

Article

LC-MS/MS and GC-MS Identification of Metabolites from the Selected Herbs and Spices, Their Antioxidant, Anti-Diabetic Potential, and Chemometric Analysis

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Abstract: Culinary herbs and spices are widely used in daily diets. Pakistan's flora is enriched with phytochemicals due to a diverse range of land. Phytochemicals, including volatile and non-volatile compounds, have captured much interest due to their numerous health advantages and significance in daily diet. The present study aimed to conduct in-depth metabolomic profiling of Pakistani-grown fenugreek leaves (*Trigonella foenum-graecum*), fennel seeds (*Foeniculum vulgare*), mint leaves (*Mentha royleana*), coriander seeds (*Coriandrum sativum*) and basil leaves (*Ocimum basilicum*) by using liquid chromatography–mass spectrometry (LC-MS/MS) and gas chromatography–mass spectrometry (GC-MS). The first study was conducted to optimize extraction using different solvents (methanol, ethanol, chloroform, acetone, and water). Total phenolic content (TPC), total flavonoid content (TFC), and total condensed tannins (TCT) were quantified along with the antioxidant and anti-diabetic activities. The highest TPC (125.42 ± 10.89 mg GAE/g) and the highest antioxidant and anti-diabetic potential were quantified in mint. Seventy-one phytochemical metabolites were identified using LC-MS/MS, while forty-nine volatile constituents were identified using GC-MS. A positive correlation was identified between phenolic contents and their biological activities. Furthermore, molecular docking helped to find drug molecules with more excellent anti-diabetic activity based on their binding affinities. This study suggests that selected herbs and spices from Pakistan have significant nutraceutical and phytopharmaceutical potential. This study could further help in drug discovery.

Keywords: herbs; spices; flavonoids; antioxidants; diabetes; volatile compounds; drug discovery; phytochemicals; human health



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1. Introduction

High blood glucose levels are a hallmark of diabetes mellitus, which ranks among the leading causes of death worldwide. The major enzyme that plays a substantial role in the absorption, hydroxylation, and digestion of sugars in the body of a human being is alpha-glucosidase (α -glucosidase). Because of this, α -glucosidase inhibition is useful for managing type 2 diabetes [1]. Utilizing natural resources to treat diabetes is becoming more popular [2,3]. To manage or suppress the consequences of diabetes, several bioactive compounds and nutraceuticals have been studied. Eliminating the frequency of pre- or post-diabetic diseases using phenolic metabolites is an effective treatment [3]. Because of this, understanding the vital function of polyphenols in food and human health requires thorough identification and phenolic metabolites characteristic evaluation. Due to their potent antioxidant and antimicrobial qualities, culinary herbs and spices are gaining more

attention from industry and research. Since prehistoric times, people have regularly used culinary herbs and spices as food ingredients. Herbs and spices have also been discovered to enhance flavor owing to preservative, sensory, and organoleptic characteristics [4]. Because of their taste, therapeutic color, and aroma effects, culinary herbs and spices are frequently employed in the industry as nutritional supplements, fortification or enrichment of various products like snacks, candies, biscuits, pickles, jams, and syrups. Due to their positive effects on health, bioactive phenolic compounds found in plant-based products are presently receiving significant consideration. Other scholars have become more interested in studying the antioxidant properties and the phenolic composition of herbs and spices frequently used due to numerous studies citing culinary herbs and spices as a source of food that are supposed to be naturally occurring in antioxidant phenolic compounds [5,6]. In recognition of numerous possible health benefits, phenolic compounds have been a desirable target in the hunt for phytochemicals believed to be advantageous for human health. It is known that culinary spices as well as herbs have been employed to address a range of illnesses, including joint inflammation, aches, bone fractures, and sprains. Additionally, they are utilized in the cosmetic, pharmaceutical, culinary, and feed industries. The widespread use of herbs and spices has increased global output.

They are frequently used as components in products that promote health, including anti-diabetic, anti-hypertensive, anti-carcinogenic, antioxidant, anti-inflammatory, anti-depressant, anti-HIV, antimicrobial, and antipyretic [7,8]. The health-promoting properties of bioactive compounds, primarily phenolics, are widely found in herbs, spices, vegetables, fruits, and other therapeutic plant species and are likewise referred to as secondary metabolites in phytochemistry. Recent studies have investigated and determined the crucial role in the prevention of disease and the promotion of wellbeing. Phenolic compounds, for instance, flavonoids, phenolic acids, other polyphenols, stilbenes, and lignans, gained much attention from food experts and nutritionists because of their numerous possible health effects. Polyphenols are currently being explored as potential antioxidants to increase the shelf life of meals that are high in lipids. Additionally, herbs and spices' phytochemicals antimicrobial components have properties that help to preserve food. These bioactive compounds have various valuable attributes, including metal chelation, free radical scavenging capacity, enzymatic activity, modulation of signal transduction pathways, and inhibition of cellular proliferation [4]. Therefore, culinary herbs and spices high in antioxidants have been praised and recommended for their use in human nutrition to promote health and wellbeing.

In addition to their elevated reactivity in biological systems, free radicals have the capability to damage or change DNA biomolecules, whereas endogenous antioxidants can reduce or eliminate their activity in the body [4]. The use of antioxidants that exist naturally in herbs, vegetables, fruits, and spices has garnered considerable attention as a potentially less costly substitute to synthetic antioxidants in conventional and contemporary therapy owing to pertains related to the negative consequences of artificially produced antioxidants. Numerous studies published recently found a link between the intake of herbs and spices and the prevalence of chronic illness in people [9].

Culinary herbs and spices comprise natural antioxidants known to lessen oxidative stress brought by increased free radical levels in tissues as well as cells [4,10]. The effect of oxidative stress, which is supposed to be chronic, has been connected to several illnesses, including heart disease, cancer, and, most significantly, accelerated aging. As a result, culinary herbs and spices have the potential to be used to avoid a variety of health issues that result from oxidative stress and different metabolic disorders in one's body. Owing to the phenolic compounds as well as the antioxidant activity of culinary herbs and spices, numerous studies have been conducted on their recognition, characterization, and investigation [3,11]. Due to their complex nature, framework, and widely used cultivars in different parts of the globe, and mainly because there aren't any commercial standards for precisely identifying and validating the bioactives in the system, comprehensive characterization of culinary herbs and spices is not yet available. A professional analytical method

called liquid chromatography–mass spectrometry (LC-MS/MS) is used in conjunction with quadrupole time of flight (QTOF) for the purpose of identifying the unidentified bioactive chemicals in diverse samples of plants, including culinary spices as well as herbs.

Although a few studies have been conducted for characterizing phytochemicals from herbs and spices, no study was conducted on these selected herbs and spices from Pakistan. Moreover, no study was conducted where the authors applied all these techniques in one study. A comprehensive characterization of the phytochemicals from the selected herbs and spices is still lacking due to the complex nature of phytochemicals and the non-availability and expense of commercial standards of all phytochemicals. Therefore, the goal of the present investigation was to research the frequently used spices and herbs (basil, mint, fennel, coriander, and fenugreek) for antioxidant and anti-diabetic potential, volatile as well as non-volatile compounds. That is why liquid chromatography–mass spectrometry–quadrupole time of flight (LC-ESI-QTOF-MS/MS) and gas chromatography–mass spectrometry (GC-MS) were used to distinguish and separate 71 phenolic compounds from selected culinary spices and herbs. Moreover, TPC, TCT, TFC, hydroxyl radical scavenging assays ($\bullet\text{OH}$ -RSA), 2,2-azobis-3-ethylbenzothiazoline-6-sulfonic acid assay (ABTS), ferric reducing antioxidant power assay (FRAP), phosphomolybdate antioxidant (PMA) assay, and ferrous ion chelating activity (FICA) were conducted to determine the antioxidant and total polyphenol contents.

Alpha-glucosidase inhibition activity was measured to investigate the anti-diabetic potential of selected herbs. In silico molecular docking further helped to investigate the role of these herbs in human health and drug discovery. This research will offer essential and precious knowledge about the powerful impact of phenolic compounds belonging to herbs on human health. Additionally, because of their potential antioxidant constituents, they will promote the utilization of culinary herbs along with spices in numerous food and feed businesses.

2. Materials and Methods

2.1. Preparation of Samples and Optimization of Extraction for Phenolic Compounds

Culinary spices and herbs were collected and grown in Rawalpindi Division. Fennel (*Foeniculum vulgare*, Mill.) and coriander (*Coriandrum sativum*) seeds in dried whole form (200 g) as well as mint (*Mentha royleana*), fenugreek (*Trigonella foenum-graecum*), and basil (*Ocimum basilicum* L.) fresh leaves (approx. 2 Kg each) were purchased from the local market in Islamabad. Samples were re-confirmed and identified by a group of 5 senior PhD and post-doctorates from the Department of Plant Sciences, Quaid-i-Azam University, Islamabad. Sweet basil, mint and fenugreek were dried in an oven at 50 °C for four days. A lab grinder was used to grind each sample. The method used for phenolic compounds extraction from culinary spices and herbs was as follows; to prepare the extracts, a 2 g sample was taken and mixed with 30 milliliters of 80% methanol, ethanol, chloroform, and acetone in Milli-Q water. The mixture was agitated for 16 h in an orbital shaker at 4 °C and 150 rpm to extract the phytochemicals. Using a Hettich Refrigerated Centrifuge, at 4 °C, the samples were then centrifuged for about 20 min at 8000 rpm. The resulting supernatant was then filtered via a 0.45 μm syringe filter, which was then chilled to $-20\text{ }^{\circ}\text{C}$ for up to seven days for further analysis. The extraction process was done in triplicate.

2.2. Quantification of Phenolic Contents in Selected Herbs and Spices

2.2.1. Determination of Total Phenolic Content

The phenolic compound profile of samples was analyzed using the previously described method by Ali et al. [3] and Bashmil et al. [12] with some modifications. Then, 25 μL of Folin Ciocalteu reagent (25% *v/v*) was taken with 200 μL of distilled water and 25 μL of sample extracts were added and incubated at room temperature for 5 min. At last, 25 μL of sodium carbonate (10% *v/v*) was added to the reaction mixture and placed in incubation again in the dark at room temperature for one hour. The absorbance of the samples was recorded at 760 nm. Quantification of the total phenolic content was carried

out by making a standard curve against gallic acid that ranged from 0–200 $\mu\text{L}/\text{mL}$ in methanol. Results were recorded using units of mg GAE per gram of dried powder.

2.2.2. Total Flavonoid Content

The flavonoid contents of the samples were analyzed using the method described by Ali et al. [4], with some modifications. The aluminium chloride (AlCl_3) colourimetric method was used to determine the TFC. Moreover, 80 μL of the sample extract was taken in 96-well plates and mixed with 80 μL of AlCl_3 solution and 120 μL of sodium acetate aqueous solution (50%). After preparation of the reaction mixture, the sample was incubated in the dark at 27 $^\circ\text{C}$ for 2.5 h, and absorbance was recorded on the spectrophotometer at 440 nm. For the quantification of flavonoid content, a standard curve was constructed against 0–50 $\mu\text{g}/\text{mL}$ of quercetin in methanol. The milligram quercetin equivalents per gram of the sample unit were used to express the results.

2.2.3. Total Tannin Content

TTC was carried out using an alteration of the Ali et al. [4] technique. Moreover, 150 μL of vanillin solution (4%), along with 25 μL of the sample solution, were added. Then, 25 μL of 32% H_2SO_4 was poured into the mixture, and it was incubated at 25 $^\circ\text{C}$ for 15 min. The absorbance was determined at 500, and the standard catechin curve (0–1000 $\mu\text{g}/\text{mL}$) was constructed. The data were given as mg CE/g.

2.3. Measurement of Antioxidant and Anti-Diabetic Potential of Selected Plants

The ABTS assay was conducted using the method of Ali et al. [13], while the FICA and FRAP activity were conducted using the methods of Ali et al. [4]. The PMA and OH-RSA assays were also conducted using the methods of Ali et al. [4] and Bashmil et al. [12]. The alpha-glucosidase inhibition activity was conducted using the methods of Ali et al. [2,3].

2.4. LC-ESI-QTOF-MS/MS Analysis

Following our previously established procedures, we were able to precisely identify and measure the phenolic metabolites from mint, coriander, fenugreek, sweet basil, and fennel [14]. Both negative and positive modes were applied using Accurate-Mass QTOF Agilent 6520. The same equipment conditions as described in Ali et al. [3] were applied to the column gradient, chromatographic, and other areas. Phytochemicals were extracted and identified using the MassHunter Workstation Software (version B.06.00) from Agilent, Santa Clara, CA, USA, using the Personal Compound Database Library (PCDL) with a library score of more than 80 and less than 5 ppm error of MS/MS spectra. Furthermore, the mass spectra of twenty-four external standards were collected, and standard equations were reported by Ali et al. [15]. Moreover, the MS/MS spectra of each compound were analyzed by referring to retention times and comparing them with published libraries (MassBank, GNPS, MoNA, HMDB and FooDB) by using MS-DIAL for double verification.

2.5. GC-HS-SPME-MS Analysis of Volatile Compounds

Headspace solid-phase microextraction (HS-SPME)—PAL RSI (CTC Analytics AG, Zwingen, Switzerland) coupled with gas chromatography–mass spectrometry 6850 series II Network GC-system (Agilent Technologies, Santa Clara, CA, USA) and a mass spectrometer (5973Network Mass Selective Detector, Agilent Technologies, USA) was used. A 30 m long DB wax capillary column from Agilent technologies with 0.25 μm film thickness and 0.25 mm international diameter in combustion with 65 μm PDMS/DVB fibre of fused silica (Sigma Aldrich, St. Louis, MO, USA) was used in this method. Helium with 60 kPa column head pressure was used as a carrier gas. The incubation time (15 min) for samples was set at 60 $^\circ\text{C}$ prior to extraction for 15 min and 6 min for desorption. Following that, a GC oven program was devised. With a starting temperature of 40 $^\circ\text{C}$ for almost 5 min, followed by an increase in temperature (190 $^\circ\text{C}$) of 5 $^\circ\text{C}$ per minute for 8 min, followed by an increase in temperature of 10 $^\circ\text{C}$ per minute for about 10 min, to

reach a temperature of 240 °C. The mass acquisition (m/z 40–360) and two minutes solvent delay time were used. One-gram dried powder with 25 μ L 4-Octanol (100 mg/L) as an internal standard was used and injected according to the procedure mentioned above. The linear retention indices (LRI) of selected compounds were calculated with alkane standards (C₇–C₂₀) through the following equation.

$$\text{LRI (selected compounds)} = 100 \times (\text{RT}_x - \text{RT}_n / \text{RT}_{(n+1)} - \text{RT}_n) + n$$

It contrasts the retention times of the chosen compound (RT_x) and alkanes (n) with n and $n + 1$ carbons that were eluted prior to and following the chosen compound (RT_n). The retention index of compounds found in particular spices and herbal products was compared to information from the NIST mass spectra database and the NIST Chemistry WebBook spectral Library (NIST 2017); by quantifying the peak area percentage of compounds, the semi-quantified culminated.

