



A multi-platform based metabolic comparison of dill (*Anethum graveolens*) and fennel (*Foeniculum vulgare*) using NMR and UPLC–QTOF/MS

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Abstract

Fennel and dill are well-known aromatic plants and are widely used commercially. They have also been recognized as a medicinal herb with numerous pharmacological activities. Thus, the interest in dill and fennel has been growing, and their industrial use is becoming more important. In this study, seeds of fennel and dill were extracted and analyzed using nuclear magnetic resonance (NMR) spectroscopy and ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC–QTOF/MS)-based metabolomics. Through 1D and 2D NMR analysis, a total of 34 primary metabolites were identified, and using UPLC–QTOF/MS, different chromatograms of dill and fennel were obtained in less than 20 min. In the multivariate statistical analysis of data obtained from NMR and MS, it was confirmed that seeds of dill and fennel discriminated clearly. Sugars and organic acids were identified as the primary metabolites contributing to significant differentiation of fennel and dill. Also, flavonoids, which were identified as secondary metabolites, significantly differentiated dill and fennel.

Keywords Metabolomics · NMR spectroscopy · Fennel · Dill · UPLC–QTOF/MS

Abbreviations

NMR	Nuclear magnetic resonance spectroscopy	RMSEE	Root mean squared error of estimation
OPLS-DA	Orthogonal partial least squares discriminant analysis	RMSEP	Root mean squared error of prediction
PCA	Principal component analysis	RT	Retention time
PLS-DA	Partial least squares discriminant	UPLC–QTOF/MS	Ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry
		VIP score	Variable importance projection score

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Introduction

Metabolomics is a research field that identifies the changes and causes of life phenomena through metabolomic analysis that occurs under various genetic, physiological and environmental conditions. Metabolomics is also considered a powerful tool in various fields, including medicines (Wishart 2016), food (Utpott et al. 2022) and environmental toxicological monitoring (Bedia 2022). This is no exception in plant metabolomics, and many things such as discrimination

of year (Yoon et al. 2019), geographical origin (Yoon et al. 2022) and species (Eisenmann et al. 2016) have become possible through metabolomics. Through metabolomics, a comprehensive qualitative and quantitative description of metabolites can be achieved.

NMR and MS are the most widely used instruments for metabolite analysis, and their unique strengths greatly contribute to metabolite analysis. NMR has high reproducibility and simple sample preparation, making it suitable for metabolite analysis (Beckonert et al. 2007, 2010). NMR allows rapid and selective analysis, but has the disadvantages of relatively low sensitivity (Sumner et al. 2003). Among the MS technology, the advantages of TOF-MS are high resolution and high mass accuracy, which makes it possible to simultaneously identify hundreds of different compounds (Deng et al. 2011). However, this system has limitations due to its poor reproducibility and sample destructive nature (Wu et al. 2021). In addition, combined with separation equipment such as UPLC enable differentiation of isomer, but analysis time is relatively long (Sumner et al. 2003).

With the development of analytical instruments such as NMR, MS and GC, research on plant metabolomics has taken off, but there are limitations in understanding plant metabolomics in depth using a single instrument. Therefore, in recent studies, multi-platform metabolomics studies using two or more instruments, rather than single instrument analysis, are being conducted, and this approach could be helpful in identifying and understanding metabolites.

Fennel (*Foeniculum vulgare*), which belongs to the Umbelliferae family, and its essential oil has been used as a remedy for carminative, digestive, lactagogue and diuretic purposes (Krizman et al. 2007). In many studies, various pharmacological activities of fennel, including anticancer (Ke et al. 2021), anti-inflammatory (Ozbek 2005), anticarcinogenic (Zaahkoui Semir et al. 2015), anti-stress and antioxidant (Koppula and Kumar 2013) and memory-strengthening effects (Joshi and Parle 2006), have been reported. Fennel has small, yellow flowers in umbels. The seeds ripen from September to October. The fruit of fennel is oval shaped dried seed. It has 3 to 5 vertical lines and its ends are slightly curved and blunt (Anka et al. 2020; Badgajar et al. 2014). Dill (*Anethum graveolens*) is a plant belonging to the Umbelliferae family as well, and it has a unique scent. Dill's unique scent comes from its fruits. The flowers of dill are yellow, arranged in umbels, and produce dried ripe fruits. The seeds are brown, flattened and over-shaped, and three vertical ridges on its back. Also, three dark lines between them, and two lines on the flat surface (Chahal et al. 2017). It has been used for centuries as an antimicrobial, anticonvulsant and antiemetic treatment (Stanojevic et al. 2015; Naseri et al. 2012). Dill is also known to have

numerous pharmacological effects such as antimicrobial (Badar et al. 2008), hyperlipidemic (Sadeghi et al. 2022), antioxidant (Kazemi 2015) and anti-diabetic activities (Haidari et al. 2020). In addition, studies have reported that dill extracts have an effect on the female and male reproductive systems in rats (Monsefi et al. 2006; Malihezaman and Sara 2007).

Fennel and dill are widely used as aromatic and medicinal herbs to treat or alleviate various ailments, and are also commonly used in various fields such as the cosmetic, food and even pharmaceutical industries due to the unique odor of their essential oils. Fennel and dill are edible plants, and their various pharmacological activities have been reported, so they are expected to be developed as promising materials in terms of industrialization.

For this reason, studies have been reported aimed at understanding the characteristics of dill and fennel, and in particular, studies have been conducted to analyze the composition of dill and fennel by considering variables such as their different parts (Hao et al. 2021), cultivation conditions (Orhan et al. 2013) and stage of maturation (Telci et al. 2009). Although there are studies on chemical analysis using instruments such as liquid chromatography, NMR and MS, most studies have been performed using GC or GC-MS due to the characteristics of the volatile oils known to be abundant in dill and fennel (Xiao et al. 2017; Stanojevic et al. 2015; Hao et al. 2021; Diaz-Maroto et al. 2005). Compared to GC analysis, NMR and MS are easier to prepare the analytical sample, and the high resolution of MS combined with LC and high reproducibility of NMR have advantages in metabolomics analysis. Performing multiple metabolite analyses using NMR and MS can help overcome their respective shortcomings and provide better metabolomics spectrum.

Dill and fennel are most commonly cultivated spice plants and belong to the same Umbelliferae family, but they belong to different species, and this difference may also affect the production of metabolites. Furthermore, these differences in metabolites may be related to their biological activities, thus it is necessary to evaluate the characteristics of metabolites in dill and fennel. As a result, in order to broaden its utility and properly utilize it, it is necessary to establish a chemical basis for distinguishing fennel from dill. Understanding of the differences between fennel and dill is important for their proper use and industrial application, but studies on this is insufficient. Therefore, the main purpose of this study was to understand their differences through a comprehensive metabolomics approach.

Materials and methods

Plant materials

Dried dill and fennel seeds were purchased from the market. Each seed with a different origin was purchased from different suppliers. The origins of the dill were China, France, the Netherlands, Italy, and Indonesia. The dill from the Netherlands was purchased from three different suppliers. Fennel originated in India, Korea, Italy, and China, and fennel from Italy was purchased from different suppliers. The seeds used in the experiment were used as non-disintegrated. All samples were grounded and homogenized to fine powder before extraction.

UPLC-QTOF/MS analyses

For the UPLC-QTOF/MS analysis, 100 mg of fine powder of dill and fennel seeds was weighed and suspended in 1 mL of solvent (70% EtOH) and vortexed at 2000 rpm for 30 min at room temperature in an Eppendorf thermomixer (Eppendorf, Westbury, NY, USA). A supernatant was obtained from the samples after centrifugation (13,500× g, 5 min at 4 °C) and diluted 20 times. All diluted samples were filtered through a syringe filter (PTFE, 0.2 µm). UPLC analysis performed using a Waters ACQUITY H-CLASS UPLC (Waters Corp., Milford, MA, USA) with a Acquity HSS C18 column (2.1 mm × 100 mm; 1.8 µm). The mobile phase was composed with solvent A (water) and solvent B (acetonitrile). The flow rate was 0.2 mL/min and the injection volume was 2 µL. The elution conditions were as follows: 0–3 min, 5–10% of B; 3–6 min, 10–20% of B; 6–9 min, 20–40% of B; 9–12 min, 40–60% of B; 12–15 min, 60–100% of B; 15–17 min, 100–5% of B. The temperature of the column oven and sample tray were maintained at 40 and 4 °C, respectively. MS analysis was performed using a Waters Xevo G2-S QTOF MS (Waters corp.), and analysis was conducted in negative ion mode. Data from mass spectrometers were acquired in the MSE acquisition mode performing alternative high- and low-energy scans. The operating MS parameters were set as follows: Source temperature: 120 °C; desolvation temperature: 550 °C; cone gas flow: 30 L/h; gas flow: 800 L/h; capillary voltage: 2.2 kV; cone voltage: 40 V. Leucine encephalin (m/z, 554.262) was used as an internal reference to obtain accurate mass measurements. Also, MS data were acquired through Masslynx 4.1 software (Waters Corp., Milford, MA, USA).

