acids were identified based on retention time of standard fatty acids (FAME mixture RM3, Merck) and expressed as percent area of individual fatty acid (Solanki *et al.*, 2024).

Statistical analysis

Statistical analysis was conducted to clearly define the relationships between phenolic acids, tocopherol isomers, and fatty acids in various seed spice genotypes. Biochemical data were systematically organized in Microsoft Excel 365 to facilitate precise sorting and the computation of essential descriptive statistics, including mean and standard deviation. Pearson's correlation coefficient (r) effectively evaluated linear relationships, categorized by correlation strength as negligible ($r < 0.4$), weak ($r = 0.4-0.59$), moderate ($r = 0.6-0.79$), and strong ($r \geq 0.8$). We rigorously tested statistical significance using two-tailed p-values ($p < 0.05$ and $p < 0.01$). The Shapiro-Wilk test was employed to confirm the normality of trait distributions prior to applying parametric tests. For data visualization, including correlation heatmaps, we utilized the "corrplot" package in R (v4.2.3), while scatter plots and regression analyses were expertly created with GraphPad Prism 9.0 (Table 4 and Figure 1).

Each sample was analysed in three replications and values are given as mean of three replications. Data of bioactive compound profiling were exported as ".txt" files to MS Excel for the creation of data matrices and CSV comma-delimited files were created for data. Multivariate analysis of data was performed using web-based MetaboAnalyst 6.0 software (Pang *et al.*, 2024). Data of seeds spices were divided in two groups i.e. Apiaceae (Apia) and other families (OF), to identify major bioactive compounds using Orthogonal Partial Least Squares—Discriminant Analysis (OPLS-DA) Variable Importance in Projection (VIP) value (Chong and Jun, 2005). Compounds with VIP values >1.0 were considered to be potentially different.

Results and Discussion

Phenolics Profiling

Phenolics profiling of seed spices is given in table-1. Chlorogenic acid is predominant in coriander (198.45 $\mu g\ g^{-1}$), fennel (549.05 µg/g), and cumin (617.4 µg/g) aligns with previous reports highlighting its role in oxidative stress mitigation and antimicrobial activity (Xu *et al.*, 2012; Olszowy-Tomczyk & Wianowska, 2023). While higher levels of caffeic acid was observed in fenugreek (>2300 $\mu g\ g^{-1}$) and nigella (407-466$\mu g\ g^{-1}$). Fenugreek and nigella seeds also had higher amount of syringic acid. However, cinnamic acid, ferulic acid, salicylic acid and resveratrol was not detected in fenugreek and nigella seeds. Caffeic acid is known to inhibit lipid peroxidation and modulate inflammatory pathways (Saphier *et al.*, 2019; Joshi *et al.*, 2022). The detection of resveratrol in coriander and fennel is notable, as this stilbene is rarely reported in seed spices. Its presence suggests potential for cardiovascular and anti-aging applications (Saad *et al.*, 2020). Syringic acid, found in fenugreek and nigella, has been linked to neuroprotective and hepatoprotective effects (Shimsa *et al.*, 2024). Similar findings were also reported by Derouich et al. in 2020. They found that the most abundant phenolic in the coriander leaf was p-coumaric acid (91.18 ± 3.39 mg/100 g DW), followed by gallic acid (29.16 ± 3.23 mg/100 g DW), caffeic acid (41.09 ± 5.14 mg/100 g DW), and chlorogenic acid (82.30 ± 7.51 mg/100 g DW).

Tocopherol Profiling

Coriander (ACr-1) and fenugreek (AFg-1) showed the highest total tocopherol content (252.47 and 281.19 µg/g oil, respectively), with all four isomers detected (Table-2). Tocopherols, particularly α- and γ-forms, are potent lipid-soluble antioxidants that stabilize cell membranes and extend shelf life in oil-rich matrices. Ajwain and cumin showed moderate β+γ-tocopherol levels but lacked α- and δ-isomers, suggesting limited antioxidant diversity. This could influence their oxidative stability and functional applications. The δ-tocopherol was observed only in coriander and cumin while α-tocopherol was observed in coriander and fenugreek oil in the present study. While all the four isomers were reported by Murli *et al.*, 2025 in cumin, coriander, fennel and ajwain with dominance of the γ- and α-tocopherol compared to β -and δ-tocopherol. In present study, β - and γ-tocopherols were present in all the seed spices. Saldeen and Saldeen (2005) reported that the blend of tocopherols naturally present in vegetable oils provides both additive and synergistic effects, supporting their wider range of beneficial biological functions. Both γ- and δ-tocopherol might be crucial for preventing lipid peroxidation and for counteracting the pro-oxidant tendencies of α-tocopherol.