2.6. Molecular Docking of the Most Abundant Phenolic Compounds

In silico molecular docking was conducted using the previously conducted method of our group [2,3,15].

2.7. Statistical Analysis

Minitab Program Version 18.0 (Minitab, LLC, State College, PA, USA) and XLSTAT-2019.1.3 (Addinsoft Inc., New York, NY, USA) were used for correlation, biplot analysis (PCA) and one-way analysis of variance (ANOVA). Heatmaps were conducted by using the MetaboAnalyst (5.0).

3. Results and Discussion

3.1. Optimization of Extraction Process of Total Phenolic Content from the Selected Plants

Different solvents are used to extract phenolic compounds from the selected herbs and spices. The results are given in Table 1.

Table 1. TPC (mg GAE/g) of different extracts of the selected herbs and spices.

Variables	Basil Leaves	Fennel Seeds	Coriander Seeds	Mint Leaves	Fenugreek Leaves
80% Methanol	26.99 ± 1.28 ^a	9.90 ± 0.27 ^a	23.02 ± 1.53 ^a	125.42 ± 10.89 ^a	17.21 ± 1.08 ^a
80% Ethanol	23.02 ± 1.53 ^b	9.61 ± 0.76 ^a	21.39 ± 1.91 ^b	116.84 ± 8.47 ^b	15.43 ± 1.21 ^b
80% Chloroform	17.02 ± 1.01 ^c	6.33 ± 0.22 ^b	19.32 ± 1.14 ^c	88.98 ± 7.32 ^c	7.86 ± 0.19 ^c
80% Acetone	25.02 ± 2.61 ^a	9.32 ± 0.52 ^a	22.15 ± 1.28 ^{ab}	123.47 ± 11.04 ^a	17.01 ± 0.32 ^a
Water	3.15 ± 0.03 ^d	1.01 ± 0.01 ^c	3.02 ± 0.04 ^d	15.53 ± 1.37 ^d	1.52 ± 0.02 ^d

According to this table given values mean ± standard deviation (SD) per dried weight where number of replicates per sample was 3 ($n = 3$). Values in each column having different superscript letters (^{a–d}) are profoundly distinct from one another at $p < 0.05$.

Five different solvent systems were used: water, 80% acetone, 80% chloroform, 80% methanol, and 80% ethanol. The highest extraction of the total phenolic content of mint leaves in terms of 80% methanol was found to be 125.42 ± 10.89 mg GAE/g. As opposed to fennel seeds, which had a minimum extraction of 9.90 ± 0.27 mg GAE/g for total phenolic content. All the treatments and various solvent extracts showed significant differences in the total phenolic contents. The study's findings also revealed important interactions between the solvents and spices. The levels of TPC obtained from fennel seeds in both solvents—ethanolic and aqueous were in the range of 1020.3 ± 11.40 to 1257.8 ± 19.0 μ g GAE/g and 1114.0 ± 6.11 to 1208.3 ± 21.36 μ g GAE/g, respectively. Similar to this, the maximum TPC for peppermint was discovered in the aqueous extract of treatment P1 (2094.7 ± 55.36 μ g GAE/g), whereas the least TPC was discovered in the ethanolic extract of treatment P1 (1824.2 ± 30.40 μ g GAE/g) [16]. According to Zheng et al. [17], spices and

herbs have significant phenolic contents. Fennel water extracts have a higher phenolic content than ethanolic extracts, according to Aliakbarlu et al. [18].

3.2. Estimation of Polyphenols from Culinary Herbs and Spices

Culinary spices, as well as herbs, are rich sources of phenolic compounds. The polyphenols' quantification in different herbs (Table 2) was performed through TPC, TCT and via TFC.

Table 2. TPC, TFC and TCT of selected culinary herbs and spices.

Variables	TPC (mg GAE/g)	TFC (mg QE/g)	TCT (mg CE/g)
Basil leaves	26.99 ± 1.28 ^b	9.35 ± 0.16 ^b	3.97 ± 0.10 ^b
Fennel seeds	9.90 ± 0.27 ^d	2.48 ± 0.21 ^d	2.88 ± 0.10 ^{bc}
Coriander seeds	23.02 ± 1.53 ^b	3.35 ± 0.26 ^{de}	11.51 ± 1.68 ^a
Mint leaves	125.42 ± 10.89 ^a	31.72 ± 1.22 ^a	3.31 ± 0.08 ^c
Fenugreek leaves	17.21 ± 1.08 ^c	4.41 ± 0.08 ^c	1.36 ± 0.08 ^d

According to this table given values mean ± SD per dried weight where number of replicates per sample was 3 (n = 3). Values in each column having different superscript letters (^{a-c}) are profoundly distinct from one another at $p < 0.05$. GAE (gallic acid equivalents); CE (catechin equivalents); QE (quercetin equivalents).

Nowadays, herbs and spices have increased attention because of their potential bioactive compounds, including phenolic acids, tannins and flavonoids, bearing immense antioxidant potential and may have advantageous effects on human health. The Folin–Ciocalteu reagent was used to determine the TPC and among the samples tested, mint leaves exhibited the highest TPC value of 125.42 ± 10.89 mg GAE/g, followed by basil leaves with a value of 26.99 ± 1.28 mg GAE/g, coriander seeds with 23.02 ± 1.53 mg GAE/g, and fennel seeds with 9.90 ± 0.27 mg GAE/g. Fenugreek leaves showed the lowest TPC value of 7.17 ± 0.10 mg GAE/g. Shan et al. [19] have also visualized a similar trend showing that TPC values for basil, fennel, and fenugreek leaves are found to be comparable to previously reported TPC of basil (26.5), parsley (13.6), and thyme (71.7), respectively. The higher values of TPC for mint were observed owing to improved extraction. These improved extractions could be due to methanol (80%).

Previously, Akhtar et al. [20] reported that the TPC (59.1 ± 5.7 mg GAE/g) of the methanolic extract of peppermint (*Mentha piperita*) from Multan, Pakistan was less than mint (*Mentha royleana*), while Kim et al. [21] reported the TPC (20.25 ± 0.85 and 9.36 ± 0.21 µg CE/g) of basil (*Ocimum basilicum*) and fennel (*Foeniculum vulgare*), respectively from Korea. Nadeem et al. [22] measured the TPC (86.6 and 191.2 mg GAE/g) of the dichloromethane and ethanolic extracts of basil (*Ocimum basilicum*) from Multan, Pakistan. Rashid et al. [23] quantified the TPC in the range of 13.46 to 59.75 mg GAE/g of basil flowers from the different regions of Punjab, Pakistan. Previously, [24] reported the TPC (1.00 ± 0.06, 1.09 ± 0.02 and 0.94 ± 0.05 mg GAE/g) of the Tunisian, Syrian, and Egyptian coriander extracted with pure methanol while Sriti et al. [25] reported the TPC (15.16 and 12.10 mg GAE/g) of the Canadian and Tunisian methanolic extract.

These variations in TPC were likely due to variations in the choice of herbs or cultivars selected for extraction and quantification of TPC in current and other studies. The vast difference among TPC values calculated in our research depicts the variety of compounds of phenolic as well as their variety capacity to decrease the Folin–Ciocalteu Reagent. Different factors, for instance, temperature combinations for extractions, solvent-to-sample ratio, solvent type and concentration, geographical location and cultivar differences, can affect the TPC of the extracts [26]. Moreover, plant parts such as leaves, roots, flowers, seeds, bark, particle size and surface area of the plant matter can also significantly affect the TPC of the plant's extracts.

The highest flavonoid content was observed in mint leaves, which had a value of 31.72 ± 1.22 mg QE/g. Basil leaves followed with a value of 9.35 ± 0.16 mg QE/g, while fenugreek leaves had a value of 4.41 ± 0.08 mg QE/g. Fennel and coriander seeds had the lowest flavonoid concentrations, with values of 3.35 ± 0.26 mg QE/g and

2.48 ± 0.21 mg QE/g, respectively. When compared to previously reported TFC values of nutmeg (2.22) and black cumin (1.73), the TFC values of fenugreek leaves, fennel seeds, and coriander seeds were found to resemble [1]. Herbs possess phenolic acids as well as flavonoids in higher proportions in comparison with other phenolic compounds. In contrast, the TCT value of coriander seeds (11.51 ± 1.68 mg QE/g) was noticeably greater than other mentioned culinary spices and herbs; for instance, TCT value for basil leaves (3.97 ± 0.10 mg QE/g), mint leaves (3.31 ± 0.08 mg QE/g), fennel seeds (2.88 ± 0.10 mg QE/g) and the lowest TPC value were observed to be of fenugreek leaves (1.36 ± 0.08 mg QE/g). The two most significant subgroups of phenolic compounds are flavonoids and tannins. Among phenolic compounds, flavonoids have earned more consideration due to their potential as antioxidants and function as a nutritional indication for many food items. Therefore, herbs' characterization with modern analytical techniques, for instance, LC-ESI-QTOF-MS/MS, can offer more reliable, accurate, and useable information on bioactive compounds, including polyphenols for applications in animal feed and human food and cosmetic industries that belong to pharmaceutical.

3.3. Antioxidant and Anti-Diabetic Activities of Selected Herbs and Spices

The antioxidant activity of herbs was further analyzed on the basis of different mechanisms, which include the radical scavenging as well as reducing power ability of the sample. Different tests such as ABTS, FICA, FRAP, •OH-RSA, PMA and RPA were performed, and the results are stated in Table 3.

Table 3. Antioxidant activities of selected culinary spices and herbs.

Variables	Basil Leaves	Fennel Seeds	Coriander Seeds	Mint Leaves	Fenugreek Leaves
ABTS (mg AAE/g)	34.70 ± 1.55^b	9.43 ± 0.35^e	19.17 ± 0.57^c	74.62 ± 0.77^a	16.23 ± 0.13^d
FICA (mg EDTA/g)	1.24 ± 0.08^{bc}	0.70 ± 0.01^c	1.88 ± 0.03^b	4.25 ± 0.05^a	0.75 ± 0.07^c
FRAP (mg AAE/g)	2.43 ± 0.22^b	0.54 ± 0.20^d	1.71 ± 0.33^c	4.35 ± 0.09^a	0.14 ± 0.05^{de}
•OH-RSA mg (AAE/g)	52.17 ± 3.46^b	23.89 ± 0.17^c	17.25 ± 1.36^{de}	61.87 ± 2.85^a	19.62 ± 0.46^d
PMA (mg AAE/g)	8.39 ± 0.35^{ab}	3.21 ± 0.93^c	8.04 ± 0.55^{ab}	8.64 ± 1.84^a	3.44 ± 0.32^c
α -glucosidase inhibition activity IC ₅₀ (μ g/mL)	3.32 ± 0.47^d	31.92 ± 2.07^a	9.41 ± 1.82^c	2.04 ± 0.03^e	15.83 ± 1.13^b

According to the above table, given values mean \pm SD per gram powder weight where the number of replicates per sample was three ($n = 3$). Values in a row with different superscript letters (^{a–e}) are profoundly distinct at p -value < 0.05 . ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid assay); FICA (ferrous ion chelating activity); FRAP (ferric reducing antioxidant power assay); •OH-RSA-hydroxyl-radical scavenging activity); PMA (Phosphomolybdate antioxidant power assay); AAE (ascorbic acid equivalents); EDTA (ethylenediaminetetraacetic acid).

The human diet uses fruits, veggies, herbs, and spices as an antioxidant chemical component source that can neutralize free radicals. Phenolic compounds are typically regarded as the active antioxidant components in culinary spices and herbs, which are supposed to provide various health benefits. Mainly, they are taken as multifunctional compounds and act as reducing agents, hydrogen ion donators, metal chelators, and free radical scavengers in biological systems [4]. A parameter used to describe the advantages of culinary spices and herbs that are eaten as food is their antioxidant ability. Numerous elements found in herbs and spices have been proposed to act as antioxidants. Numerous bioactive substances, mainly phenolic compounds like flavonoids, tannins, procyanidins, coumarins, phenolic acids, stilbenes, xanthenes, lignans, as well as other polyphenols, have been mentioned as potential therapeutic agents for neutralizing free radicals [27].

The ABTS, FICA, FRAP, •OH-RSA and PMA assays have been utilized to determine the free radical scavenging activity of various bioactive compounds, especially those of polyphenols [28]. The ABTS assay is generally considered to be the least expensive. This is because the ABTS assay uses a synthetic chromogenic substrate, which is relatively inexpensive to produce compared to the other assays. The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay is also considered to be a versatile and reliable method for evaluating

the antioxidant potential of a variety of samples, including foods, dietary supplements, and biological samples. The ABTS assay is also relatively simple to perform as the ABTS assay is based on the reaction between ABTS and a substance that has antioxidant activity. This reaction produces a blue–green color, which can be measured spectrophotometrically. The antioxidant activity of the substance being evaluated is inversely correlated with the color intensity. From Table 1, ABTS values of mint leaves (74.62 ± 0.77 mg AAE/g), basil leaves (34.70 ± 1.55 mg AAE/g), coriander seeds (19.17 ± 0.57 mg AAE/g), fennel seeds (9.43 ± 0.35 mg AAE/g), fenugreek leaves (16.23 ± 0.13 mg AAE/g) are estimated significantly higher at $p < 0.05$ than other listed herbs. Mint, fennel, and fenugreek values of ABTS were found to be close to those of sage (73.78), cumin (9.34), and black cardamom (6.41), which had previously been reported by Ali et al. [4,10]. Previously, many researchers have looked into the phenolic components and antioxidant potential of several spices and herbs, including Patra et al. [29], Wojdyło et al. [5], Przygodzka et al. [30], Dvorackova et al. [26], and Muhammad et al. [31]. A higher value of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) represents a greater antioxidant capacity of the tested sample. In other words, a higher ABTS value indicates that the sample has a more remarkable ability to prevent oxidative damage and neutralize free radicals. The ABTS values for the mint were found to be greater in other studies [5].