NMR data acquisition and data processing

For the NMR analysis, polar metabolites in seeds of fennel and dill were extracted using solvent of 50% methanol

(v/v). Samples were vortexed for 5 min and then extracted ultrasonically for 30 min. After centrifugation, a supernatant of the extract was filtered using a 0.22 µm PVDF membrane filter. The solvent of the filtered extract was eliminated using a Speedvac (EYELA, Tokyo, Japan). After elimination of the solvent, 560 µL of deuterated sodium phosphate buffer containing 2 mM of 3-(trimethylsilyl)-propionic-2,2,3,3-d₄ (TSP-d₄) was added to dissolve the metabolites for the NMR experiment. TSP-d₄ was used for calibration of the chemical shift (δ 0.00) and quantification of the metabolites. For the NMR measurement, samples were transferred to a 5 mm NMR tube. Samples were measured using a Bruker Avance 700 spectrometer (Bruker Biospin, Rheinstetten, Germany) with a cryogenic triple-resonance probe at a frequency of 700.40 MHz for ¹H and a temperature of 298 K. One-dimensional (1D) ¹H-NMR spectra were recorded with a pulse sequence of Carr–Purcell–Meiboom–Gill (Bruker; cpmgpr1d) for suppression of high molecular weight metabolites and water signals, 64 scans, relaxation delay of 2 s, and acquisition time of 1.802 s. The data of two-dimensional (2D) ¹H–¹H correlation spectroscopy (COSY) and ¹H–¹³C heteronuclear single quantum coherence spectroscopy (HSQC-DEPT) were acquired to confirm the identification of metabolites. The phase and baseline of NMR spectra were manually corrected with TOPSPIN (4.1.0; Bruker Biospin, Rheinstetten, Germany). Metabolites were identified and quantified using Chenomx NMR Suite 8.4 Professional (Chenomx Inc, Canada) with the metabolite library database and 2D data.

Data analysis and multivariate analysis

For multivariate statistical analysis of the data obtained through UPLC-QTOF/MS analysis, data peak acquisition and metabolite alignment were performed using Progenesis QI software (Waters Co.). All data collected through TOF/MS were processed into data including ion intensity, m/z and retention time through a series of processes: Chromatographic peak alignment, peak picking, normalization and compound identification. All NMR spectra were binned using Chenomx NMR Suite 8.4 Professional for the multivariate statistical analyses. The data obtained through NMR analysis were processed. The binning area of spectra was from 0.5 to 10 ppm with a binning size of 0.001 ppm and normalization was performed to the total area. The binning data were aligned using an icoshift algorithm of MATLAB (The MathWorks, Natick, MA, USA). SIMCA 15.0.2 software (Umetrics, Umeå, Sweden) for the multivariate statistical analysis of the processed data and binning results. Principal component analysis (PCA) was conducted to show the distribution of unsupervised samples. Partial least squares discriminant analysis (PLS-DA) and orthogonal

partial least squares discriminant analysis (OPLS-DA) were performed to show group clustering. Univariate and multivariate statistical analyses of metabolites were performed using MetaboAnalyst 5.0 (<https://www.metaboanalyst.ca>).

Results

NMR based primary metabolites analysis

In this study, NMR spectroscopy was used to compare the differences in the primary metabolites of fennel and dill. In order to identify as many primary metabolites as possible, two-dimensional ^1H – ^1H correlation spectroscopy (COSY) ^1H – ^{13}C heteronuclear single quantum coherence spectroscopy (HSQC) NMR spectra, as well as one-dimensional proton NMR spectra, were acquired from fennel and dill (Fig. S1). A total of 34 primary metabolites obtained using NMR were identified, including amino and organic acids. These metabolites are annotated in the NMR spectra of Fig. 1. In this study, an untargeted metabolomics approach was performed; however, it is difficult to directly interpret the results for data acquired in this way. Therefore, multivariate analysis was employed to visualize significant information from the data.

In the partial least squares discriminant analysis (PLS-DA), the plots were separated respectively according to their

group ($R^2\text{X}=0.585$, $R^2\text{Y}=0.965$, $Q^2=0.888$) (Fig. 2A). To verify the over-fitting of the PLS model, 200 permutations were performed (Fig. 2B). As a result of the permutation tests, the Q^2 and R^2 values of the original model were higher than all of the permuted models; therefore, it was determined that the PLS model was valid. The root mean squared error of estimation (RMSEE) and the root mean squared error of prediction (RMSEP) were used to assess the performance validation of the PLS model (Fig. 2C, D). In the Y-prediction plot, the RMSEE and RMSEP were 0.103 and 0.028, respectively. These results indicate significant statistical differences in the metabolites of fennel and dill. In order to show the metabolites contributing to the differences between fennel and dill, the S-line plot of OPLS-DA was analyzed (Fig. 3).

The fennel extract contained a greater amount of sugars, including glucose and sucrose, and amino acids, including valine, isoleucine, threonine and alanine. Meanwhile, the dill extract contained a greater amount of organic acids, such as acetate, malate and citrate. In addition, the dill extract also contained more glycerophosphocholine and betaine. Univariate and multivariate statistical analyses of the quantified metabolites were also conducted. A variable importance in projection (VIP) score plot was obtained (Fig. 4). Components with a VIP score of 1.5 or higher have important meaning in variation. In the VIP score plot, citrate, adenosine, betaine, arginine and glycerophosphocholine were the