Fatty Acid Composition

Fatty acid composition of seed spices given in Table 3 revealed that fenugreek oil is rich in linoleic (46–48%) and linolenic acids (15–21%) and nigella oil is good source of linoleic (47–49%) and linolenic acids (18–20%). Linoleic and linolenic acids are essential fatty acids and known for their anti-inflammatory and cardioprotective roles (Ahmad *et al.*, 2013; Mata-Pérez *et al.*, 2015). In the Tunisian and Iranian origin nigella seed oil, linoleic acid (50.3–49.2%), followed by oleic acid (25.0–23.7%) and palmitic acid (17.2–18.4%) were reported major fatty acids. While, Myristic, myristoleic, palmitoleic, margaric, margaroleic, stearic, linolenic, arachidic, eicosenoic, behenic and lignoceric acids were also detected (Cheikh-Rouhou *et al.*, 2007). Similarly, Saxena *et al.*, 2017b and Hossain *et al.*, 2024 also reported linoleic acid as major fatty acid followed by oleic, palmitic and stearic acid in nigella genotypes of India and Bangladesh.

In the present study, we could not identify petroselinic acid (PA), a major fatty acid of Apiaceae family using wax column. Petroselinic acid is the less-common monounsaturated isomer of oleic acid and considered as rare fatty acid. Sriti *et al.*, 2011 identified and quantified nine fatty acids in coriander seed oil, among them petroselinic acid was observed 74–77% of the total fatty acids, followed by linoleic, oleic and palmitic acids, accounting for 12–13%, 4–6% and 3%, respectively. Total fatty acids. In the fenugreek, linoleic acid (34–54%), linolenic acid (27–46%), stearic acid (0–5%), and palmitic acid methyl ester (7–13%) were reported major fatty acids in 13 genotypes by Saxena *et al.* (2017a). In the cumin, Sharma *et al* (2016) reported oleic acid as major fatty acid (88.1 -88.2 %) followed by palmitic (7.5%) palmitoleic (1.2 %) and stearic (1.1 %) using HP 5 MS. Thus these results showed that separation of of PA is requires highly polar capillary column.

Hajib *et al* (2023) reported concentrations of PA in different seed spices oils. For instance, anise seed oil contains 10.4% to 75.6% PA, while coriander seed oil ranges from 1% to 81.9%. Caraway seed oil shows two reported ranges: 28.5% to 57.6% and 41.3% to 61.8%. Other notable sources (Aćimović, (2017) include celery seed oil (49.4% to 75.6%), dill seed oil (79.9% to 87.2%), fennel seed oil (43.1% to 81.9%), and parsley seed oil (35% to 75.1%). Nguyen *et al.* (2020) reported 70 to 80% of PA in total fatty acids of Apiaceae fruits. Several investigations showed that this fatty acid has a wide range of biological potentials, including antidiabetic, antibacterial, and antifungal activities. In cosmetics, PA alone or in association with other active compounds has interesting applications as an anti-inflammatory agent for the treatment of skin, hair, and nail disorders.

Based on the bioactive profiling of seed spices, the genotypes were categorized into four functional clusters reflecting their dominant phytochemical traits (Table 5). The "antioxidant-rich" cluster included genotypes such as ACr-1 (coriander), AF-1 (fennel), and AFg-1 (fenugreek), which exhibited high levels of tocopherols and detectable amounts of resveratrol. Compounds known for their potent antioxidant and nutraceutical properties (Saxena *et al.*, 2023). The "PUFA-dense" cluster, comprising AN-20 (nigella) and AFg-2 (fenugreek), was characterized by elevated levels of polyunsaturated fatty acids, particularly linoleic and linolenic acids, which are essential for cardiovascular and metabolic health (ICAR-NRCSS, 2012; Ahmad *et al.*, 2013). The "phenolic-dominant" cluster included AA-1 (ajwain) and GC-4 (cumin), both of which showed high concentrations of chlorogenic and caffeic acids—phenolics associated with antimicrobial and anti-inflammatory activities (Xu *et al.*, 2012). Lastly, the "low-diversity" cluster, represented by AA-2 (ajwain) and AF-2 (fennel), exhibited a relatively narrow phytochemical spectrum, suggesting limited nutraceutical potential under the studied parameters. These functional groupings provide a strategic framework for selecting seed spice genotypes for targeted breeding, value addition, and functional food development.