The results of the study reveal that mint leaves had the greatest FRAP value of 4.35 ± 0.05 mg AAE/g, which is being followed by basil leaves with a value of 2.43 ± 0.22 mg AAE/g. In contrast, fennel, coriander, and fenugreek leaves exhibited the lowest FRAP values with measurements of 0.54 ± 0.20 mg AAE/g, 1.71 ± 0.33 mg AAE/g, and 0.14 ± 0.05 mg AAE/g, respectively. The FRAP values obtained from coriander, fennel, and fenugreek leaves were found to be in line with the FRAP values reported for black pepper (0.10), cardamom (0.15), cumin (0.21), fennel (0.53), and nutmeg (0.81) [4]. Flavonoids and antioxidant activity have a favourable association which indicates that the primary components that act as antioxidants are flavonoids [4].

The FICA numbers of fennel leaves (0.70 ± 0.01 mg AAE/g), fenugreek leaves (0.75 ± 0.07 mg AAE/g) and coriander seeds (1.88 ± 0.03 mg AAE/g) had the lowest FICA value, followed by higher FICA values for mint leaves (4.25 ± 0.05 mg AAE/g) and basil (1.24 ± 0.08 mg AAE/g). It was discovered that the FICA values derived in this study from basil, coriander, fennel, and fenugreek leaves were quite similar to the FICA values reported for cinnamon (1.32), cumin (1.13), fennel (1.17), nutmeg (1.05), and star anise (0.63) [4]. The herbal compound's FICC is essential because it lowers the quantity of transition metals needed to catalyze lipid peroxidation during that process. To stabilize the oxidized form of metal ions, chelating agents, which are secondary antioxidants, create s-bonds together with metal during the reaction [16,18]. This reduces the redox potential. By converting hydrogen as well as lipid peroxides to free radicals through the Fenton reaction, it is thought that ferrous ions enhanced lipid peroxidation. Ferrous ions' breakdown of lipid hydroperoxides into alkoxyl and peroxy radicals resulted in a rise in lipid peroxidation. In this test, ferrozine and ferrous ions create a complex bond, but the herbal extracts inhibit the formation of the complex. Thus, herbal extracts offer a defense against oxidative harm by reducing ferrous ions.

The scavenging capacity of herb extracts was calculated from the analysis of $\bullet\text{OH}$ -RSA. The maximum value of $\bullet\text{OH}$ -RSA was detected in mint leaves (61.87 ± 2.85 mg AAE/g), followed by basil leaves (52.17 ± 3.46 mg AAE/g), fennel seeds (23.89 ± 0.17 mg AAE/g), and fenugreek leaves (19.62 ± 0.46 mg AAE/g), while the minimum was found in coriander seeds (17.25 ± 1.36 mg AAE/g). The $\bullet\text{OH}$ -RSA values found in the leaves of basil, coriander, fennel, and fenugreek leaves were identical to those found in allspice (52.17), cinnamon (17.25), nutmeg (23.89), and cumin (9.62). Among the most reactive species, hydroxyl radicals ($\bullet\text{OH}$) attack nearly every molecule in the biological system, resulting in lipid peroxidation, significant biological damage, and DNA damage. As a result, herbal extracts' ability to scavenge $\bullet\text{OH}$ radicals could offer a significant defense against biological damage caused by these free radicals. The molybdenum (VI) to molybdenum (V)

reduction capacity by an antioxidant phenolic compound and the resulting development of a green molybdenum (V)/phosphate complex is measured using the Phosphomolybdenum antioxidant (PMA) assay. According to the PMA assay findings, mint leaves (8.64 ± 1.84 mg AAE/g), basil leaves (8.39 ± 0.35 mg AAE/g) and coriander seeds (8.04 ± 0.55 mg AAE/g) have considerably greater total antioxidant activity than the other listed spices and herbs, while fennel seeds, fenugreek and basil leaves have considerably lower total antioxidant activity (3.21 ± 0.93 , 3.44 ± 0.32 mg AAE/g, respectively). The present study found that the PMA values of all five herbs and spices examined were equivalent to those previously reported for clove (8.45), fennel (8.16), allspice (8.08), thyme (7.93 mg AAE/g), fenugreek (7.61 mg AAE/g), cardamom (6.48 mg AAE/g), and black cardamom (5.72 mg AAE/g).

Alpha-glucosidase IC_{50} ($\mu\text{g/mL}$) is a measure of the concentration of an inhibitor required to inhibit 50% of the activity of the enzyme α -glucosidase. The lower the IC_{50} value, the more potent the inhibitor is in inhibiting the activity of the enzyme. In this present study comparing the inhibitory activity of five selected herbs and spices against α -glucosidase, results showed that among all mentioned herbs and spices extracts, mint leaves showed stronger and more potent inhibition activity with $IC_{50} = 2.04 \pm 0.03$ $\mu\text{g/mL}$ in comparison with the selected herbs and spices, then comes basil leaves which also exhibited strong inhibitory activity against α glucosidase with IC_{50} values of 3.32 ± 0.47 $\mu\text{g/mL}$. The IC_{50} values for α -glucosidase inhibitory activities of coriander seeds, fenugreek leaves and fennel seeds were 9.41 ± 1.82 , 15.83 ± 1.13 and 31.92 ± 2.07 $\mu\text{g/mL}$, respectively, that were referred to as moderate α -glucosidase inhibitory properties. According to Alqahtani et al. [32], who conducted α -glucosidase inhibitory activities of fenugreek and coriander seeds, which showed low α -glucosidase inhibitory activity with IC_{50} value in the range of 6.05 ± 0.67 $\mu\text{g/mL}$ to 17.86 ± 2.22 $\mu\text{g/mL}$ for fenugreek and 19.31 ± 1 $\mu\text{g/mL}$ to 42.57 ± 2.13 $\mu\text{g/mL}$ for coriander seeds.

Antioxidants in culinary spices and herbs are vital for disabling ROS by impeding their generation [33]. According to reports, antioxidant activity varies in selected spices and herbs because of their complex makeup of bioactive compounds, which predominantly depend on the extraction method. Depending on the cultivar type, area, and climatic conditions, the bioactive compounds, mainly polyphenols, are in each herb and spice. There is a collection of techniques to determine the antioxidant potential of culinary spices and herbs, each with advantages and disadvantages. No method has been developed that accurately depicts phenolic compounds' nearly equal antioxidant capacity because of their complex nature, multiple reactions, and mechanisms. In the past, different studies have determined the radical scavenging abilities of different spices and herbs [34–39]. The results from that analysis depicted that spices and herb extracts possess various scavenging abilities subject to their capability to bind reactive herbs in the biological systems.

Our findings revealed that the antioxidant activity of each spice and herb has varying tendencies depending upon the polyphenols concentration and method chosen for its quantification. Numerous *in vitro* assays can be utilized to assess the antioxidant potential of selected spices and herbs, whereas the recognition and characterization of these specific antioxidant or phenolic compounds can be attained by using LC-MS. Total polyphenols in herbs and their antioxidant capacities are abundantly apparent. A more thorough investigation is necessary to determine and confirm the polyphenols' fundamental role in antioxidant potential while reducing or eliminating the influence of non-phenolic compounds.

3.4. Correlation of Biological Activities and Phenolics

Pearson's association test was performed to estimate the correlation between the obtained results of conducted assays and phenolic contents in spices and herbs (Table 4).

Table 4. Pearson’s correlation between phenolic contents and antioxidant activities.

Variables	TPC	TFC	TCT	ABTS	FICA	FRAP	•OH-RSA	PMA
TFC	0.99							
TCT	−0.12	−0.22						
ABTS	0.97	0.99	−0.13					
FICA	0.97	0.93	0.13	0.93				
FRAP	0.89	0.90	0.12	0.95	0.92			
•OH-RSA	0.78	0.85	−0.30	0.89	0.69	0.87		
PMA	0.10	0.11	0.67	0.26	0.25	0.50	0.32	
α-Glu	0.98	0.97	−0.04	0.96	0.96	0.95	0.83	0.21

Values in bold are different from 0 with a significance level of $\alpha = 0.1$.

It has been reported that total phenolics, as well as total flavonoids, are believed to be accountable for antioxidant activities. Since polyphenols are vital antioxidant agents in spices and herbs, this study considered TFC, TPC, and TCT in selected spices and herbs. The value of TPC was identified, ranging from 9.90 to 125.42 mg GAE/g (Table 2). TFC and antioxidant activities (FRAP, ABTS, FICA, and •OH-RSA) showed a highly significant correlation ($p \leq 0.01$), but TPC and ABTS showed a substantial correlation having $r^2 = 0.97$ ($p \leq 0.05$). Surprisingly, TCT and TPC showed a negative correlation. Previously, Kam et al. [40] also reported that flavonoids have greater antioxidant capability than tannins. It is recorded that total phenolic and flavonoids are major players in the antioxidant values of plant foods [19,41,42]. Numerous variables, such as the variety of samples analyzed, the values, and the various antioxidant assessments, have an impact on the correlation. In the current study, the ABTS was highly correlated with FRAP, FICA, •OH-RSA and alpha-glucosidase, while •OH-RSA was highly correlated with alpha-glucosidase inhibition activity. A similar trend was stated by Kim et al. [21] when it was discovered that flavonoids, which are phenolic compounds, are the key contributors to antioxidant activity. Previously, a positive correlation had been stated between the antioxidant, total flavonoids and total polyphenols contents of herbs [42].

After analyzing the findings, it is suggested that polyphenolic components in herbal extracts may also be the cause of the antioxidant activity. Simple phenols interact with the Folin–Ciocalteu reagent even though they have no significant antioxidant effects. Along with many other compounds found in each spice and herb extract, various phenolic compounds also display varying antioxidant potentials depending on their structural makeup, as well as antagonistic and synergistic behavior.

A biplot analysis (Figure 1) demonstrates that phenolic acids and flavonoids positively correlate with antioxidant activities (ABTS, FRAP, •OH-RSA and FICA) and anti-diabetic activity (alpha-glucosidase inhibition activity). Overall, the biplot shows a variety of bioactive compounds in herbs.

The results of this study exhibited an increase in the concentration of phenolic compounds in spices and herbs with notable antioxidant activity. Furthermore, LC-ESI-QqQ-MS/MS can better quantitatively analyze various phenolic constituents in spices and herbs. They can deliver better knowledge about the relationship among phenolic compounds, their antioxidant activities, and their structure.

3.5. LC-MS Characterization of Phenolic Compounds from Culinary Spices and Herbs

The phenolic compounds from culinary herbs and other spices were screened, characterized, and verified using the LC-ESI-QTOF-MS/MS. The base peak chromatograms of the selected herbs and spices are given in Figure S2. The behavior of these phenolic compounds as antioxidants and their potential health advantages are gaining more and more attention. Remarkably, the interest in phenolic compounds from culinary spices and herbs received a lot of attention because of their powerful health benefits. For this purpose, 71 phenolic compounds were recognized using LC-ESI-QTOF-MS along with their accurate mass, retention times (RT), mass error and molecular formula (Table 5). The MS/MS spectra

of each compound were analyzed by referring to retention times and comparing them with published libraries (MassBank, GNPS, MoNA, HMDB and FooDB) by using MS-DIAL. Peaks were also authenticated and validated from masses and chemical formulas. The given compounds depict different classes, including phenolic acid derivatives (hydroxycinnamic and hydroxybenzoic acids), flavonoids, other polyphenols, lignans, and stilbenes.

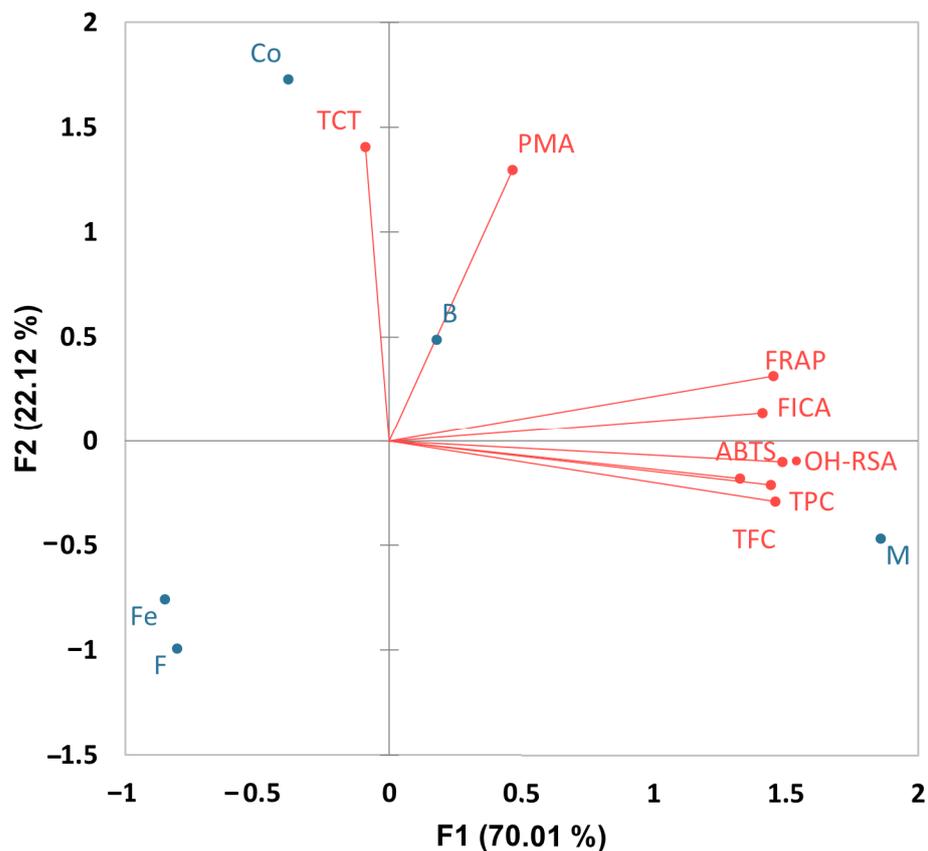


Figure 1. Biplot analysis of the phenolics (TPC, TFC, TCT) and their antioxidant capacities (ABTS, FRAP, •OH-RSA, PMA, and FICA) of selected culinary herbs and spices.