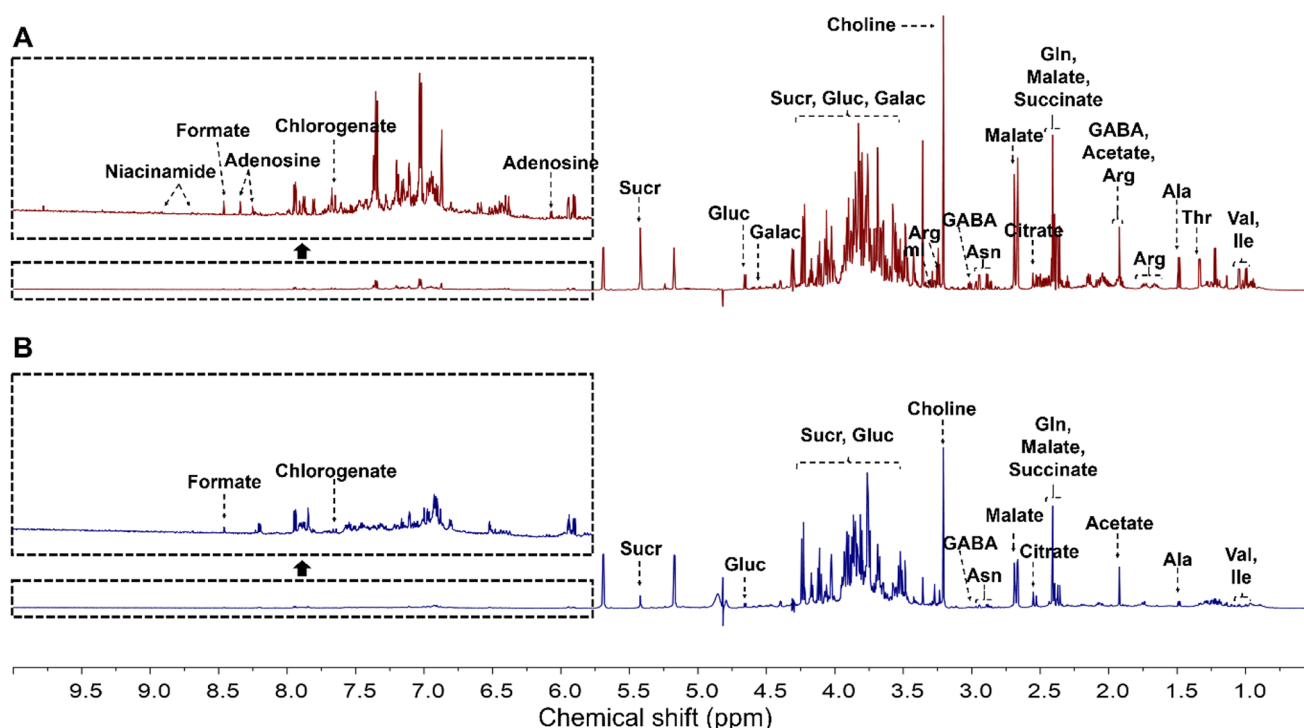


Fig. 1 Representative ^1H -NMR spectra of (A) fennel and (B) dill with annotation. [Ala: alanine, Arg: arginine, Asn: asparagine, GABA: 4-aminobutyrate, Galac: galactose, Gln: glutamine, Gluc: glucose, Ile: isoleucine, ml: myo-inositol, Sucr: sucrose, Thr: threonine, Val: valine]

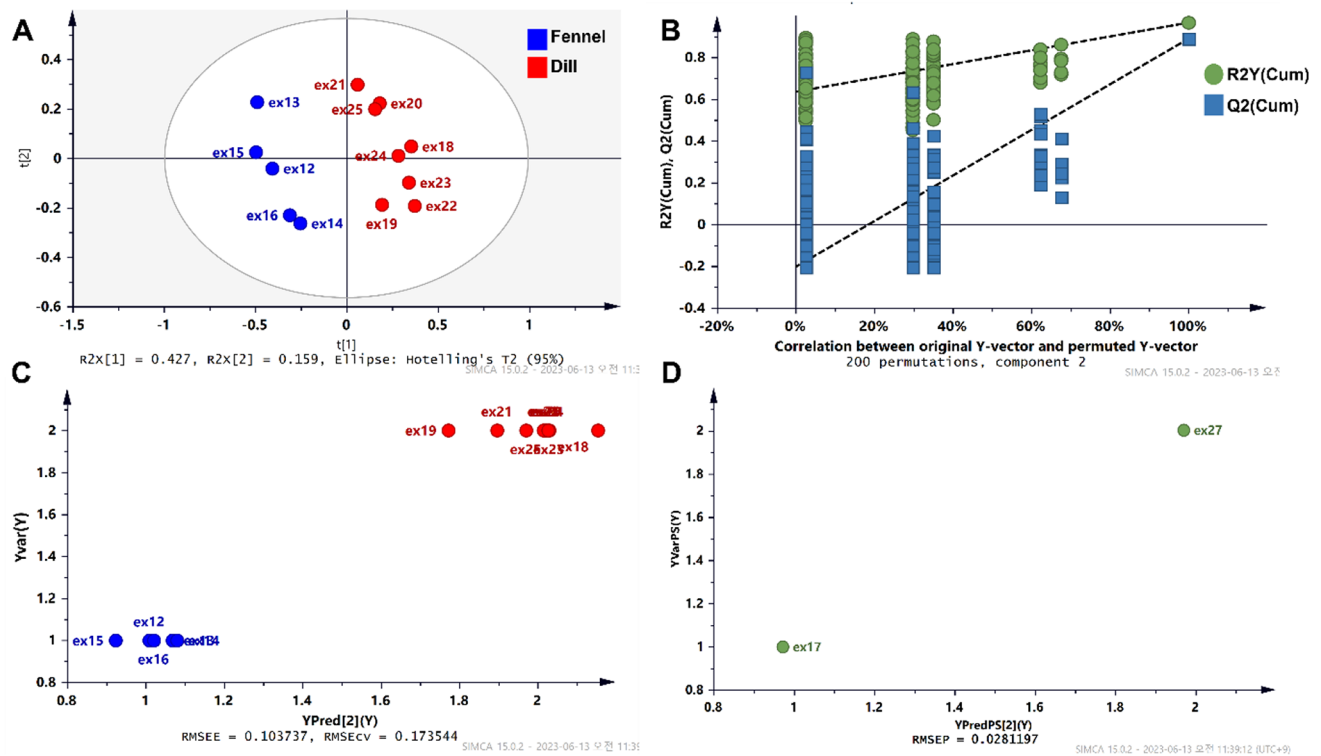


Fig. 2 Multivariate statistical analyses of NMR spectra from fennel and dill. (A) PLS-DA score plot ($R^2X=0.585$, $R^2Y=0.965$, $Q^2=0.888$), (B) two hundred permutation tests on the PLS model, (C) RMSEE on the PLS model and (D) RMSEP on the PLS model

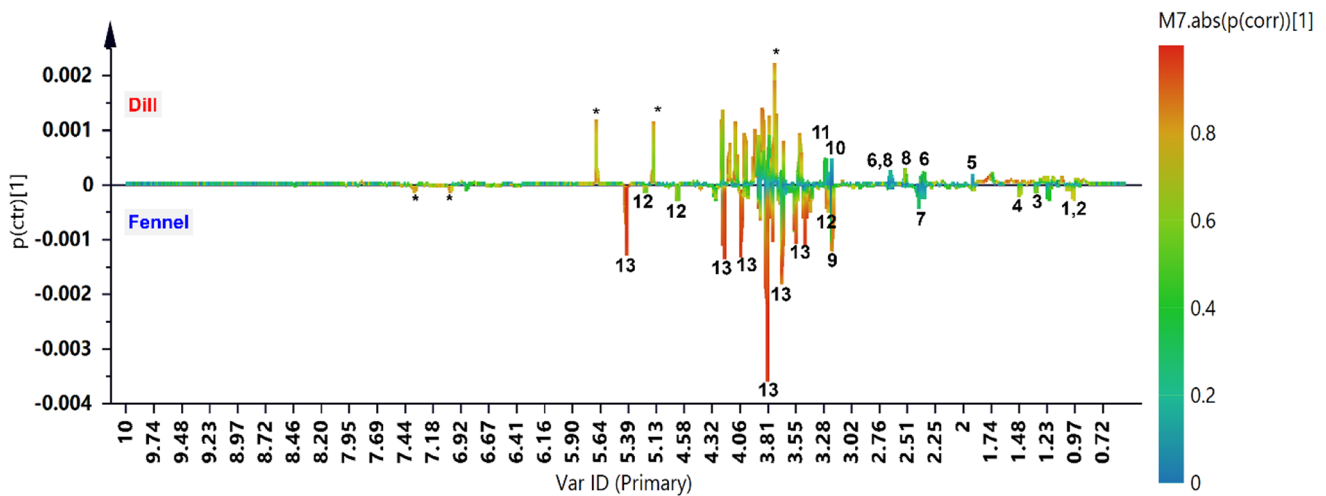
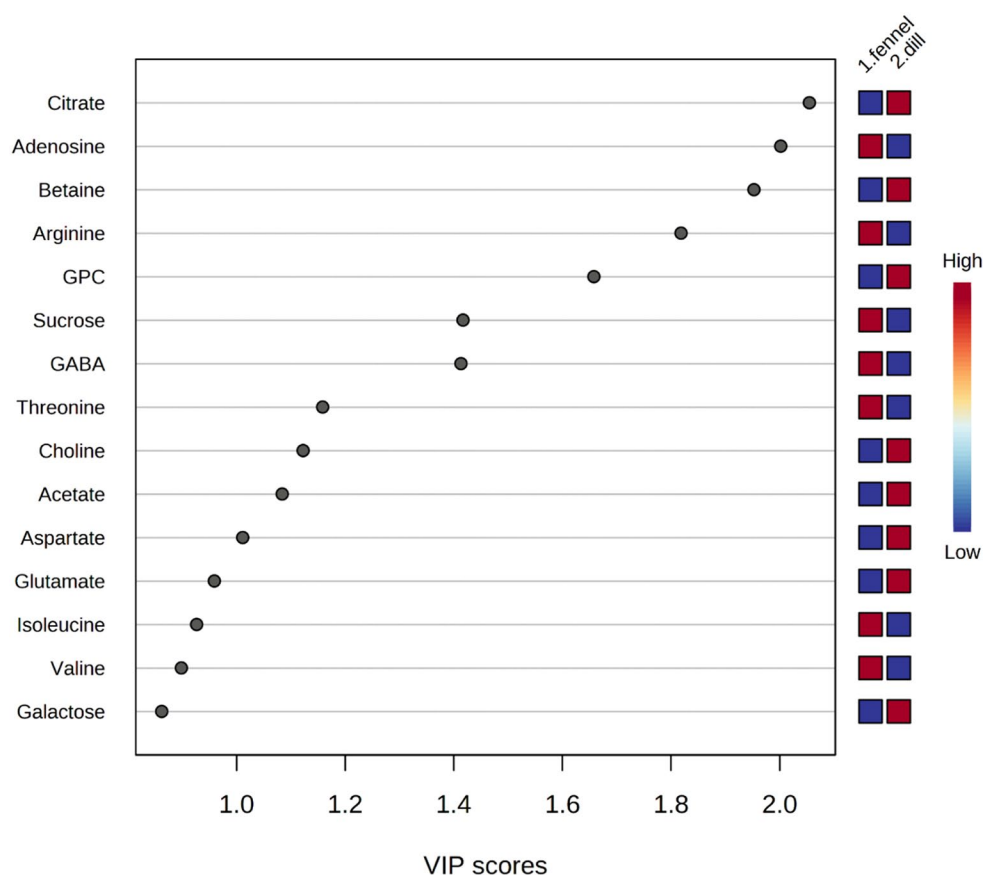