A multivariate analysis using Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) revealed biochemical differences between Apiaceae genotypes (the Apia group) and other seed spice families (the OF group). VIP scores identified linoleic acid, syringic acid, palmitic acid, and stearic acid as key contributors to group separation. These acids were higher in OF genotypes such as nigella and fenugreek. In contrast, caffeic acid and arachidic acid were more prevalent in Apiaceae members such as coriander, fennel, ajwain, and cumin. This suggests unique phytochemical accumulation within each family and highlights variation in antioxidant and lipid-related traits. The distinct clustering pattern further supports the hypothesis of differing biosynthetic orientations among seed spice families (Figure 2). Genotype relationships were assessed using Pearson's linkage in a hierarchical clustering analysis based on bioactive compound profiles (Figure 3). The resulting dendrogram of seed spice genotypes revealed two main clusters. The Apiaceae, or "Apia" group, was formed by the first cluster, which included cumin (GC-4), fennel (AF-1, AF-2), ajwain (AA-1, AA-2), coriander (Acr-1, Acr-2), and a mixed genotype. The second cluster, "OF," included fenugreek (AFG-1, AFG-3) and nigella (AN-1, AN-20). Branch lengths supported the OPLS-DA results by indicating intra-family biochemical similarity.

The observed correlations among phenolic acids, tocopherols, and fatty acids in seed spices suggest coordinated biosynthetic or stress-response mechanisms. A strong positive correlation between chlorogenic acid and β+γ-tocopherol ($r = +0.82$) indicates that genotypes like coriander and ajwain may co-accumulate these antioxidants, potentially enhancing oxidative stability. This is consistent with findings by (Olszowy-Tomczyk and Wianowska, 2023), who reported synergistic antioxidant effects of chlorogenic acid and tocopherols in model systems, particularly under oxidative stress conditions.

Similarly, the positive correlation between caffeic acid and linoleic acid ($r = +0.76$) suggests that polyunsaturated fatty acid (PUFA)-rich genotypes like fenugreek and nigella may also accumulate phenolic antioxidants to counter lipid peroxidation. This aligns with studies showing that caffeic acid protects PUFA-rich matrices from oxidative degradation, such as in infant milk formulas and nanoemulsions (Saphier *et al.*, 2019; El-Maksoud *et al.*, 2025).

The moderate correlation between resveratrol and α-tocopherol ($r = +0.68$) in coriander and fennel supports the hypothesis of antioxidant synergy. Resveratrol has been shown to enhance the efficacy of other antioxidants, including tocopherols, in various food and nutraceutical systems (Saad *et al.*, 2020; Kumar *et al.*, 2019;Shahidi & Ambigaipalan, 2015).

A notable correlation between linolenic acid and total phenolics ($r = +0.71$) suggests that genotypes with higher unsaturation levels may upregulate phenolic biosynthesis as a defence against oxidative stress. This is supported by transcriptomic studies in Arabidopsis, where linolenic acid-responsive genes were linked to ROS signaling and phenolic metabolism under abiotic stress (Mata-Pérez *et al.*, 2015).

Conversely, the weak negative correlation between δ-tocopherol and syringic acid (r = –0.45) may reflect divergent roles or compartmentalization of these compounds. While -tocopherol is lipid-soluble and membrane-associated, syringic acid is more hydrophilic and may function in aqueous cellular compartments. Although direct studies on this interaction are limited, syringic acid has been shown to exhibit potent antioxidant and anti-inflammatory effects independently (Shimsa *et al.*, 2024).

Conclusion

This study highlights the biochemical diversity among seed spices, with coriander and fennel showing high nutraceutical potential due to the presence of resveratrol and tocopherols. Fenugreek and nigella were rich in caffeic acid and polyunsaturated fatty acids, suggesting antioxidant and anti-inflammatory benefits. These findings support the valorization of seed spices not only as culinary agents but also as functional food ingredients. These groups can guide targeted extraction strategies depending on the desired functional trait—antioxidant potential, oil quality, or nutraceutical value.