3.5.1. Flavonoids

Flavonoids are bioactive compounds that belong to the most prevalent group of secondary metabolites found in herbs as well as spices, with various health benefits. Due to its anti-mutagenic, anti-inflammatory, anti-carcinogenic, anti-cancer, antimicrobial, and antioxidative capabilities, it is the most prevalent group of secondary metabolites, and a necessary component of functional, nutraceutical, cosmetics, medicinal, and pharmaceutical uses [43]. In addition to their health benefits, flavonoids contribute to the sensory properties of herbs and spices, providing unique flavors and colors. The diversity of flavonoids found in herbs and spices ensures that there are many options for incorporating these beneficial compounds into the diet. Our study identified 28 flavonoids, including 05 flavanols, 04 flavanones, 07 flavones, 05 flavonols, and 07 Isoflavonoids.

Table 5. LC-MS/MS characterization of phytochemicals in selected plants.

No.	Proposed Compounds	Molecular Formula	RT (min)	ESI +/-	Theoretical (m/z)	Observed (m/z)	MS/MS	Mass Error (ppm)	Samples
Flavonoids									
Flavanols									
1	Epigallocatechin-3'-glucuronide	C ₂₁ H ₂₂ O ₁₃	7.331	[M-H] ⁻	481.0987	481.0989	305	0.4	F, B
3	Catechin	C ₁₅ H ₁₄ O ₆	14.158	[M-H] ⁻	289.0717	289.0701	245, 205	-4.5	B, Ci, F, M
2	(+)-Gallocatechin	C ₁₅ H ₁₄ O ₇	16.244	[M-H] ⁻	305.0667	305.0659	245	-2.5	M, Co, B
4	Procyanidin trimer C1	C ₄₅ H ₃₈ O ₁₈	24.969	[M-H] ⁻	865.1985	865.1972	739, 713, 695, 577, 451	-1.5	B, M, Fe
5	Procyanidin B2	C ₃₀ H ₂₆ O ₁₂	27.396	[M-H] ⁻	577.1351	577.1353	451, 425, 407, 289	0.3	B, M, F
Flavanones									
6	Hesperidin	C ₂₈ H ₃₄ O ₁₅	31.486	[M-H] ⁻	609.1825	609.1805	301	-3.3	B, M, Co, Fe, F
7	Neohesperidin	C ₂₇ H ₃₂ O ₁₅	36.849	[M-H] ⁻	595.1668	595.1665	459, 287, 151	-0.6	M, F, B
8	Didymin	C ₂₈ H ₃₄ O ₁₄	41.407	[M-H] ⁻	593.1876	593.1872	447, 285, 151	-0.7	Fe, M, B
9	6-Geranylaringenin	C ₂₅ H ₂₈ O ₅	54.360	[M-H] ⁻	407.1864	407.1878	287, 243, 159, 119	3.4	Co, B, Fe
Flavones									
10	Diosmin	C ₂₈ H ₃₂ O ₁₅	17.572	[M-H] ⁻	607.1668	607.1697	300, 299	4.8	Fe, F, B, M
11	Apigenin 6,8-di-C-glucoside	C ₂₇ H ₃₀ O ₁₅	21.708	[M-H] ⁻	593.1512	593.1525	449, 287	2.2	F, Co, M, B
12	Rhoifolin	C ₂₇ H ₃₀ O ₁₄	26.583	[M-H] ⁻	577.1563	577.1554	431, 269	-1.6	B, M, F
13	Apigenin 7-O-glucuronide	C ₂₁ H ₁₈ O ₁₁	32.733	[M-H] ⁻	445.0776	445.0770	269	-1.8	F, M, Fe
14	Apigenin 6-C-glucoside (Isovitexin)	C ₂₁ H ₂₀ O ₁₀	33.72	[M-H] ⁻	431.0983	431.1001	269	4.2	F
15	Chrysoeriol 7-O-glucoside	C ₂₂ H ₂₂ O ₁₁	35.196	[M-H] ⁻	461.1089	461.1085	299	-0.9	F, B, Fe
16	Cirsilineol	C ₁₈ H ₁₆ O ₇	37.596	[M-H] ⁻	343.0823	343.0815	327, 255, 241	-2.3	Fe, M
Flavanols									
17	Quercetin 3'-O-glucuronide	C ₂₁ H ₁₈ O ₁₃	17.998	[M+H] ⁺	479.0820	479.0801	303	-4.5	B
18	Rutin	C ₂₇ H ₃₀ O ₁₆	24.423	[M-H] ⁻	609.1461	609.1466	301, 300, 271	0.9	F, M, B
19	Myricetin 3-O-rhamnoside	C ₂₁ H ₂₀ O ₁₂	28.522	[M-H] ⁻	463.0882	463.0887	317	1.1	B, M, Co
20	Quercetin 3-O-arabinoside	C ₂₀ H ₁₈ O ₁₁	30.943	[M-H] ⁻	433.0776	433.0756	301	-4.6	B, M, F
21	Isorhamnetin	C ₁₆ H ₁₂ O ₇	36.254	* [M-H] ⁻	315.0510	315.0523	300, 151, 107	4.1	M
22	Quercetin	C ₁₅ H ₁₀ O ₇	40.120	[M-H] ⁻	301.0349	301.0332	179, 151	-4.2	F, B, M
23	3,7-Dimethylquercetin	C ₁₇ H ₁₄ O ₇	43.569	* [M-H] ⁻	329.0667	329.0658	314, 299, 271	-2.7	B

Table 5. Cont.

No.	Proposed Compounds	Molecular Formula	RT (min)	ESI +/-	Theoretical (m/z)	Observed (m/z)	MS/MS	Mass Error (ppm)	Samples
Isoflavonoids									
24	Equol 4'-O-glucuronide	C ₂₁ H ₂₂ O ₉	17.955	[M-H] ⁻	417.1191	417.1191	399, 241	0.0	M
25	3'-O-Methylequol	C ₁₆ H ₁₆ O ₄	18.413	[M-H] ⁻	271.0976	271.0964	255, 149, 121	-4.4	M
26	6''-O-Acetylglycitin	C ₂₄ H ₂₄ O ₁₁	26.070	[M-H] ⁻	487.1246	487.1248	283, 267, 59	0.6	F, M
27	3'-Hydroxygenistein	C ₁₅ H ₁₀ O ₆	32.762	* [M-H] ⁻	285.0404	285.0418	267, 251, 201, 177	4.9	M
28	3'-Hydroxydaidzein	C ₁₅ H ₁₀ O ₅	36.949	[M-H] ⁻	269.0455	269.045	241, 224, 213, 181	-1.9	B, F, M
29	3',4',7-Trihydroxyisoflavanone	C ₁₅ H ₁₂ O ₅	47.982	[M-H] ⁻	271.0612	271.0611	239, 135, 121	-0.4	M
30	Violanone	C ₁₇ H ₁₆ O ₆	48.320	[M-H] ⁻	315.0874	315.0866	300, 285, 135	-2.5	M
Phenolic Acids									
Hydroxybenzoic acids									
31	Gallic acid 4-O-glucoside	C ₁₃ H ₁₆ O ₁₀	10.099	[M-H] ⁻	331.067	331.0666	169, 125	-1.2	M, Co, F, B
32	Gallic acid	C ₇ H ₆ O ₅	10.544	* [M-H] ⁻	169.0142	169.0144	125	1.2	B, M, F, Fe, Co
33	Syringic acid	C ₉ H ₁₀ O ₅	12.284	* [M-H] ⁻	197.0455	197.0459	182, 163, 138, 123, 95	2	B, F, M, Fe
34	Protocatechuic acid 4-O-glucoside	C ₁₃ H ₁₆ O ₉	12.569	[M-H] ⁻	315.0721	315.0727	153	1.9	F, M, B
35	3-O-Methylgallic acid	C ₈ H ₈ O ₅	14.587	* [M-H] ⁻	183.0299	183.0292	139, 123	-3.8	M, F, B
36	Protocatechuic acid	C ₇ H ₆ O ₄	14.784	* [M-H] ⁻	153.0193	153.0197	109	2.6	F, M, B, Fe
37	4-Hydroxybenzoic acid 4-O-glucoside	C ₁₃ H ₁₆ O ₈	16.589	* [M-H] ⁻	299.0772	299.0786	255, 137	4.7	*F, Ci, B
38	Punicafolin	C ₄₁ H ₃₀ O ₂₆	20.341	[M-H] ⁻	937.0952	937.0967	169, 125	1.7	F, B, M
39	Ellagic acid	C ₁₄ H ₆ O ₈	23.790	[M-H] ⁻	300.999	300.9977	284, 257	-4.3	Fe, M, B, F
40	Valoneic acid dilactone	C ₂₁ H ₁₀ O ₁₃	30.649	[M-H] ⁻	469.0048	469.0052	425, 301, 139	0.9	M
41	p-Hydroxybenzoic acid	C ₇ H ₆ O ₃	37.172	* [M-H] ⁻	137.0244	137.0242	93	-1.5	F, Co, B, M, Fe
Hydroxycinnamic acids									
42	3-Caffeoylquinic acid	C ₁₆ H ₁₈ O ₉	13.005	* [M-H] ⁻	353.0878	353.0867	191, 179, 161	-3.1	Fe, B, F, M
43	Ferulic acid	C ₁₀ H ₁₀ O ₄	13.817	[M-H] ⁻	193.0506	193.0504	178, 149, 134	-1.0	Fe, Co, F, B, M
44	Caffeoyl glucose	C ₁₅ H ₁₈ O ₉	14.764	[M-H] ⁻	341.0878	341.0878	179	0.0	B, M
45	Caffeoyl aspartic acid	C ₁₃ H ₁₃ NO ₇	17.247	[M-H] ⁻	294.0619	294.0631	179	4.1	M
46	p-Coumaric acid 4-O-glucoside	C ₁₅ H ₁₈ O ₈	17.313	[M-H] ⁻	325.0929	325.0923	163	-1.8	B, F, M

Table 5. Cont.

No.	Proposed Compounds	Molecular Formula	RT (min)	ESI +/-	Theoretical (m/z)	Observed (m/z)	MS/MS	Mass Error (ppm)	Samples
47	<i>p</i> -Coumaric acid	C ₉ H ₈ O ₃	18.320	* [M–H] [–]	163.04	163.0405	119	3.1	F, Co, B, M
48	Caffeic acid	C ₉ H ₈ O ₄	21.084	[M–H] [–]	179.035	179.0346	135	–2.2	B, F, M
49	Sinapic acid	C ₁₁ H ₁₂ O ₅	21.339	* [M–H] [–]	223.0612	223.0615	193, 179, 149, 134	1.2	M, Fe, F, B
50	3- <i>p</i> -Coumaroylquinic acid	C ₁₆ H ₁₈ O ₈	23.499	[M–H] [–]	337.0929	337.0939	191, 119	3.0	F, B, M
51	3-Feruloylquinic acid	C ₁₇ H ₂₀ O ₉	24.897	[M–H] [–]	367.1034	367.1030	193, 191	–1.6	F, M, B
52	Chicoric acid	C ₂₂ H ₁₈ O ₁₂	30.115	* [M–H] [–]	473.0725	473.0736	293, 311	2.3	B, M
53	1,5-Dicaffeoylquinic acid	C ₂₅ H ₂₄ O ₁₂	30.921	[M–H] [–]	515.1195	515.1197	191, 179, 135	0.7	B, F, M, Fe
54	Rosmarinic acid	C ₁₈ H ₁₆ O ₈	33.313	[M–H] [–]	359.0772	359.0776	197, 179, 161, 135	1.1	M, B, F
55	Cinnamic acid	C ₉ H ₈ O ₂	38.22	[M–H] [–]	147.0451	147.046	103	6.1	F, B, M, Fe
Other compounds									
56	Scopoletin	C ₁₀ H ₈ O ₄	8.681	[M–H] [–]	191.035	191.035	175, 147	0.0	M
57	Pyrogallol	C ₆ H ₆ O ₃	8.573	[M–H] [–]	125.0244	127.0256	107, 97, 79	2.1	B, M, F
58	Trigonelline	C ₇ H ₇ NO ₂	14.621	[M–H] [–]	138.0549	138.0553	120, 94, 92	5.0	M, F
59	<i>p</i> -Anisaldehyde	C ₈ H ₈ O ₂	15.526	* [M–H] [–]	135.0451	135.0445	122, 109	1.7	B, F, M
60	Diosgenin	C ₂₇ H ₄₂ O ₃	16.716	[M+H] ⁺	415.3212	415.3212	271, 253, 157	0.0	F
61	Sagerinic acid	C ₃₆ H ₃₂ O ₁₆	26.198	[M–H] [–]	719.1612	719.1610	359, 197, 179, 161	–1.2	B, M, Fe
62	Esculin	C ₁₅ H ₁₆ O ₉	20.426	[M–H] [–]	339.0721	339.0728	177	2.2	F, M
63	Esculetin	C ₉ H ₆ O ₄	20.473	[M–H] [–]	177.0193	177.0196	133, 105	–4.4	F, B
64	Coumarin	C ₉ H ₆ O ₂	22.521	[M+H] ⁺	147.0441	147.0441	103	0.0	M, B, F
65	2-Hydroxybenzaldehyde	C ₇ H ₆ O ₂	24.656	* [M–H] [–]	121.0295	121.0292	92, 77	–2.5	Co, F, B
66	Umbelliferone	C ₉ H ₆ O ₃	26.399	* [M–H] [–]	161.0244	161.0242	133	–1.6	F, M
67	Rosmanol	C ₂₀ H ₂₆ O ₅	26.775	[M–H] [–]	345.1707	347.1708	301	0.2	F, Fe
68	Carnosic acid	C ₂₀ H ₂₈ O ₄	44.680	[M–H] [–]	331.1915	331.1903	287	–4.5	B, M, F
69	Carnosol	C ₂₀ H ₂₆ O ₄	46.724	[M–H] [–]	329.1758	329.1760	285	0.6	B, M
70	[6]-Gingerol	C ₁₇ H ₂₆ O ₄	49.633	[M–H] [–]	293.1758	293.1769	293	3.6	B, M
71	Carvacrol	C ₁₀ H ₁₄ O	58.621	[M–H] [–]	149.0972	149.0966	131, 105	4.4	B, M, F

RT stands for “retention time”. Culinary herbs and spices were presented with abbreviations: basil leaves (B), mint leaves (M), fennel seeds (Fe), fenugreek leaves seeds (F), and coriander seeds (Co). Compounds with an asterisk (*) were found in negative and positive modes.