Fig. 3 S-line plot from the OPLS-DA score plot from samples of dill and fennel based on the NMR data. Positive resonances are correlated to dill and negative resonances are correlated to fennel with annotation. Color of the line indicates the significance of correlation. [1,

valine; 2, iso-leucine; 3, threonine; 4, alanine; 5, acetate; 6, malate; 7, succinate; 8, citrate; 9, choline; 10, GPC; 11, betaine; 12, glucose; 13, sucrose; * unknown]

Fig. 4 Variable importance in projection (VIP) plot of the 15 most important primary metabolites in the PLS-DA score plot based on NMR data from fennel and dill using MetaboAnalyst



best markers to discriminate dill and fennel. As in the S-line plot, citrate, betaine and glycerophosphocholine were identified as important metabolites of dill in the VIP score plot, and sucrose, which was identified to have significant meaning of fennel in the S-line plot, also showed a VIP score of over 1.2. The VIP score plot indicates that the two components showed discrimination potential.

UPLC-QTOF/MS based primary metabolites analysis

UPLC-QTOF/MS analysis was performed to analyze the secondary metabolites of fennel and dill. The negative ion mode had a better ionization efficiency than the positive ion mode, so it was considered suitable for secondary metabolite profiling of dill and fennel seeds. The chromatograms obtained from negative ion mode are represented in supplementary data (Fig. S2).

The secondary metabolites of fennel and dill were compared by multivariate statistical analysis of chromatograms obtained by UPLC-QTOF/MS (Fig. 5). In the PCA score plot, dill and fennel were separated according to the group ($R^2X=0.823$, $Q^2=0.492$). Additionally, in the PLS-DA score plot, fennel and dill were discriminated without overlapping ($R^2X=0.576$, $R^2Y=0.986$, $Q^2=0.943$) (Fig. 5A, B).

The S-plot obtained through the OPLS-DA score plot ($R^2X=0.632$, $R^2Y=0.996$, $Q^2=0.959$) was analyzed to identify the metabolites that differentiate fennel from dill (Fig. 5C). Variables with high p-values (corr) in the S-plot have significant meaning in distinguishing between two different groups (Fig. 5D). In the S-plot, the metabolites with RT and m/z values of 6.24 and 563.1410, respectively, were confirmed as the most significant metabolites in Dill. Meanwhile, the metabolites with RT and m/z values of 9.4 and 680.2053, respectively, were identified as the most significant metabolites in fennel. Table 1 shows the RT and m/z values of the metabolites with significant p-values in the S-plot, including the most significant in fennel and dill. To identify the significant secondary metabolites that distinguish between dill and fennel more clearly, statistical analysis was performed on 1000 metabolites with high scores in the PLS-DA-based VIP score plot (Fig. 6). Figure 6A shows the correlation matrix of these 1000 metabolites. In the correlation analysis, the columns of the heat map represent the secondary metabolites acquired from fennel and dill, also, colors ranging from blue to red indicate a concentration of metabolites; the closer to red, the greater the concentration of metabolites, indicating a positive correlation, while closer to blue represents the opposite. Fennel and dill showed distinct patterns in the metabolite correlation analysis. For the

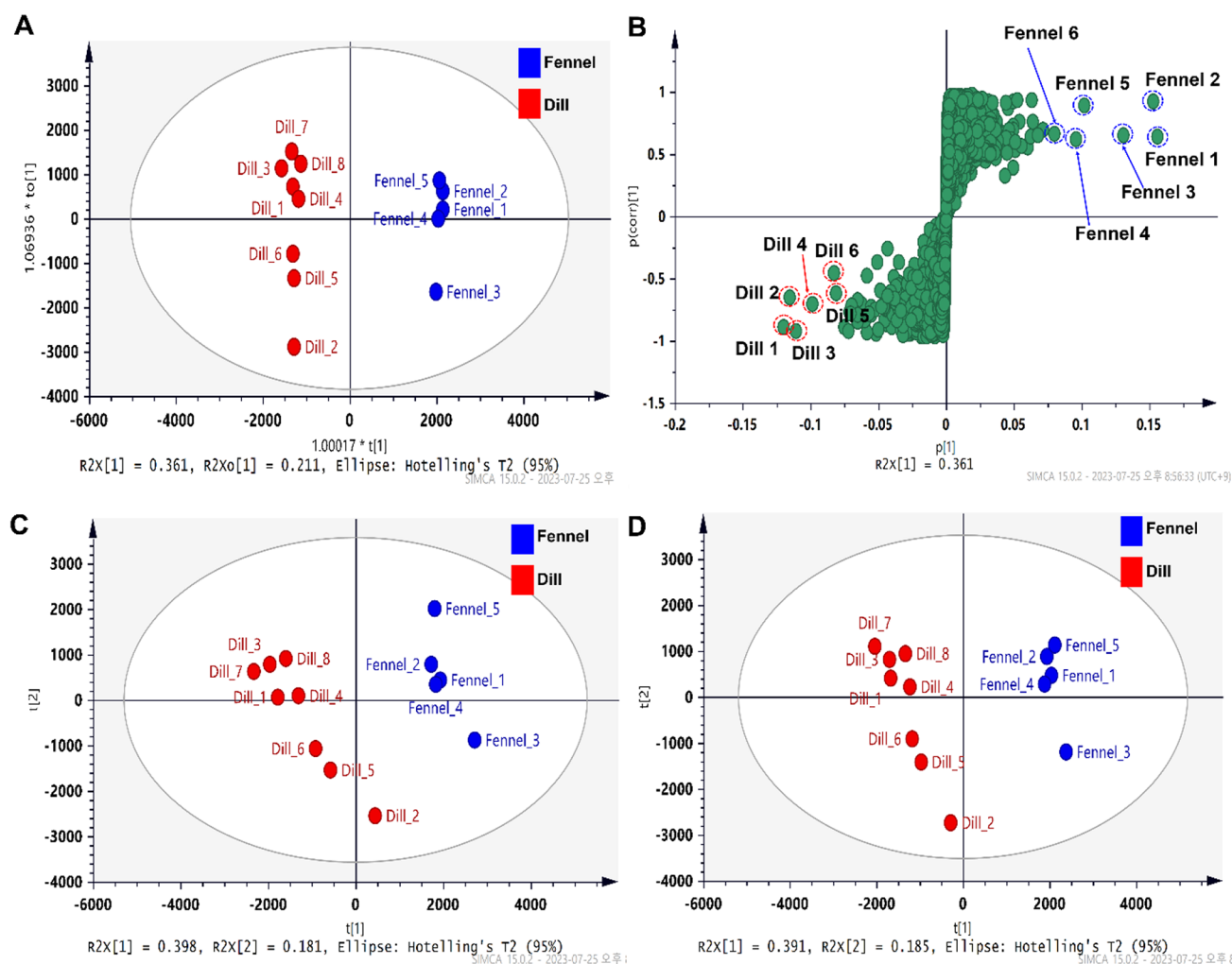


Fig. 5 Multivariate statistical analyses of UPLC-QTOF/MS data from fennel and dill. (A) PCA score plot ($R^2X=0.823$, $Q^2=0.492$), (B) PLS-DA score plot ($R^2X=0.576$, $R^2Y=0.986$, $Q^2=0.943$), (C) OPLS-DA score plot ($R^2X=0.632$, $R^2Y=0.996$, $Q^2=0.959$) and (D) S-line plot