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Table 4: Correlation Study for Phytochemical Diversity

Trait Pair	Correlation Coefficient (r)	Interpretation
Chlorogenic acid vs. β+γ-Tocopherol	0.82	Strong positive correlation
Caffeic acid vs. Linoleic acid	0.76	High caffeic acid linked with PUFA content
Resveratrol vs. α-Tocopherol	0.68	Antioxidant synergy in coriander and fennel
Linolenic acid vs. Total Phenolics	0.71	Suggests oxidative stress adaptation
δ-Tocopherol vs. Syringic acid	-0.45	Weak negative correlation

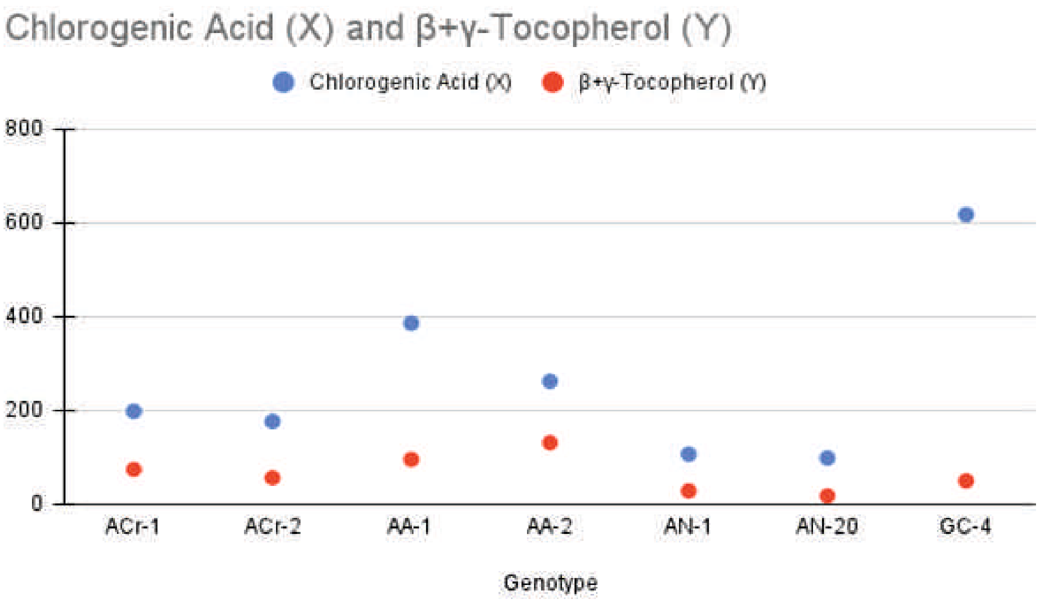

Fig 1. Scatter Plot – Chlorogenic Acid vs. + -Tocopherol

Table 2. Tocopherol content of seed spices (μg g^{-1} oil)

Seed spice	Genotype	δ-Tocopherol	B + γ-Tocopherol	α-Tocopherol	Total Tocopherols
Coriander	ACr-1	26.92	74.54	151.01	252.47
	ACr-2	7.81	56.54	133.80	198.15
Fenugreek	AFg-1	0	277.62	3.57	281.19
	AFg-2	0	200.33	4.53	204.86
Ajwain	AA-1	0	95.81	0	95.81
	AA-2	0	131.3	0	131.3
Nigella	AN-1	0	28.64	0	0
	AN-20	0	18.24	0	28.64
Cumin	GC-4	2.25	50.12	0	52.3

Value given as 0 was not detected

Table 3. Fatty Acid Profile (% of total FAME) of seed spices

Seed Spices	Genotype	Myristic acid	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Arachidic acid	Lignoceric acid
Coriander	ACr-1	0.09	3.04	0.52	43.82	10.42	0.13	0.09	0.57
	ACr-2	3.29	3.88	0.57	54.89	12.59	0.14	0.06	0.23
Fenugreek	AFg-1	0	10.32	3.10	14.79	46.42	21.36	0.53	0.23
	AFg-2	0	11.81	3.24	16.38	48.40	15.24	0.21	0.27
Ajwain	AA-1	0.08	0.42	0	3.57	1.67	0	0	0.23
	AA-2	0.11	0.43	0	3.00	1.65	0	0	0.25
Nigella	AN-1	0.22	10.50	1.66	18.04	47.70	0.02	0.07	0
	AN-20	0.26	10.95	1.66	20.20	49.61	0.12	0.11	0
Fennel	AF-1	0	1.51	0.31	15.23	3.68	0	0	0.32
	AF-2	0	1.60	0.32	17.49	3.56	0	0	0.30
Cumin	GC-4	2.81	0.51	0	5.57	3.12	0	0	0.29

Value given as 0 was not detected

Table 5. Functional Clusters of Seed Spices

S.N.	Cluster Type	Genotypes Included	Dominant Traits
1.	**Antioxidant-rich**	Coriander (ACr -1), Fennel (AF-1), Fenugreek (AFg-1)	High tocopherols, resveratrol, caffeic acid
2.	**PUFA-dense**	Nigella (AN -20), Fenugreek (AFg-2)	High linoleic and linolenic acids
3.	**Phenolic-dominant**	**Ajwain (AA -1), Cumin (GC-4)**	**High chlorogenic and caffeic acids, low tocopherols**
4.	**Low-diversity (minimalist)**	Ajwain (AA -2), Fennel (AF - 2)	Narrow phytochemical spectrum