Flavanols

A subclass of flavonoids known as flavanols is present in a variety of plant-based foods, particularly spices and other herbs. They are renowned for their antioxidant properties and have been associated with various health benefits, such as improving heart health and cognitive function. Flavanols are also found in herbs and spices such as cinnamon, parsley, and thyme [44]. Moreover, (+)-gallocatechin (compound 3) and (+)-Catechin (compound 2) are the most abundant flavanols in nature [45]. All compounds from 1 to 5 are detected in negative modes of ionization ($[M-H]^-$). Compound 1 (epigallocatechin-3'-glucuronide, $C_{21}H_{22}O_{13}$) was tentatively recognized at m/z 481.0987 in basil and fenugreek leaves, which was confirmed through MS [46]. Epigallocatechin-3'-glucuronide has been shown to increase beneficial gut bacteria while reducing harmful bacteria, improving gut health and potentially reducing the risk of diseases, for instance, inflammatory bowel disease and colorectal cancer [47]. It also has anti-cancer as well as anti-inflammatory properties, inhibiting the growth and proliferation of cancer cells and potentially preventing certain types of cancer. While primarily found in green tea [48,49], (+)-gallocatechin (compound 3, $C_{15}H_{14}O_7$) was putatively characterized in coriander seeds and basil leaves at m/z 305.0659 and 305.0667. (+)-gallocatechin is a flavonoid that aids in shielding cells from oxidative stress and free radical damage, which can contribute to the development of chronic diseases such as cancer, heart disease, as well as neurodegenerative disorders [50]. Additionally, research has suggested that (+)-gallocatechin may have anti-cancer along with anti-inflammatory properties [48]. Compound 2 was characterized as catechin having chemical formula $C_{15}H_{14}O_6$ at m/z 289.0701 in basil, coriander, fenugreek, and mint leaves. Catechin is a flavonoid compound found in various herbs and spices extensively studied for its potential health-promoting effects. This compound possesses potent antioxidant properties that aid in shielding cells from oxidative stress and free radical damage [51]. Additionally, catechin has been observed to exhibit anti-inflammatory effects and may also enhance cognitive function and positively impact brain health [52].

Procyanidin dimer B2 and trimer C1 are tannins that may protect the heart and cardiovascular system due to their significant antioxidant potential [53]. Compound 4 was putatively identified in basil, fennel, and mint leaves at m/z 865.1972 and designated as procyanidin trimer C1. Procyanidin dimer B2 (compound 5, $C_{30}H_{26}O_{12}$) was tentatively known at m/z 577.1313 in negative ionization mode in basil, fenugreek, and mint leaves [54].

Flavanones

Compounds 6–9 were also detected with the negative mode of ionization. Compound 6 (Hesperidin, $C_{28}H_{34}O_{15}$) was found at m/z 609.1805 in basil, mint, coriander and fennel leaves extract [55]. In addition to its anti-inflammatory and antioxidant properties, hesperidin has anti-allergic effects that can help reduce symptoms of allergies [56]. Furthermore, some studies have suggested that hesperidin may positively impact cognitive function, have beneficial neuro-pharmacological effects, and have potential benefits for cardiovascular health by reducing inflammation and improving blood flow [57]. Compound 7 was identified at m/z 595.1629 in negative mode and putatively categorized as neoeriocitrin in mint, fenugreek, and basil leaves. In addition to research on its potent antioxidant properties, neoeriocitrin also exhibits anti-inflammatory effects, making it a potential natural remedy for conditions such as inflammatory bowel disease and arthritis [58]. This compound has also been shown to positively affect cardiovascular health, reducing blood pressure and cholesterol levels. Furthermore, neoeriocitrin has been suggested to have neuroprotective effects, potentially reducing the risk of cognitive decline and neurodegenerative diseases [59,60]. A precursor ion of compound 8 (m/z 593.1872 and 593.1876) was identified in basil, fennel and mint leaves as didymin in negative mode [61]. A flavonoid compound called didymin is present in a variety

of citrus fruits, herbs, and spices that offer unique health benefits. This compound has neuroprotective effects and antioxidant, anti-inflammatory, and anti-diabetic properties [62]. Additionally, didymin has been shown to have advantageous effects on blood sugar levels, making it a potential natural remedy for managing diabetes. Studies have also suggested that didymin may benefit skin health, because it has been demonstrated to shield against UV-induced harm [63]. 6-geranylneringenin (compound 9) was characterized in coriander, basil, and fennel leaves at m/z 407.1878 and 407.1864. Previously, lemon and mint were said to have significant antioxidant potential.

Flavones

Five flavones (compounds 10–16) were detected in this study with negative ionization mode. Compound 10 was characterized as diosmin ($C_{28}H_{32}O_{15}$) at m/z 607.1697 in fennel, fenugreek, basil, and mint leaves [64]. With its potent antioxidant properties, inflammation and oxidative stress can be reduced by diosmin, potentially lowering the risk of chronic diseases, for instance, heart disease, cancer, and diabetes. Additionally, diosmin has been shown to have advantageous effects on venous and skin health and analgesic and anti-inflammatory effects [65]. Compound 11 (apigenin 6,8-di-C-glucoside, m/z 593.1525) was identified in fenugreek, coriander, mint, and basil leaves. Compound 12 (m/z 577.1524) was putatively recognized as rhoifolin in the negative mode in basil, fenugreek, and mint leaves extract [66]. Rhoifolin's unique health benefits include reducing inflammation, protecting against cardiovascular diseases, improving cognitive function, and aiding in the prevention and treatment of cancer [67]. It has also been found to have potential therapeutic effects in diabetes and skin disorders. Some studies also suggested its potential to improve liver function, alleviate symptoms of allergies, and have a protective effect on the skin, as well as anti-inflammatory effects. Compound 13 was identified in fennel, fenugreek, and mint leaves with a precursor ion at $[M-H]^-$ m/z 445.0740 and was designated as (apigenin 7-O-glucuronide— $C_{21}H_{18}O_{11}$) [68,69]. Compound 14 was identified at m/z 431.1001 and 431.0983 in negative mode as well as tentatively categorized as apigenin 6-C-glucoside in fenugreek leaves extract only. Compound 15 with $[M-H]^-$ at m/z 461.1085 was putatively designated as chrysoeriol 7-O-glucoside, which was detected in only fennel, fenugreek and basil leaves. Cirsilineol (compound 16, $C_{18}H_{16}O_7$) was only tentatively known at m/z 343.0802 in negative ionization mode in fennel seeds. Cirsilineol is a flavone found in several plants, including the popular herb thyme. This compound has been found to have numerous possible health advantages, for instance antioxidant, anti-inflammatory as well as antimicrobial properties [70]. Cirsilineol has been demonstrated to lessen inflammation in the body, which may aid in lowering the chance of developing chronic illnesses, including cancer and cardiovascular disease. Some studies have suggested that cirsilineol acts as a natural insecticide and exhibits nootropic effects, which means it may help enhance cognitive function.

Flavonols

In this current study, compounds 17–23 were identified in basil, fenugreek, coriander, and mint leaves, which are considered to fall under flavonols in negative as well as positive ionization mode ($[M-H]^-$ / $[M+H]^+$). Compound 17 with $[M+H]^+$ at m/z 479.0801 was putatively designated as quercetin 3'-O-glucuronide, which was detected in only basil leaves. Quercetin 3'-O-glucuronide is a metabolite of quercetin, a flavonoid that is found in many plant-based foods. Quercetin 3'-O-glucuronide has potent antioxidant and anti-inflammatory properties, immune-boosting properties, and potential therapeutic effects in the treatment of diabetes [71]. It may also have unique benefits in improving exercise performance and recovery and protecting the liver against damage caused by toxins [72]. Compound 18 was characterized as rutin ($C_{27}H_{30}O_{16}$) at m/z 609.1466 in fenugreek, basil, and mint leaves. Compound 19 was identified in basil, coriander, and mint leaf with a precursor ion at $[M-H]^-$ m/z 463.0887 and 483.0882 was designated as myricetin

3-*O*-rhamnoside (C₂₁H₂₀O₁₂). Prior reports noted the remarkable antioxidant properties of mint and lemon. Compound **20** was identified at *m/z* 433.0756 in the negative mode which was tentatively categorized as quercetin 3-*O*-arabinoside (C₂₀H₁₈O₁₁) in basil, mint and fenugreek leaves only. Compound **21** was characterized as Isorhamnetin (C₁₆H₁₂O₇) at *m/z* 609.1466 in mint leaves. Isorhamnetin has potent anti-cancer and anti-inflammatory properties, cardiovascular benefits, reduced inflammation and oxidative stress, and improved blood pressure and cholesterol levels. Isorhamnetin may also have neuroprotective effects and may help in the treatment of neurological disorders, for instance, Alzheimer's disease. Additionally, It has been discovered to control blood sugar levels and has skin-protective properties, potentially preventing premature aging and protecting against UV damage [73]. Compound **22** (quercetin, *m/z* 301.0332) was identified in mint, basil, and fenugreek leaves. Compound **23** (*m/z* 329.0658—3,7-dimethylquercetin) was identified in basil leaves only in the negative mode of ionization.

3.5.2. Isoflavonoids

Based on MS/MS data, seven isoflavonoid compounds (from **24–30**) were discovered in this investigation in the negative mode of ionization. Compounds **24** and **25** showed [M–H][−] at *m/z* 417.1191 and 271.0964 were putatively identified as equol 4'-*O*-glucuronide and 3'-*O*-methylequol, respectively, in mint leave only. Compound **26** (6''-*O*-acetylglycitin, *m/z* 487.1278) was identified in mint and fenugreek leaves. A precursor ion of compounds **27**, **29**, **30** (*m/z* 285.0418, 271.0611, 315.0866) was identified in mint leaves as 3'-hydroxygenistein, 3',4',7-trihydroxyisoflavanone and violanone, respectively, in negative mode. A previous study also reported about this compound as violanone has been found to have specific properties that make it potentially valuable for medicines, for instance, its anti-inflammatory and antimicrobial effects. Its chemical structure also allows it to readily bind to another molecule, which may have implications for potential use in drug development. Compound **28**, having chemical formula C₁₅H₁₀O₅, was identified at *m/z* 269.0450 in the negative mode, which was tentatively categorized as 3'-hydroxydaidzein in fenugreek seeds and basil plus mint leaves.

3.5.3. Phenolic Acids

They are widely distributed secondary aromatic plant compounds with potential health benefits. In our research, 25 phenolic acids were identified using LC-ESI-QTOF-MS/MS. Phenolic substances are classified as phenolic acids if they contain a carboxyl group. In-depth research has been conducted on the phenolic acids' antioxidant, anti-bacterial, anti-cancer, anti-inflammatory and cardiovascular qualities. Both herbs and spices contain phenolic acid species. This study discovered the two phenolic acid subclasses, hydroxybenzoic acids (11) and hydroxycinnamic acids (14) [74].

Hydroxybenzoic and hydroxycinnamic acids are potent phenolic compounds in various herbs and spices that offer unique health benefits. These compounds exhibit strong antioxidant properties, helping to reduce oxidative stress as well as inflammation in the body, which can lead to chronic diseases. Moreover, hydroxybenzoic acids and hydroxycinnamic acids have anti-inflammatory effects, which may help to further lower the chance of developing chronic illnesses.

Hydroxybenzoic Acids

In the present work, we characterized ten hydroxybenzoic acids. Compound **31** (gallic acid 4-*O*-glucoside) was characterized in negative mode at *m/z* 331.0666 in mint, fenugreek, basil leaves, and coriander seeds [75]. Yisimayili et al. [76] also mentioned this compound, gallic acid, in their study [76]. Compounds **32** (gallic acid, C₇H₆O₅) and **40** (*p*-hydroxybenzoic acid, C₇H₆O₃) with [M–H][−] at *m/z* 169.0144 and 137.0242, respectively, were commonly identified in basil, mint, fenugreek leaves, fennel, and coriander seeds. Gallic acid is one of nature's most pervasive phytochemicals with strong anti-cancer, anti-allergic, anti-inflammatory, anti-asthmatic, anti-mutagenic, and neuroprotective prop-

erties [77]. Compounds **33** (m/z 197.0459) and **36** (m/z 153.0197) were discovered as syringic acid and protocatechuic acid [78] found commonly in fennel seeds, fenugreek leaves, mint, and basil leaves. Syringic acid is considered to be a phenolic acid that occurs naturally in fruits, vegetables, and herbs like grapes, strawberries, walnuts, and rosemary. It has potential health benefits, including antioxidant and anti-inflammatory properties, and may improve heart health via lowering blood pressure, minimizing the danger of blood clots, and enhancing lipids levels [79]. Syringic acid may also have anti-cancer, anti-bacterial, and antiviral effects and has the potential as a natural food preservative [80]. Two more compounds, **34** and **35**, were characterized at m/z 315.0727, 183.0292, which were tentatively detected as protocatechuic acid 4-*O*-glucoside and 3-*O*-methylgallic acid in the negative mode in basil, mint, and fenugreek leaves. In contrast, compound **38** (ellagic acid, $C_{14}H_6O_8$) was identified in fennel seeds, basil, and mint leaves with $[M-H]^-$ at m/z 300.9977. It possesses various potent health advantages, for instance, anti-cancerous, anti-inflammatory, antioxidant, anti-aging, antimicrobial, antiulcerogenic, and anti-depressant qualities [81]. Additionally, it has cardio-protective and neuro-protective properties. Some research suggests that ellagic acid may enhance insulin sensitivity and assist in controlling blood sugar levels, potentially benefiting people with diabetes or those at risk of developing the disease. Compound **37** (m/z 299.0786—4-hydroxybenzoic acid 4-*O*-glucoside) was identified in coriander seeds and fenugreek basil leaves. Compound **40** (valoneic acid dilactone, $C_{21}H_{10}O_{13}$) was identified in the negative mode at m/z 469.0052 and found only in mint leaves. It has potential health benefits, for instance, anti-inflammatory effects, antioxidant activity and antimicrobial properties [82]. Valoneic acid dilactone has been studied for its potential to prevent cellular damage and chronic diseases like cancer, diabetes, and heart disease. It may have hepatoprotective effects via lowering oxidative stress and inflammation in the liver, which can help to prevent liver injury and disease [83].