Table 1 Distinguished metabolites of dill and fennel identified by OPLS-DA based S-line plot, which is result of multivariate statistical analysis of secondary metabolites obtained through UPLC-QTOF/MS

Component	RT	Observed m/z	Component	RT	Observed m/z
Dill 1	6.24	563.1410 m/z	Fennel 1	9.94	680.2053n
Dill 2	5.84	461.0727 m/z	Fennel 2	0.79	342.1164n
Dill 3	6.74	470.1667 m/z	Fennel 3	5.51	477.0677 m/z
Dill 4	6.11	491.0833 m/z	Fennel 4	9.94	679.2842 m/z
Dill 5	0.77	1008.3372n	Fennel 5	0.79	684.2322n
Dill 6	0.77	504.1696n	Fennel 6	8.63	842.2571n

1000 significant metabolites, a VIP score plot was obtained. The VIP score plot for the top 20 metabolites is displayed in Fig. 6B. The RT and m/z values of these metabolites are also displayed. In the VIP score plot, higher values of metabolite in fennel and dill are displayed in red, and vice versa in blue. Half of the 20 metabolites were identified as significant metabolites in dill, whereas the other half showed significant meaning in fennel. Among the metabolites shown

to be significant in dill, the metabolite with RT and m/z values of 10.85 and 325.0719 showed the highest VIP score. Meanwhile, the RT and m/z values of the metabolite with the highest VIP score in fennel were identified as 6.85 and 605.6797, respectively.

In the VIP score plot obtained by sorting 1000 significant metabolites, the two metabolites with the highest scores in fennel were observed in previous studies, but the compounds were confirmed as “unknown.” Meanwhile, the metabolite that showed the highest VIP score in dill was confirmed to have the same molecular ion $[M-H]^-$ value as hexaoxaicosanediol in a previous study (Rashed et al. 2021).

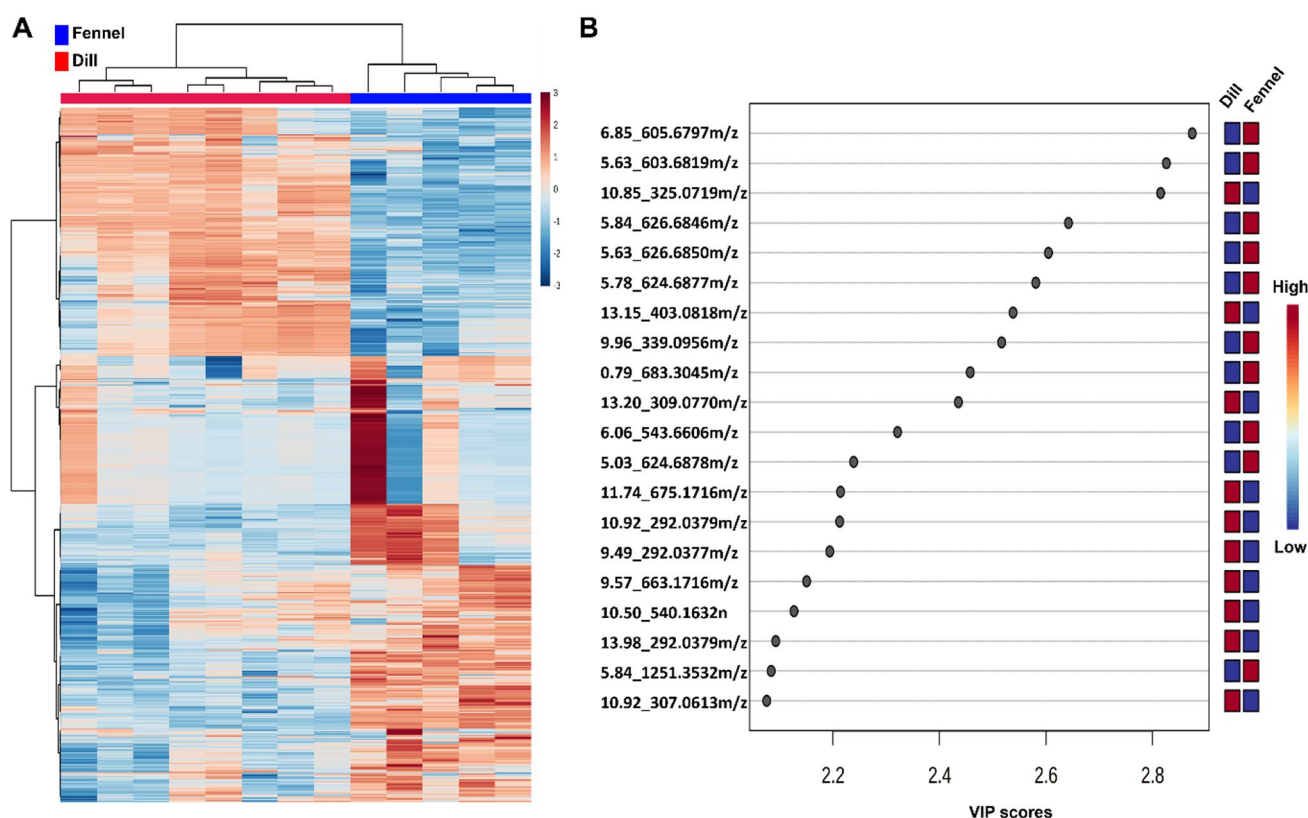


Fig. 6 Multivariate statistical analyses of 1000 metabolites with high scores in the PLS-DA-based VIP score plot for secondary metabolites of fennel and dill acquired by UPLC-QTOF/MS: (A) Correlation analysis and (B) VIP score plot of the 20 most important secondary metab-

olites. Each column in (A) indicates an observed secondary metabolite, color of the cell indicates the significance of correlation and the Y-axis of (B) represents the measured mass values and retention times of significant secondary metabolites

Discussion

In this study, multi-platform metabolomics was conducted to discriminate fennel and dill using UPLC-QTOF/MS and NMR spectroscopy. In the NMR analysis, sucrose and glucose were confirmed to be the significant metabolites in fennel. Moreover, this is consistent with a previous study reported by Cataldi et al., which reported that the main sugars in fennel are glucose and fructose (Cataldi et al. 1998). In plants, sucrose serves as an important energy source, osmotic solute and signaling molecule. Moreover, glucose is converted from sucrose. In a previous study on the nutritional composition of fennel, sugars were identified as glucose and fructose, and in particular, all parts of fennel showed high glucose content (Barros et al. 2010). The utilization of sucrose in plants is very important, and recent studies have focused on the roles of sugars and sugar polymers in determining seed vigor (Wang et al. 2018). Sucrose was identified as a significant primary metabolite in fennel, and previous studies have also reported that sucrose has the highest content among the carbohydrates in fennel (Pencheva et al. 2022).

On the contrary, organic and amino acids were confirmed as the important metabolites of dill. These two ingredients have a positive association in linking the spicy and bitter tastes of dill. A study on the nutritional composition of fennel found that the most abundant biochemical composition in fennel seeds of seven cultivars was sugar (Abdesslem et al. 2022). In a previous study by Castro-Alves et al., organic acids are known to significantly contribute to the sensory properties of dill. In a partial network analysis of the sensory characteristics of dill, amino acids appeared a positive correlation with the sour taste of dill, and organic acids showed a positive correlation with amino acids, pungency, and bitterness. Also, compared with outdoor grown dill, sensory quality of greenhouse grown dill was improved when it was exposed to eustress. The improvement of sensory quality of dill such as bitterness and pungency was related to the accumulation of organic acids and amino acids (Castro-Alves et al. 2021). Fennel and dill are considered important crops due to their unique aroma and taste, and metabolites with significant meaning in fennel and dill are also expected to affect sensory properties such as aroma.