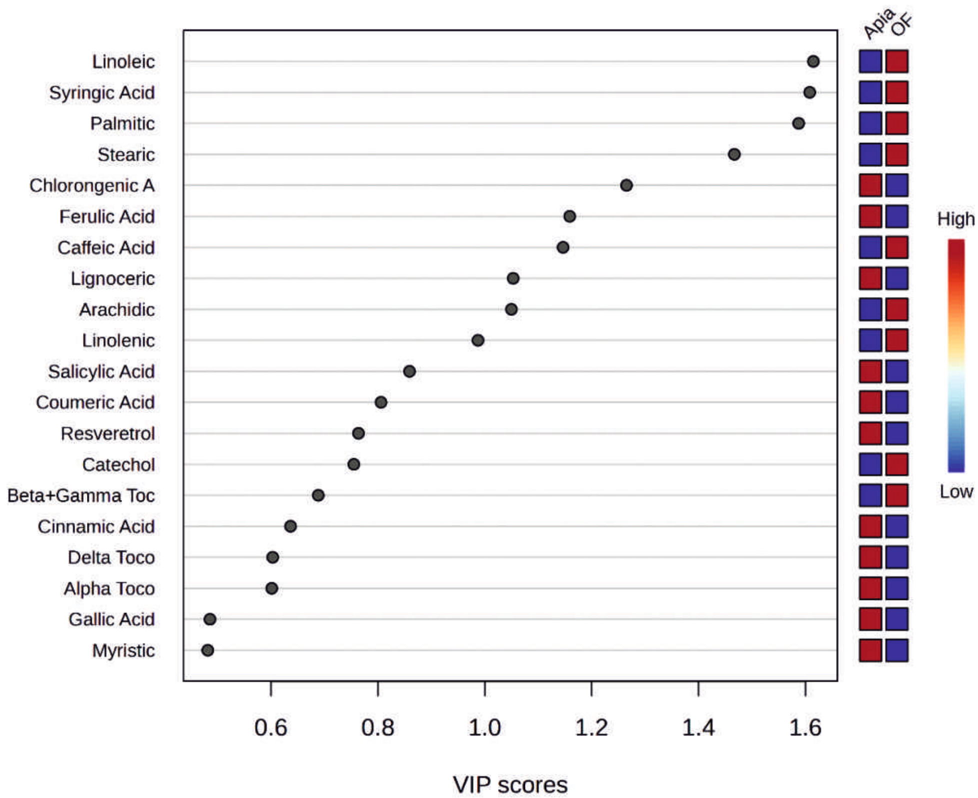

Fig 2: Orthogonal Partial Least Squares—Discriminant Analysis (PLS-DA) Variable Importance in Projection (VIP) score of major bioactive compounds of seed spices comparison of Apiaceae (Apia) and other families (OF)

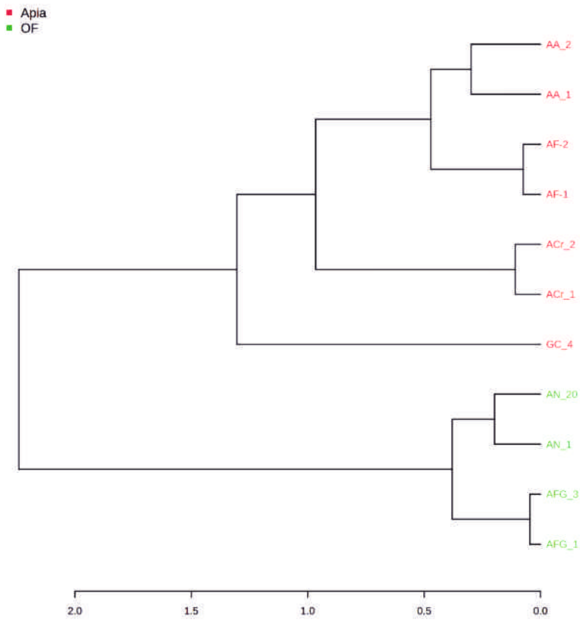

Fig 3: Dendrogram analysis (Pearson-average) of seed spices genotypes based on the bioactive compounds profile showing hierarchical clustering

necessary facilities to conduct this research work.

Conflict of interest

The authors declare that the research was conducted beyond any commercial or financial affairs that could be taken as a potential conflict of interest.

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