Hydroxycinnamic Acids

In this study, compounds **42–55** belong to the class of hydroxybenzoic acids. Compound **42** was tentatively identified as ferulic acid predominantly found in fennel and coriander seeds, basil, fenugreek, and mint leaves. Ferulic acid has been studied for its potential to improve cardiovascular health by reducing blood pressure, inhibiting the formation of blood clots, and improving lipid levels in the blood [84]. Additionally, some research suggests that ferulic acid may have anti-cancer, antioxidant, anti-inflammatory, and antimicrobial properties [85]. Ferulic acid has been shown to have photoprotective effects, helping to protect the skin from UV damage and reduce the risk of skin cancer [86]. Compounds **44**, **52** and **54** with $[M-H]^-$ at m/z 294.0631, 473.0736, and 359.0776 were putatively designated as caffeoyl glucose, chicoric acid and rosmarinic acid, respectively, which were detected in basil and mint leaves. Chicoric acid and rosmarinic acid have potential health benefits, including antioxidant, anti-inflammatory, antimicrobial, and anti-diabetic effects. It can reduce inflammation in the body, improve blood sugar levels, improve digestion, and protect against cellular damage caused by oxidative stress. Additionally, some research suggests that chicoric acid may help to improve cognitive function and protect against neurodegenerative diseases like Alzheimer's and Parkinson's [87]. Rosmarinic acid may have the potential as a natural allergy treatment by reducing inflammation in the airways and improving respiratory function [88]. While four more Compounds, **46**, **47**, **49** and **50**, were detected in fenugreek, basil and mint leaves and identified to be 3-feruloylquinic acid, caffeic acid, *p*-coumaric acid 4-*O*-glucoside, 3-*p*-coumaroylquinic acid with the negative mode of ionization at m/z 367.1062, 179.0346, 325.0923 and 337.0939, respectively. Caffeic acid has several potential health benefits, including anti-cancer, antioxidant, anti-inflammatory, anti-diabetic and antimicrobial properties [89]. Compound **45** (m/z 294.0631—caffeoyl aspartic acid) was determined in mint leaves only in a negative mode of ionization. Compounds **42** (m/z 353.0867), **53** (m/z 515.1165), **48** (m/z 223.0626) and **55** (m/z 147.0460) were putatively recognized as 3-caffeoylquinic acid, 1,5-dicafeoylquinic acid, sinapic acid and cinnamic acid, respectively, in fennel seeds and basil, fenugreek, and mint leaves

extract in negative mode. Cinnamic acid has several potential health advantages, including anti-cancer, antioxidant, anti-diabetic, anti-inflammatory, and antimicrobial properties [90]. Some studies have suggested that cinnamic acid may have potential as a natural sunscreen ingredient, as it can help to absorb UV radiation and protect the skin from damage [91]. *P*-coumaric acid (compound 47) was found in $[M-H]^-$ mode at m/z 163.0405.

3.5.4. Other Compounds

Eleven additional compounds (compounds 55–71) were identified from different culinary herbs and spices. Scopoletin (compound 55) was found in $[M-H]^-$ mode at m/z 147.0438. It has several potential health benefits, including antioxidant, anti-depressant, anti-diabetic, anti-inflammatory, antimicrobial, natural pain reliever, and anxiety reliever properties. Scopoletin may also have the potential to be a natural treatment for skin conditions like eczema and psoriasis, as it has been shown to have anti-inflammatory effects on the skin [92]. Compound 58 (trigonelline), an alkaloid, was tentatively identified in mint and fenugreek. Previously, it was identified in coffee arabica [93]. Diosgenin (compound 60), a steroidal saponin, was putatively identified in fenugreek. Diosgenin has several pharmacological benefits, as reported by Jesus et al. [94]. Three more compounds, esculin (compound 62— $C_{15}H_{16}O_9$), esculetin (compound 63— $C_9H_6O_4$) and pyrogallol (compound 57— $C_6H_6O_3$), were identified commonly in fenugreek, basil, and mint leaves at m/z 339.0738, 177.0196, 127.0383 and 477.3279, respectively, in the negative mode of ionization. Esculin and esculetin are natural compounds found in several herbs and plants, including horse chestnut, chicory, and celery. They have several potential health benefits, including anti-inflammatory, anti-diabetic, antioxidant, and anti-tumor properties. Additionally, some research suggests that esculin might be useful as a natural remedy for venous insufficiency, a condition characterized by the improper functioning of vein valves in the legs, as it can help to improve blood flow and reduce swelling [95]. Some research suggests that esculetin may have the potential as a natural treatment for osteoporosis [96]. Compound 66 was tentatively known as umbelliferone by the precursor ion $[M-H]^-$ at m/z 161.0242 in basil, mint leaves, and fennel seeds. Compound 58 (*p*-anisaldehyde) was considered at m/z 135.0445 in negative mode from basil and fenugreek [97]. Compounds 67 and 68, rosmanol ($C_{20}H_{26}O_5$ — m/z 347.1847) and carnosic acid ($C_{20}H_{28}O_4$ — m/z 331.1903) were tentatively characterized from fennel and basil, respectively. In the past, cinnamon, oregano, rosemary, and thyme were all said to contain carnosic acid [98]. In addition, carnosol, rosmanol, and carnosic acid were found in culinary herbs and spices with substantial antioxidant potential [99]. Carnosic acid has several potential health benefits, including antioxidant, anti-diabetic, anti-inflammatory, and antimicrobial properties. Additionally, some research suggests that carnosic acid might be useful as a natural remedy for Alzheimer's disease, as it has been shown to protect against beta-amyloid plaques that accumulate in the brain, alongside playing a role in contributing to the development of the disease [100]. Furthermore, it might have value as a natural remedy for obesity because it may aid in reducing deposits of fat and enhancing metabolic processes [101]. While some of the health benefits of rosmanol are shared in addition to the natural compounds present in herbs and spices, such as its antioxidant, anti-diabetic, antimicrobial, and anti-inflammatory properties, some potential unique benefits are explicitly associated with rosmanol. For example, some research has suggested that rosmanol may have potential as a natural treatment for hair loss, as it can help to stimulate hair growth by promoting the proliferation of hair follicle cells [102]. Additionally, rosmanol could possess the ability to function as an all-natural remedy for ageing skin [103]. Compound 65 was identified at m/z 121.0292 in negative mode as well as putatively categorized as 2-hydroxybenzaldehyde in fenugreek, basil leaves and in coriander seeds. In negative mode, compound 70 ([6]-gingerol) was described at m/z 299.2222 in fennel and fenugreek leaves. There are various potential health advantages including anti-diabetic, anti-inflammatory, anti-cancer, antioxidant, as well as antimicrobial properties. Ovarian cancer, breast cancer, as well as pancreatic cancer

are just a few forms of the cancers that [6]-gingerol may be able to treat naturally [104]. Phytochemicals are beneficial for human and animal health [105].

3.6. Quantification and Semi-Quantification of Volatile and Non-Volatile Compounds

A heatmap was generated for the most abundant volatile compounds (Figure 2A, Table S2), while twenty-eight phenolic compounds were quantified using LC-MS/MS (Figure 2B, Table S1). GC-MS analysis was conducted for the detailed profiling of volatile constituents from selected culinary spices and herbs (sweet basil, mint, fenugreek, fennel, and coriander), and 49 compounds were identified. The GC-MS total ion chromatograms (TIC) of the selected herbs and spices are given in Figure S1.

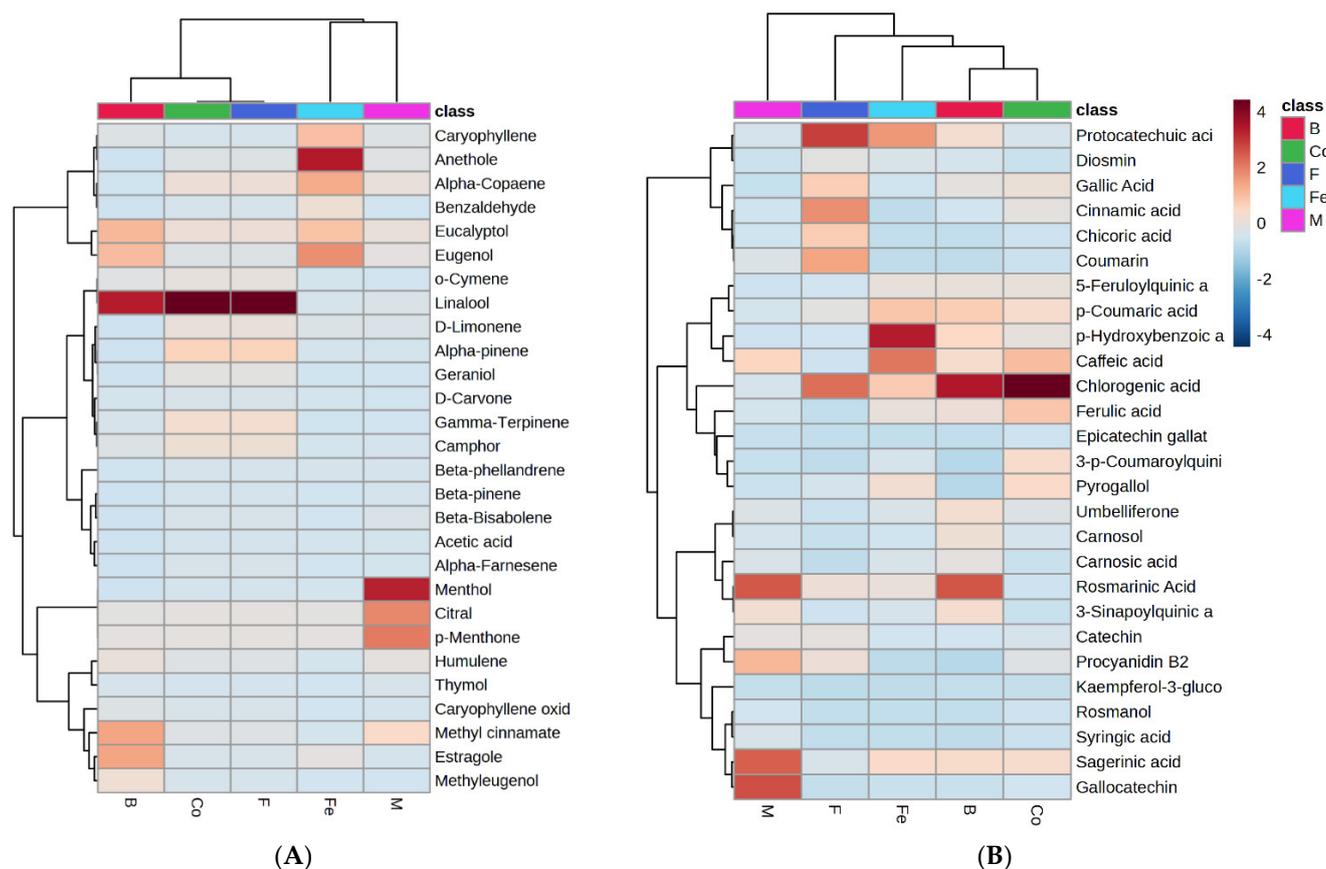


Figure 2. Heatmap analysis of the concentration of volatile (A) and non-volatile phenolic components (B) in basil (B), mint (M), fenugreek (F), fennel (Fe), and coriander (Co).

The highest number of phenolic compounds were quantified in basil (twenty-four compounds), mint (twenty-one compounds), fennel and fenugreek (fourteen compounds), and coriander (twenty-one compounds), respectively. The three major compounds (caffeic acid, *p*-coumaric acid, and chlorogenic acid) are found in almost all five selected culinary spices and herbs but in different quantities. For instance, the highest amount of *p*-coumaric acid and chlorogenic acid were quantified in basil ($144.63 \pm 4.72 \mu\text{g/g}$) and ($384.07 \pm 12.39 \mu\text{g/g}$), respectively, while the lowest amount of *p*-coumaric acid was found in coriander ($22.43 \pm 1.07 \mu\text{g/g}$) and the least amount of chlorogenic acid was observed to be in fennel ($29.07 \pm 0.98 \mu\text{g/g}$). The highest amount of caffeic acid was quantified in mint ($157.82 \pm 8.62 \mu\text{g/g}$) and lowest in fenugreek ($11.66 \pm 0.58 \mu\text{g/g}$). Moreover, the highest amount of rosmarinic acid and 3-sinapoylquinic acid was quantified in mint ($416.16 \pm 19.13 \mu\text{g/g}$) and ($114.73 \pm 2.41 \mu\text{g/g}$), respectively and the lowest values for these compounds were observed in fennel as ($16.49 \pm 2.04 \mu\text{g/g}$) and ($8.93 \pm 0.30 \mu\text{g/g}$), respectively. 5-feruloylquinic acid and epicatechin gallate are the phe-

nolic compounds observed to be quantified only in basil with values of $(80.20 \pm 5.11 \mu\text{g/g})$ and $(17.41 \pm 1.73 \mu\text{g/g})$, respectively. Gallic acid was quantified in the range of 7 to $74 \mu\text{g/g}$ in basil, fennel, coriander, and fenugreek. The following phenolic compounds (rosmanol, umbelliferone, sagerinic acid, syringic acid and gallic acid) are only observed and quantified in mint and basil. Rosmanol, sagerinic acid, syringic acid and gallic acid have shown higher quantification values for mint as $(26.52 \pm 0.88 \mu\text{g/g})$, $(402.63 \pm 16.81 \mu\text{g/g})$, $(44.82 \pm 3.83 \mu\text{g/g})$, $(430.61 \pm 19.62 \mu\text{g/g})$, respectively, and lower quantification values for basil as $(16.45 \pm 6.03 \mu\text{g/g})$, $(113.31 \pm 9.29 \mu\text{g/g})$, $(14.91 \pm 0.62 \mu\text{g/g})$, $(25.03 \pm 2.41 \mu\text{g/g})$. In contrast, umbelliferone showed the opposite trend as it has a higher quantification value for basil $(102.63 \pm 24.94 \mu\text{g/g})$ and a lower quantification value for mint $(52.41 \pm 4.62 \mu\text{g/g})$. *p*-hydroxybenzoic acid and kaempferol-3-glucoside were quantified only in basil and fennel and the highest amount for these two compounds were quantified in basil $(124.83 \pm 15.83 \mu\text{g/g})$ and $(19.09 \pm 0.83 \mu\text{g/g})$ while the lowest was measured in fennel $(73.39 \pm 5.74 \mu\text{g/g})$ and $(2.59 \pm 0.18 \mu\text{g/g})$, respectively. On the other hand, cinnamic acid was quantified in mint $(22.40 \pm 1.68 \mu\text{g/g})$, basil $(36.87 \pm 3.20 \mu\text{g/g})$, coriander $(14.08 \pm 1.24 \mu\text{g/g})$ and fenugreek $(125.13 \pm 13.81 \mu\text{g/g})$. Syringic acid, kaempferol, caffeic acid, protocatechuic acid, gallic acid, coumaric acid, rosmarinic acid, cinnamic acid, carnosic acid, gallic acid and other flavonoids have previously been measured in a variety of herbs. In contrast, GC-MS analysis of culinary spices and herbs for volatile compounds indicates that menthol, citral, *p*-menthone and methyl cinnamate were found in higher concentrations in mint. The highest concentration of linalool was found in fenugreek, coriander, and basil. Anethol, alpha-copaene, eugenol, eucalyptol was measured in higher concentration in fennel (Figure 2A).