In this UPLC-QTOF/MS-based metabolomics study, the secondary metabolites of fennel and dill also showed

significant differences. Although they are similar aromatic plants, it was confirmed that they are distinct due to obvious differences in metabolomics. In the multivariate statistical analysis of the data obtained via TOF/MS analysis, the RT and m/z values of the significant metabolites in fennel and dill were obtained. Among the significant metabolites of fennel identified in the S-plot, the metabolite with a molecular ion $[M-H]^-$ at m/z 477.067 was identified as quercetin-O-glucuronide (Crescenzi et al. 2021, 2022). In addition, metabolites contributing to distinguishing between dill and fennel were identified through S-Plot analysis. The metabolites with a molecular ion $[M-H]^-$ at m/z 461.0727 and a molecular ion $[M-H]^-$ at m/z 491.0833 were identified as kaempferol-3-O-glucuronide and iso-rhamnetin-3-O-glucuronide, respectively (El-Zaedi et al. 2017). These secondary metabolites identified in fennel and dill are classified as flavonoids. Flavonoids are secondary metabolites and they assist in the transmission of pollen or seeds to plants by giving them color, flavor and aroma (Kose and Quattrocchio 1994). Flavonoids consist of two phenyl rings connected to a heterocyclic ring. Quercetin and kaempferol have the same backbone (3-hydroxy flavone backbone) and the only difference between quercetin and kaempferol is that quercetin has an additional hydroxyl group at the C3' position in ring B (Kumar and Pandey 2013; Sharma et al. 2018). Quercetin is synthesized from kaempferol, and this flavonoid hydroxylation step is performed by cytochrome P450 reductase (CPR) and flavonoid monooxygenase (FMO) (Stahlhut et al. 2015; Kubina et al. 2021). Quercetin not only promotes plant physiological processes such as seed germination and pollen growth, but also induces proper growth of plants. It also acts as a powerful antioxidant, providing plant tolerance against environmental stress by strengthening several physiological functions (Singh et al. 2021). Additionally, a previous study showed that quercetin supplementation can improve seed germination and vigor under osmotic stress (Yang et al. 2021).

Quercetin, a flavonoid compound, has been confirmed as a major component of fennel in a previous study, and in the study, fennel seed extract showed high antioxidant and antimicrobial activity (Salami et al. 2016). In addition, a previous study that isolated 12 compounds from fennel waste also reported that isolation of quercetin-O-glucuronide, which was identified in this experiment. And these compounds showed powerful radical scavenging activity (Parejo et al. 2004). According to study by Crescenzi, in terms of COX enzyme inhibitory activity of the main metabolites of fennel, quercetin has anti-inflammatory activity that can inhibit COX-2 enzymes more than other metabolites (Crescenzi et al. 2022). Additionally, a study on the extraction method of fennel seeds indicated that quercetin glucuronide had a higher content than other quercetin compounds regardless

of the extraction method (Malin et al. 2022). These research results could help interpret the various pharmacological effects of fennel and indicate its potential as a natural pharmaceutical ingredient.

In a previous study by Giordano et al. that investigated the flavonoid content of four types of microgreens, including dill, it was confirmed that the content of kaempferol-3-dihexoside in dill was higher than that of luteoline or quercetin (Giordano et al. 2022). Also, a previous study using water and ethanol extracts of dill showed that dill extract inhibited the interaction between SARS-CoV-2's S-protein and ACE2 receptor. In that study, among the 10 compounds identified in dill extract, the relative ionic strength of kaempferol glucuronide had a higher relative ion intensity, which was compatible with the results of our study (Choe et al. 2023). Additionally, in a previous study that confirmed the content of flavonoids and phenolic compounds in dill extract, kaempferol showed higher content than apigenin and quercetin when acids were excluded, and showed antimicrobial activity against food-borne microorganisms (Mashraqi 2023). These results seem to be related to the traditional use of dill.

Dill seed extract has been confirmed to cause infertility in previous studies using animal models. This appears to be because kaempferol, the main component of dill, has phytoestrogenic properties that promote cell growth, differentiation, and general homeostasis of reproductive and other (Monsefi et al. 2014). Kaempferol is also known to act as a powerful antioxidant in plants by removing the reactive oxygen species generated by biotic and abiotic stresses (Mierziak et al. 2014). In addition, previous study on interaction and stability between dill-derived compounds and target proteins involved in antimicrobial and antioxidant activities reported that kaempferol exhibited better binding energies with the proteins (Hadi et al. 2024). However, it is known that the kaempferol overaccumulation caused by flavonoid 3' hydroxylase tt7 mutants disrupt the outer integument differentiation of seed coat and diminishes seed longevity (Ninoles et al. 2022). Therefore, it can be seen that kaempferol is an important factor in seed longevity and development.

In our study, kaempferol and quercetin were identified as important components in dill and fennel, respectively, and previous studies have reported that they are closely related to the pharmacological actions of dill and fennel (Crescenzi et al. 2022; Monsefi et al. 2014; Hadi et al. 2024). Some studies on the composition and content of dill and fennel differed from ours (Malin et al. 2022; Choe et al. 2023), but this appears to be due to differences in methods such as the variety of ingredients used in the experiment and the extraction method. As a result, it seems necessary to conduct

additional studies on the flavonoid compounds, which are the main components of dill and fennel, identified in our study.

Conclusion

Fennel and dill, which are representative aromatic herb belonging to the Umbelliferae family, are thought to have different chemical compositions because they belong to different gens and species. In this study, we identified differences in the primary and secondary metabolites of fennel and dill using NMR and UPLC-QTOF/MS. The differences in their chemical composition are considered to also affect their physiological activity results. Therefore, the metabolomics research used in this study is expected to provide new values about the components of fennel and dill and be helpful for their appropriate industrial use.

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Author contributions DYL conceived and designed the experiments. B-RC and DY. wrote the paper. J-KJ contributed to the plant material preparation. DY performed the NMR experiments, B-RC and W-CS performed UPLC-QTOF/MS experiments. DYL reviewed and edited the paper, and SL man-aged the research project. All authors helped prepare the paper and approved the final version.

Declarations

Conflict of interest All authors confirm no conflict of interest.

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References

Abdesslem SB, Elbaz M, Boulares M, Moussa OB, Timoumi M, Hassouna M (2022) Value adding search among a selection of