3.7. Distribution of Phytochemicals in Selected Herbs and Spices

The Venn diagram is a powerful tool to investigate the distribution of a diverse range of phytochemicals in various samples. We conducted Venn diagrams of volatile (A) and non-volatile phytochemicals (B) in basil (B), mint (M), fenugreek (F), fennel (Fe), and coriander (Co) (Figure 3).

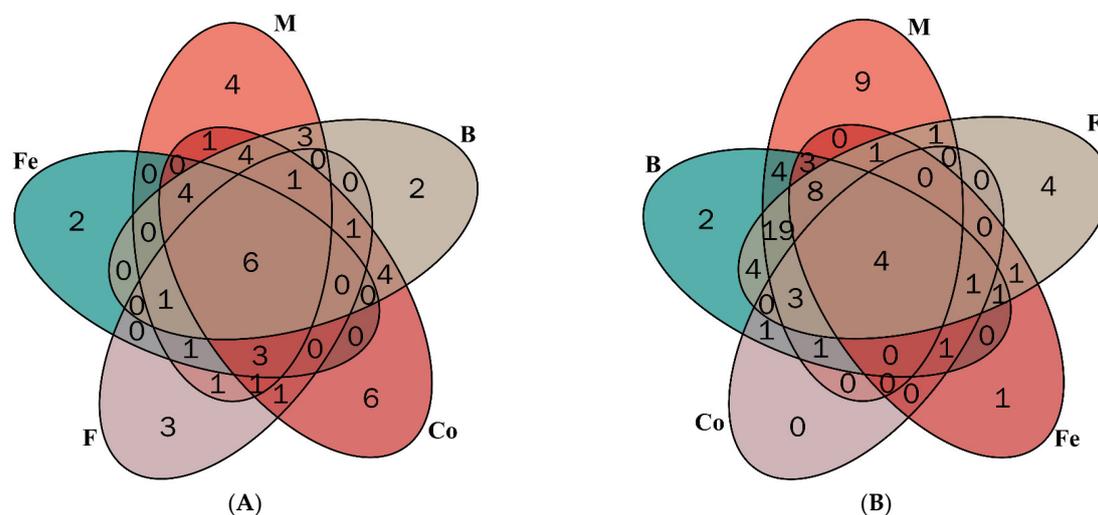


Figure 3. Distribution of volatile (A) and non-volatile phytochemicals (B) in basil (B), mint (M), fenugreek (F), fennel (Fe), and coriander (Co).

A total of six volatile compounds (eucalyptol, o-cymene, linalool, caryophyllene, anethole, and eugenol) were identified in all five herbs and spices (Figure 3A). Only two unique volatile compounds were identified in basil and fennel, while three, four and six unique compounds were identified in fenugreek, mint, and coriander, respectively. Four compounds (β -phellandrene, α -copaene, D-carvone and benzaldehyde, 4-methoxy) were overlapped in basil, mint, fennel, and coriander. Mint was shown to have the

greatest, most distinct, non-volatile chemical compounds (Figure 3B). One, two and four distinct non-volatile substances were identified in fennel, basil, and fenugreek, respectively. Four compounds (hesperidin, gallic acid, *p*-hydroxybenzoic acid, as well as ferulic acid) were identified in all these five herbal plants. The highest number of compounds (19) are overlapped in sweet basil, mint, and fenugreek.

3.8. Molecular Docking of Abundant Phenolic Compounds

The functions of various phenolic compounds in α -glucosidase inhibitory activities were predicted by employing in silico molecular docking. Using computational methods, such as molecular docking, it is possible to accurately forecast the affinities as well as modes of binding of a target molecule (or ligand) to a specific protein (or receptor). Figure 4A,B shows the predicted 2D and 3D binding geometries of chlorogenic acid (A) and punicafofin (B) in the α -glucosidase protein (5NN8), respectively. The calculated binding energies are given in Table S3.

Punicafofin was docked to the protein structure of 5NN8. During the molecular docking process, punicafofin was placed in the binding site of 5NN8 and allowed to interact with the surrounding amino acid residues. PHE 649 is considered to be a hydrophobic amino acid. Punicafofin also established hydrophobic bonds with the amino acid residues, such as PHE 525, ALA 284, and TRP 481. Chlorogenic acid (Figure 4A) created a single hydrogen bond with each of the ASP 404 as well as ASP 518, another one with a molecule of water, two hydrogen bonds with ASP 282, and one with the hydrophobic PHE 649, which refers to as π - π stacking with this same compound. Quercetin 3-(2-galloylglucoside) formed hydrogen bonds with water molecules, the positively charged ARG 600, the negatively charged ASP 518, the negatively charged ASP 282, ASP 616 (negatively charged), the negatively charged ASP 404, and two hydrophobic bonds with PHE 525 and ALA 284; and PHE 525, TRP 481, as well as PHE 649 connecting with Pi-Pi bonding. Myricitrin, in addition to quercitrin, formed one hydrogen bond association with hydrophobic ALA 284, negatively charged ASP 616, and EDO 1024, while two bonds of hydrogen with the negatively charged ASP 282. They possessed a single Pi-Pi binding with TRP 481 and a kind of double Pi-Pi stacking hydrophobically associated with PHE 525. Specifically, rutin formed four collective hydrogen bonds: one with ASP 404, two with ASP 616, and a final one with ASP 518. Additionally, it interacted with one amino acid residue, i.e., TRP 481, via Pi-Pi stacking. Twelve hydrogen bonds were formed by acarbose: two with ASP 404, ASP 518, SER 523, and ASP 282; it also forms three OH groups taken from molecules of water that additionally formed hydrogen bonds with ASP 281 as well as ASP 645. Naringin formed hydrogen bonding associations with EDO 1024, PHE 525, ASP 282, LEU 678, as well as ARG 281, along with one Pi-Pi stacking contact with TRP 481. Additionally, diosmin formed four hydrogen connections with ASP 282, which is negatively charged, and one with the similarly negatively charged ASP 616. The binding energies of punicafofin, rutin, acarbose, procyanidin B2, punicalagin, myricitrin, 3-feruloylquinic acid, taxifolin, diosmin, chlorogenic acid, quercetin-3-*O*-arabinoside, naringin, 3-*p*-coumaroylquinic acid, myricetin, apigenin 8-*C*-glucoside, quercetin, kaempferol, ellagic acid, carnosic acid, isorhamnetin, luteolin, hesperetin, (-)-epicatechin, gallic acid, protocatechuic acid, 3-*O*-sinapoylquinic acid, Isorhamnetin, caffeic acid, pyrogallol, *p*-hydroxybenzoic acid, *p*-coumaric acid and cinnamic acid in 5NN8 were calculated as -12.16, -11.14, -10.56, -10.05, -9.95, -9.59, -9.32, -9.13, -8.77, -8.17, -7.59, -7.58, -7.38, -6.92, -6.44, -6.01, -5.94, -5.91, -5.68, -5.67, -5.47, -5.36, -5.10, -4.96, -4.91, -4.89, -4.87, -4.80, -4.30, -3.80, and -3.04, respectively. Punicafofin is expected to have a higher binding affinity than the other chosen phenolic compounds. Rutin and procyanidin B2 have a stronger ability of α -glucosidase-inhibition than other non-anthocyanin flavonoids. It is interesting to note that 3-feruloylquinic acid has a greater affinity for binding than diosmin, taxifolin, quercetin, naringin, chlorogenic acid, myricetin, isorhamnetin, as well as luteolin. Comparatively, quercetin, hesperetin, myricetin, luteolin, and isorhamnetin, along with (-)-epicatechin, have lower binding affinities than 3-*p*-coumaroylquinic acid. The calculated glide energy

of myricitrin, punicafofin, rutin, chlorogenic acid, acarbose, procyanidin B2, punicalagin, 3-feruloylquinic acid, diosmin, quercetin-3-*O*-arabinoside, naringin, 3-*p*-coumaroylquinic acid, myricetin, apigenin 8-*C*-glucoside, quercetin, kaempferol, ellagic acid, carnosic acid, isorhamnetin, luteolin, hesperetin, (–)-epicatechin, protocatechuic acid, 3-*O*-sinapoylquinic acid, isorhamnetin, caffeic acid, pyrogallol, *p*-hydroxybenzoic acid, taxifolin, *p*-coumaric acid, gallic acid and cinnamic acid in 5NN8 were observed as 63.23, –71.81, –69.71, –58.68, –58.26, –56.13, –72.90, 59.38, –54.35, 51.48, –55.25, –46.44, –43.43, –40.31, –39.43, –33.74, –24.60, –32.63, –31.91, –36.15, –25.08, 31.65, –29.18, 30.62, –34.55, –30.58, –23.12, 22.92, –62.84, 21.38, –30.17 and 19.21 kcal/mol, respectively.

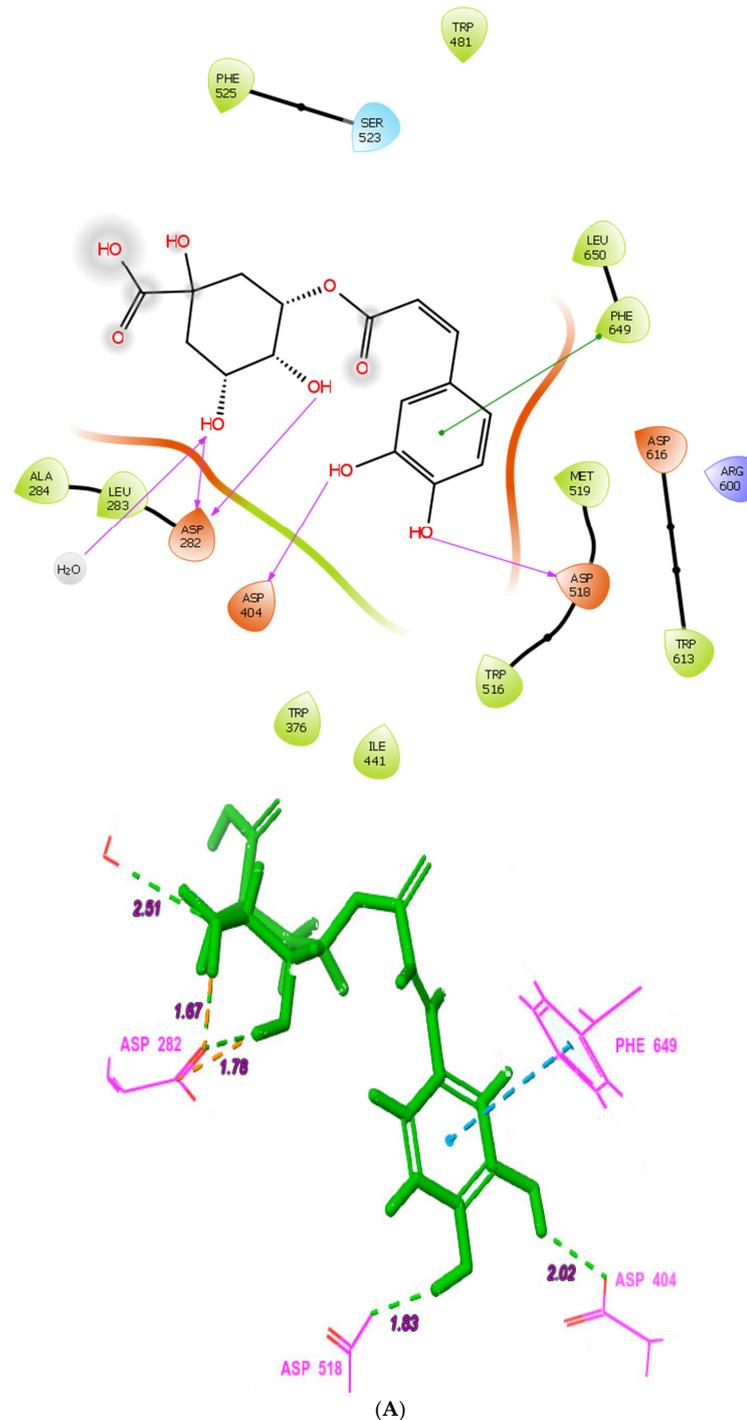


Figure 4. Cont.