- Tunisian fennel (*Foeniculum vulgare* Mill.) Cultivars: nutritional composition, chlorophyll and β -carotene contents of fennel seeds. *J OASIS AGRIC SUSTAIN DEV* 4(2):110–116. <https://doi.org/10.56027/JOASD.spiss152022>
- Anka ZM, Gimba S, Nanda A, Salisu L (2020) Phytochemistry and pharmacological activities of *Foeniculum vulgare*. *IOSR J Pharm* 10(1):1–10
- Badar N, Arshad M, Farooq U (2008) Characteristics of *Anethum graveolens* (Umbelliferae) seed oil: extraction, composition and antimicrobial activity. *Int J Agric Biol* 10(3):329–332
- Badgular SB, Patel VV, Bandivdekar AH (2014) *Foeniculum vulgare* Mill.: a review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. *Biomed Res Int* 2014(1):842674. <https://doi.org/10.1155/2014/842674>
- Barros L, Carvalho AM, Ferreira IC (2010) The nutritional composition of fennel (*Foeniculum vulgare*): shoots, leaves, stems and inflorescences. *LWT-FOOD SCI TECHNOL* 43(5):814–818. <https://doi.org/10.1016/j.lwt.2010.01.010>
- Beckonert O, Keun HC, Ebbels TM, Bundy J, Holmes E, Lindon JC, Nicholson JK (2007) Metabolic profiling, metabolomic and metabolomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. *Nat Protoc* 2(11):2692. <https://doi.org/10.1038/nprot.2007.376>
- Beckonert O, Coen M, Keun HC, Wang Y, Ebbels TM, Holmes E, Lindon JC, Nicholson JK (2010) High-resolution magic-angle-spinning NMR spectroscopy for metabolic profiling of intact tissues. *Nat Protoc* 5(6):1019–1032. <https://doi.org/10.1038/nprot.2010.45>
- Bedia C (2022) Metabolomics in environmental toxicology: Applications and challenges. *Trends Environ Anal Chem*. <https://doi.org/10.1016/j.teac.2022.e00161>
- Castro-Alves V, Kalbina I, Nilsen A, Aronsson M, Rosenqvist E, Jansen MAK, Qian M, Ostrom A, Hyotylainen T (2021) Integration of non-target metabolomics and sensory analysis unravels vegetable plant metabolite signatures associated with sensory quality: a case study using dill (*Anethum graveolens*). *Food Chem*. <https://doi.org/10.1016/j.foodchem.2020.128714>
- Cataldi TRI, Margiotta G, Zamboni CG (1998) Determination of sugars and alditols in food samples by HPAEC with integrated pulsed amperometric detection using alkaline eluents containing barium or strontium ions. *Food Chem* 62(1):109–115. [https://doi.org/10.1016/S0308-8146\(97\)00154-4](https://doi.org/10.1016/S0308-8146(97)00154-4)
- Chahal KK, Kumar A, Bhardwaj U, Kaur R (2017) Chemistry and biological activities of *Anethum graveolens* L.(dill) essential oil: a review. *J Pharmacogn Phytochem* 6(2):295–306
- Choe U, Li Y, Liu Z, Yao Y, He X, Sun J, Wu X, Pehrsson P, Zhang Y, Gao B, Wang TTY, Yu LL (2023) Chemical compositions of Dill (*Anethum graveolens* L.) water and ethanol extracts and their potential in blocking the SARS-CoV-2 entry into cells and free radical scavenging capacities. *ACS Food Sci Technol* 3(10):1654–1662. <https://doi.org/10.1021/acsfoodscitech.3c00206>
- Crescenzi MA, D'Urso G, Piacente S, Montoro P (2021) LC-ESI/LTQ-Orbitrap/MS metabolomic analysis of fennel waste (*Foeniculum vulgare* Mill.) As a byproduct rich in bioactive compounds. *Foods* 10(8):1893. <https://doi.org/10.3390/foods10081893>
- Crescenzi MA, D'Urso G, Piacente S, Montoro P (2022) UPLC-ESI-QTRAP-MS/MS analysis to quantify bioactive compounds in fennel (*Foeniculum vulgare* Mill.) Waste with potential anti-inflammatory activity. *Metabolites* 12(8):701. <https://doi.org/10.3390/metabo12080701>
- Deng XJ, Yang HQ, Li JZ, Song Y, Guo DH, Luo Y, Du XN, Bo T (2011) Multiclass residues screening of 105 veterinary drugs in meat, milk, and egg using ultra high performance liquid chromatography tandem quadrupole time-of-flight mass spectrometry. *J*

- Liq Chromatogr Relat Technol 34(19):2286–2303. <https://doi.org/10.1080/10826076.2011.587224>
- Diaz-Maroto MC, Diaz-Maroto HIJ, Sanchez-Palomo E, Perez-Coello MS (2005) Volatile components and key odorants of fennel (*Foeniculum vulgare* Mill.) And Thyme (*Thymus vulgaris* L.) oil extracts obtained by simultaneous distillation–extraction and supercritical fluid extraction. *J Agric Food Chem* 53(13):5385–5389. <https://doi.org/10.1021/jf050340+>
- Eisenmann P, Ehlers M, Weinert CH, Tzvetkova P, Silber M, Rist MJ, Luy B, Muhle-Goll C (2016) Untargeted NMR spectroscopic analysis of the metabolic variety of new apple cultivars. *Metabolites* 6(3):29. <https://doi.org/10.3390/metabo6030029>
- El-Zaedi H, Calin-Sanchez A, Nowicka P, Martinez-Tome J, Noguera-Artiaga L, Burlo F, Wojdylo A, Car-bonell-Barrachina AA (2017) Preharvest treatments with malic, oxalic, and acetylsalicylic acids affect the phenolic composition and antioxidant capacity of coriander, dill and parsley. *Food Chem* 226:179–186. <https://doi.org/10.1016/j.foodchem.2017.01.067>
- Giordano M, Petropoulos SA, Kyriacou MC, Graziani G, Zarrelli A, Rouphael Y, El-Nakhel C (2022) Nutritive and Phytochemical Composition of Aromatic Microgreen Herbs and spices belonging to the Apiaceae Family. *Plants* 11(22):3057. <https://doi.org/10.3390/plants11223057>
- Hadi N, Drioiche A, Bouchra EM, Baammi S, Abdelaziz Shahat A, Tagnaout I, Radi M, Remok F, Bouzoubaa A, Zair T (2024) Phytochemical analysis and evaluation of antioxidant and Antimicrobial properties of essential oils and seed extracts of Anethum graveolens from Southern Morocco: in Vitro and in Silico Approach for a natural alternative to synthetic preservatives. *Pharmaceuticals* 17(7):862. <https://doi.org/10.3390/ph17070862>
- Haidari F, Zakerkish M, Borazjani F, Ahmadi Angali K, Amoochi Foroushani G (2020) The effects of Anethum graveolens (dill) powder supplementation on clinical and metabolic status in patients with type 2 diabetes. *Trials* 21:1–11. <https://doi.org/10.1186/s13063-020-04401-3>
- Hao Y, Kang J, Guo X, Yang R, Chen Y, Li J, Shi L (2021) Comparison of nutritional compositions and essential oil profiles of different parts of dill and two fennel cultivars. *Foods* 10(8):1784. <https://doi.org/10.3390/foods10081784>
- Joshi H, Parle M (2006) Cholinergic basis of memory-strengthening effect of *Foeniculum vulgare* Linn. *J Med Food* 9(3):413–417. <https://doi.org/10.1089/jmf.2006.9.413>
- Kazemi M (2015) Phenolic profile, antioxidant capacity and anti-inflammatory activity of Anethum graveolens L. essential oil. *Nat Prod Res* 29(6):551–553. <https://doi.org/10.1080/14786419.2014.951934>
- Ke W, Wang H, Zhao X, Lu Z (2021) *Foeniculum vulgare* seed extract exerts anti-cancer effects on hepatocellular carcinoma. *Food Funct* 12(4):1482–1497. <https://doi.org/10.1039/D0FO02243H>
- Koppula S, Kumar H (2013) *Foeniculum vulgare* Mill (Umbelliferae) attenuates stress and improves memory in Wister rats. *Trop J Pharm Res* 12(4):553–558. <https://doi.org/10.4314/tjpr.v12i4.17>
- Kose RE, Quattrocchio F (1994) The flavonoid biosynthetic pathway in plants: function and evolution. *BioEssays* 16(2):123–132. <https://doi.org/10.1002/bies.950160209>
- Krizman M, Baricevic D, Prosek M (2007) Determination of phenolic compounds in fennel by HPLC and HPLC-MS using a monolithic reversed-phase column. *J Pharm Biomed Anal* 43(2):481–485. <https://doi.org/10.1016/j.jpba.2006.07.029>
- Kubina R, Iriti M, Kabala-Dzik A (2021) Anticancer potential of selected flavonols: Fisetin, keampferol, and quercetin on head and neck cancers. *Nutrients* 13:845. <https://doi.org/10.3390/nu13030845>
- Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. *Sci World J* 2013:162750. <https://doi.org/10.1155/2013/162750>
- Malihezaman M, Sara P (2007) Effects of aqueous extract of Anethum graveolens on male reproductive system of rats. *J Biol Sci* 7(5):815–818
- Malin V, Elez Garofulic I, Repajic M, Zoric Z, Pedisic S, Sternisa M, Smole Mozina S, Dragovic-Uzelac V (2022) Phenolic characterization and Bioactivity of Fennel seed (*Foeniculum vulgare* Mill.) Extracts isolated by microwave-assisted and conventional extraction. *Processes* 10(3):510. <https://doi.org/10.3390/pr10030510>
- Mashraqi A (2023) Induction role of chitosan nanoparticles to Anethum graveolens extract against food-borne bacteria, oxidant, and diabetic activities in vitro. *Front Microbiol* 14:1209524
- Mierziak J, Kosyn K, Kulma A (2014) Flavonoids as important molecules of plant interactions with the environment. *Molecules* 19(10):16240–16265. <https://doi.org/10.3390/molecules191016240>
- Monsefi M, Ghasemi M, Bahaoddini A (2006) The effects of Anethum graveolens L. on female reproductive system. *Phytother Res* 20(10):865–868. <https://doi.org/10.1002/ptr.1959>
- Monsefi M, Lohrasbi P, Abpaikar Z, Bakhtiari S (2014) Anti-implantation and anti-fertility potentials of Anethum graveolens L. extracts in rats. *Toxicol Environ Chem* 96(9):1402–1413. <https://doi.org/10.1080/02772248.2015.1027204>
- Naseri M, Mojab F, Khodadoost M, Kamalinejad M, Davati A, Choopani R, Hasheminejad A, Bararpour Z, Sharia-tapanahi S, Emtiazy M (2012) The study of anti-inflammatory activity of oil-based dill (*Anethum graveolens* L.) extract used topically in formalin-induced inflammation male rat paw. *Iran J Pharm Res* 11(4):1169–1174
- Ninoles R, Arjona P, Hashim A, Bueso E, Serrano R, Molina I, Gadea J (2022) Kaempferol over-accumulation in the flavonoid 3' hydroxylase tt7 mutant disrupts seed coat outer integument differentiation and compromises seed longevity. *bioRxiv* 2022(03):483417. <https://doi.org/10.1101/2022.03.08.483417>
- Orhan IE, Senol FS, Ozturk N, Celik SA, Pulur A, Kan Y (2013) Phytochemical contents and enzyme inhibitory and antioxidant properties of Anethum graveolens L. (dill) samples cultivated under organic and conventional agricultural conditions. *Food Chem Toxicol* 59:96–103. <https://doi.org/10.1016/j.fct.2013.05.053>
- Ozbek H (2005) The anti-inflammatory activity of the *Foeniculum vulgare* L. essential oil and investigation. *Int J Pharmacol* 50(4):329–331
- Parejo I, Viladomat F, Bastida J, Schmeda-Hirschmann G, Burillo J, Codina C (2004) Bioguided isolation and identification of the nonvolatile antioxidant compounds from fennel (*Foeniculum vulgare* Mill.) Waste. *J Agric Food Chem* 52(7):1890–1897. <https://doi.org/10.1021/jf030717g>
- Pencheva M, Petkova Z, Dincheva I, Kostova I, Damyanova S, Stoyanova A, Gaceu L (2022) Phytochemical and biological profiles of fennel fruits (*Foeniculum vulgare* Mill. Var. Dulce Mill). *Carpathian J Food Sci Technol* 14(4):28–49. <https://doi.org/10.3430/2/crpfst/2022.14.4.3>
- Rashed DM, Emad AM, Ali SF, Ali SS, Farag MA, Meselhy MR, Satter EA (2021) UPLC-PDA-ESI/MS metabolic profiling of dill shoots bioactive fraction; evidence of its antioxidant and hepatoprotective effects in vitro and in vivo. *J Food Biochem* 45(6):e13741. <https://doi.org/10.1111/jfbc.13741>
- Sadeghi M, Kabiri S, Amerizadeh A, Heshmat-Gahdarijani K, Masoumi G, Teimouri-Jervekani Z, Amirpour A (2022) Anethum graveolens L.(dill) effect on human lipid profile: an updated systematic review. *Curr Probl Cardiol* 47(11):01072. <https://doi.org/10.1016/j.cpcardiol.2021.101072>
- Salami M, Rahimmalek M, Ehteman MH (2016) Inhibitory effect of different fennel (*Foeniculum vulgare*) samples and their phenolic compounds on formation of advanced glycation products and comparison of antimicrobial and antioxidant activities. *Food*