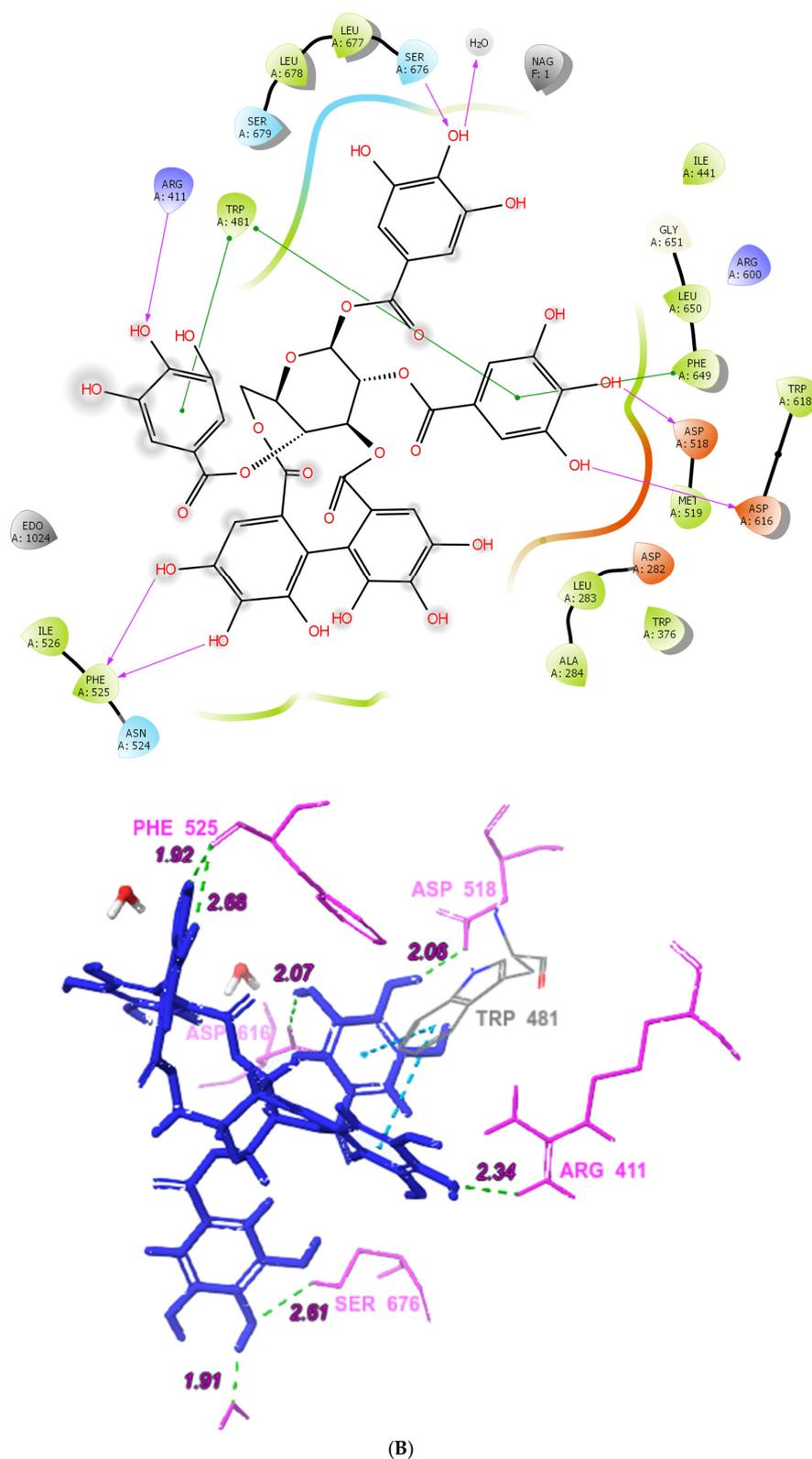


Figure 4. Molecular docking 2D and 3D view of chlorogenic acid (A) and punicafolin (B).

3.9. Chemometrics Analysis of Abundant Metabolites

Phytochemicals in selected herbs and spices were analyzed to locate their abundance and relationship with each other. Chemometrics analysis is given in Figure 5.

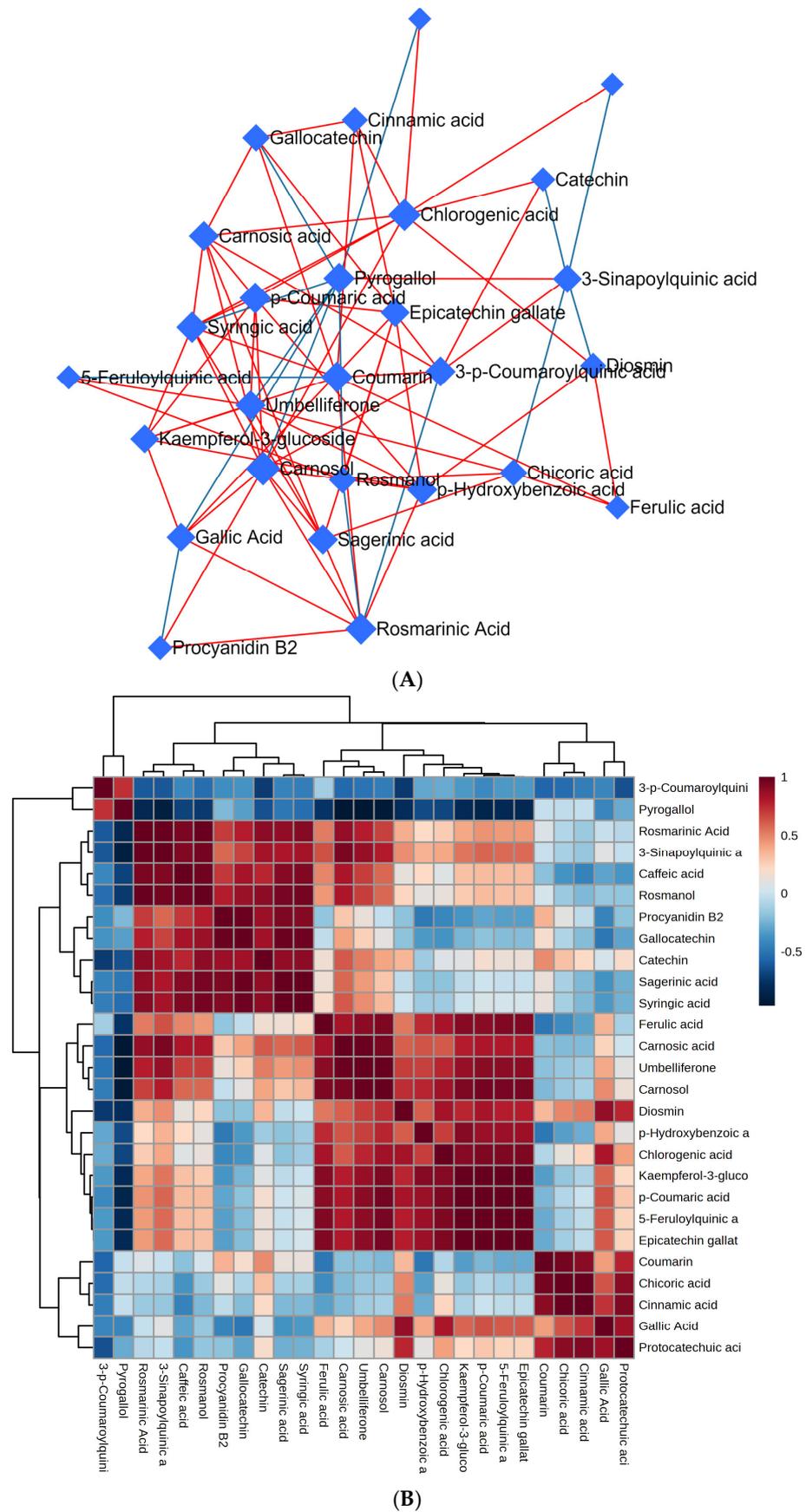
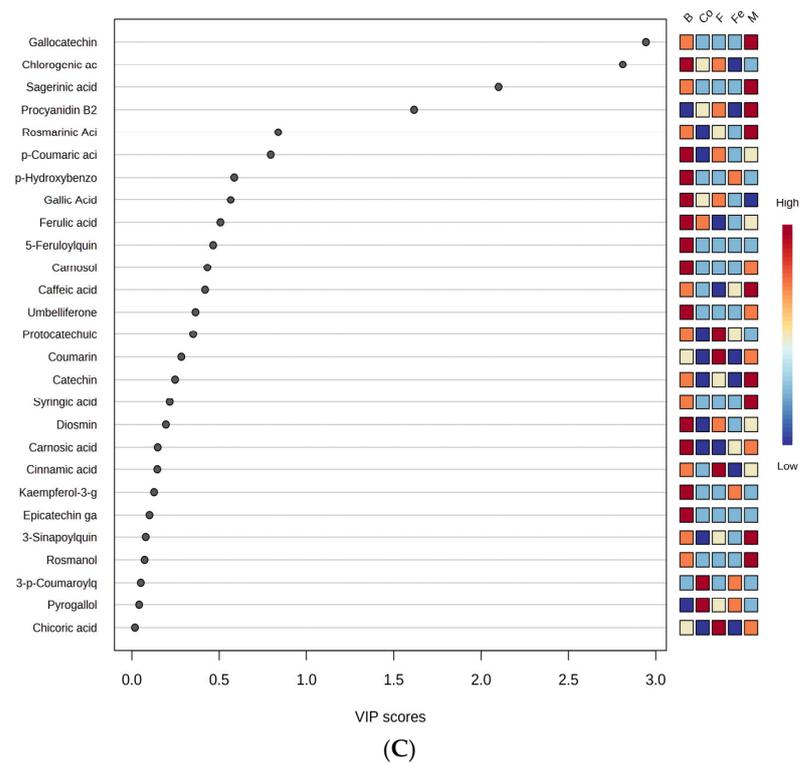


Figure 5. Cont.



Top 25 compounds correlated with the Rosmarinic Acid

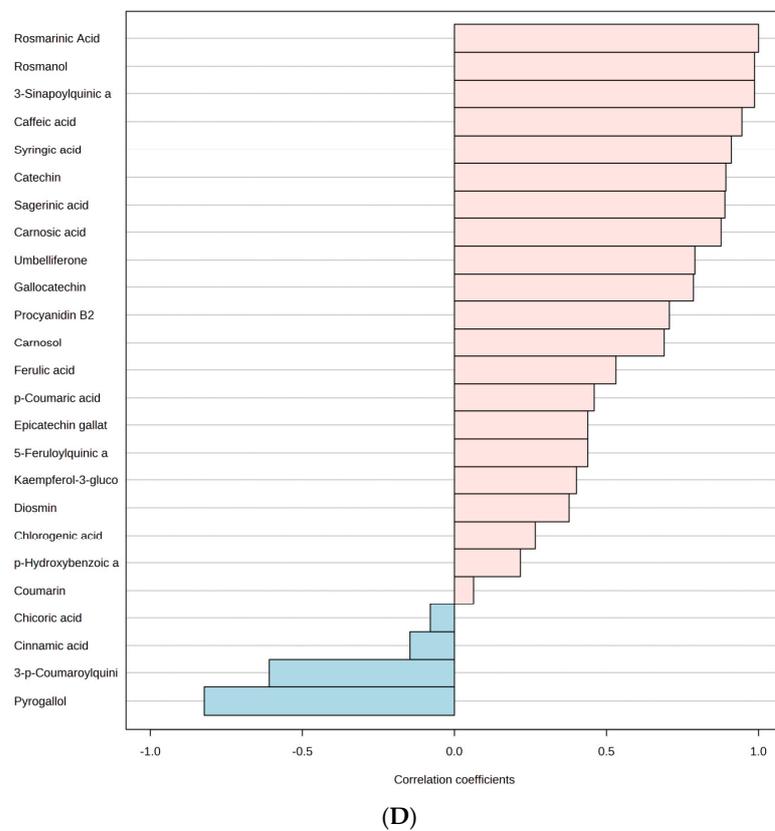


Figure 5. Chemometrics analysis of phytochemicals in selected herbs and spices, including basil (B), mint (M), fenugreek (F), fennel (Fe), and coriander (Co). Debiased Sparse Partial Correlation Network (A), Spearman's correlation heatmap (B), Partial Least Squares Discriminant Analysis VIP score (C), Correlation coefficients between rosmarinic acid (D).

Debiased Sparse Partial Correlation Network Figure 5A clearly indicates a strong networking of rosmarinic acid, sagerinic acid and carnosol with all other abundant phytochemicals. Spearman's correlation (Figure 5B) and correlation coefficients (Figure 5D) indicate that rosmarinic acid has the strongest interaction with 3-caffeoylquinic acid, caffeic acid, syringic acid, rosmanol and catechin. Figure 5C indicates the concentration and VIP score of individual phytochemicals in selected herbs and spices. Gallocatechin has the highest VIP score and is observed in higher concentrations in mint. It is observed that selected herbs and spices contain a diverse range of phytochemicals that are beneficial for human health.

4. Conclusions

These selected herbs and spices have a considerable concentration of phenolic contents, having predominant antioxidant and anti-diabetic potential. Moreover, these five tested herbs and spices have higher antioxidant activities, total phenolic, and total flavonoid content. A total of 71 phenolic compounds have been reported in our study. LC-ESI-QTOF-MS/MS was used for the identification and characterization of these phenolic compounds. We believe it is the first study that has been comprehensively conducted for metabolomic profiling of selected herbs and spices. The comprehensive screening and identification of polyphenols through LC-ESI-QTOF-MS/MS in selected herbs is a significant contribution of this work. Chlorogenic acid, *p*-hydrobenzoic acid, rosmarinic acid, sagerinic acid, and gallocatechin are supposed to be the most prevalent phenolic compounds. A novel steroidal saponin (diosgenin) was also identified in this study. Molecular docking helped us to clear that condensed tannins have greater anti-diabetic activity. These herbs and spices having higher phenolic compounds could be rich sources of antioxidant and anti-diabetic compounds than many other plants reported somewhere in other studies. A significant boost to human wellness as well as nutrient intake may come from the widespread use of these herbs and spices. Herbs are recommended for use in the human and animal diet, as well as in the pharmaceutical and nutraceutical industries, because of their extensive anti-radical characteristics. Awareness should be carried out for its bioavailability and use for therapeutic and nutritional values. The studies on *in vivo* bioavailability and bioaccessibility must be directed to industrialize the secondary metabolites. This study will help to explore these phytochemicals in drug discovery.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pr11092721/s1>, Figure S1: Total Ion Chromatograms (TIC) of fenugreek, mint, coriander, fennel, and basil using GC-MS are presented; Figure S2: Base peak chromatograms (BPC) of mint, fennel, basil, coriander, and fenugreek in positive and negative modes; Table S1: Quantification/Semi quantification of abundant phenolic compounds ($\mu\text{g/g}$); Table S2: Quantification/Semi quantification of abundant phenolic compounds ($\mu\text{g/g}$); Table S3: The calculated binding energies of selected compounds.

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