- Chem 213:196–205. <https://doi.org/10.1016/j.foodchem.2016.06.070>
- Sharma A, Sharma P, Tuli HS, Sharma AK (2018) Phytochemical and pharmacological properties of flavonols. *eLS* 2018:1–12. <https://doi.org/10.1002/9780470015902.a0027666>
- Singh P, Arif Y, Bajguz A, Hayat S (2021) The role of quercetin in plants. *Plant Physiol Biochem* 166:10–19. <https://doi.org/10.1016/j.plaphy.2021.05.023>
- Stahlhut SG, Siedler S, Malla S, Harrison SJ, Maury J, Neves AR, Forste J (2015) Assembly of a novel biosynthetic pathway for production of the plant flavonoid fisetin in *Escherichia coli*. *Metab Eng* 31:84–93. <https://doi.org/10.1016/j.ymben.2015.07.002>
- Stanojevic LP, Radulovic NS, Djokic TM, Stankovic BM, Ilic DP, Cakic MD, Nikolic VD (2015) The yield, composition and hydrodistillation kinetics of the essential oil of dill seeds. *Ind Crops Prod* 65:429–436. <https://doi.org/10.1016/j.indcrop.2014.10.067>
- Sumner LW, Mendes P, Dixon RA (2003) Plant metabolomics: large-scale phytochemistry in the functional genomics era. *Phytochemistry* 62(6):817–836. [https://doi.org/10.1016/S0031-9422\(02\)00708-2](https://doi.org/10.1016/S0031-9422(02)00708-2)
- Telci I, Demirtas I, Sahin A (2009) Variation in plant properties and essential oil composition of sweet fennel (*Foeniculum vulgare* Mill.) Fruits during stages of maturity. *Ind Crops Prod* 30(1):126–130. <https://doi.org/10.1016/j.indcrop.2009.02.010>
- Utpott M, Rodrigues E, de Oliveira Rios A, Mercali GD, Flores SH (2022) Metabolomics: an analytical technique for food processing evaluation. *Food Chem* 366:130685. <https://doi.org/10.1016/j.foodchem.2021.130685>
- Wang L, Patrick JW, Ruan YL (2018) Live long and prosper: roles of sugar and sugar polymers in seed vigor. *Mol Plant* 11(1):1–3
- Wishart DS (2016) Emerging applications of metabolomics in drug discovery and precision medicine. *Nat Rev Drug Discov* 15(7):473–484. <https://doi.org/10.1038/nrd.2016.32>
- Wu X, Liu Y, Guo J, Wang J, Li M, Tan Y, Zheng Q, Feng Y (2021) Differentiating Pu-erh raw tea from different geographical origins by ¹H-NMR and U-HPLC/Q-TOF-MS combined with chemometrics. *J Food Sci* 86(3):779–791. <https://doi.org/10.1111/1750-3841.15624>
- Xiao Z, Chen J, Niu Y, Chen F (2017) Characterization of the key odorants of fennel essential oils of different regions using GC–MS and GC–O combined with partial least squares regression. *J Chromatogr B* 1063:226–234. <https://doi.org/10.1016/j.jchromb.2017.07.053>
- Yang J, Zhang L, Jiang L, Zhan YG, Fan GZ (2021) Quercetin alleviates seed germination and growth inhibition in *Apocynum Venetum* and *Apocynum pictum* under mannitol-induced osmotic stress. *Plant Physiol Biochem* 159:268–276. <https://doi.org/10.1016/j.plaphy.2020.12.025>
- Yoon D, Choi BR, Ma S, Lee JW, Jo IH, Lee YS, Kim GS, Kim S, Lee DY (2019) Metabolomics for age discrimination of Ginseng using a Multiplex Approach to HR-MAS NMR spectroscopy, UPLC–QTOF/MS, and GC × GC–TOF/MS. *Molecules* 24(13):2381. <https://doi.org/10.3390/molecules24132381>
- Yoon D, Shin WC, Oh SM, Choi BR, Lee DY (2022) Metabolomics in environmental toxicology: integration of multiplatform metabolomics and multivariate analysis for geographical origin discrimination of *Panax ginseng*. *Food Res Int* 159:111610. <https://doi.org/10.1016/j.foodres.2022.111610>
- Zaahkouk Semir AM, Aboul-Ela EI, Ramadan MA, Bakry S, Mhany Ahmed BM (2015) Anti-carcinogenic activity of methanolic extract of fennel seeds (*Foeniculum vulgare*) against breast, colon, and liver cancer cells. *Int J Adv Res* 3(5):1525–1537

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