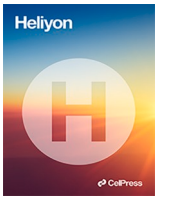
Heliyon 9 (2023) e21392

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Improved antioxidant activities of spice require enrichment of distinct yet closely-related metabolic pathways

Sonia Gandhi [a], Manas Ranjan Saha [b], Priyankar Dey [a,*]

[a] Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, Punjab, India
[b] Department of Bio-sciences, Habibpur Teachers Training College, Malda, West Bengal, India

ARTICLE INFO

Keywords:
Antioxidant
Apiaceae
GC-MS
Metabolic engineering
Metabolomics
Spice

ABSTRACT

Improved biosynthesis of commercially and pharmacologically relevant phytometabolites through genetic and metabolic engineering is a lucrative strategy for crop improvement. However, identifying appropriate biosynthetic pathways pertaining to specific bioactivities has been challenging since the major metabolic pathways remain closely interconnected. Here we propose a reverse association strategy in which, based on the phytochemical profile, putative target metabolic pathways could be identified for increased production of phytochemicals. Dried seed fruits of *Coriandrum sativum*, *Trachyspermum ammi*, *Cuminum cyminum,* and *Foeniculum vulgare* (family Apiaceae) were subjected to untargeted gas chromatography-mass spectrometry-based phytochemical profiling followed by evaluation of the overall antioxidant profile using multiple antioxidant assays. Using bioinformatics approaches, specific phytochemical classes and the enrichment of their respective biosynthetic pathways were identified. Collectively, the data suggest enrichment of isoprenoids and fatty acids biosynthetic pathways. The close association of metabolic pathways with antioxidant capacities indicated a need for enrichment of specific yet closely-related metabolic pathways to achieve an improved quality of spices for better antioxidant effects.

1. Introduction

The makeup of secondary metabolites, particularly aroma compounds in spice plants, are strongly associated with their therapeutic efficacy and are potential targets for crop improvement. Insight into the metabolic engineering of flavor compounds from spice plants through the identification of both genes and enzymes involved in the biosynthesis of spice flavors can be achieved. In order to identify specific genes or enzymes involved in the biosynthesis pathway or to modulate the aroma property to meet different practical demands of plant-derived products in the food, perfume, or medicine industries, research on flavor compounds of spice plants is primarily focused on their chemical characterization along with biosynthesis mechanisms. In addition to being an excellent source for traditional medicines, pharmaceuticals, nutraceuticals, aromatherapy, preservatives, drinks, natural colors, fragrances, cosmetics, and botanical insecticides, spice plant components are also usually regarded as safe [1]. Due to the high degree of structural diversity, terpenes, phenols, coumarins, flavonoids, and alkaloids are among the metabolites responsible for the majority of the health benefits associated with spices, including flavoring, antimicrobial, antioxidant, anticancer, anti-inflammatory, wound healing, skin penetration,

* Corresponding author.
E-mail addresses: soniagandhi0111@gmail.com (S. Gandhi), manas.bot@gmail.com (M.R. Saha), priyankar.dey@thapar.edu, priyankardey28@gmail.com (P. Dey).

https://doi.org/10.1016/j.heliyon.2023.e21392
Received 8 May 2023; Received in revised form 9 October 2023; Accepted 20 October 2023
Available online 21 October 2023

management of diabetes, insecticidal properties for plant disease, and antibiotic replacement in animal feed [2,3].

Since the phytochemical profile of plants is partly dependent on the biosynthetic pathways, metabolic and genetic engineering strategies have been employed to improve the yield of phytochemicals with pharmacological and industrial value. For instance, using a heterologous S-linalool synthase gene placed under the transcription regulation of the tomato late-ripening-specific E8 promoter, increased production of S-linalool and 8-hydroxylinalool and their accumulation in ripening tomato fruits were achieved [4]. In line, similar strategies are being employed to produce commercially viable phytochemicals in unrelated organisms, like the production of curcumin in engineered *E. coli* [5]. Two main approaches may be used in metabolic engineering to increase the production of natural products [6]. One, increasing the synthesis of an entire class of chemicals in food crops is frequently advantageous that potentially improves plant's capacity for environmental adaptation or increases the total nutritional content (e.g., antioxidant activities) of a food product. Two, targeting certain chemicals within a biosynthetic pathway is frequently required to boost the production of a single product for use in pharmaceutical or nutraceutical applications. Typical metabolic engineering strategies to achieve these outcomes include endogenous or foreign enzyme overexpression, transcription factor overexpression, gene silencing, genome editing, and co-overexpression [7]. However, for these strategies to be implemented, the identification of target biosynthetic pathways is a prerequisite to increase the yield of the desired compound(s), which is a challenging task since, due to the common carbon pool, most of the plant biosynthetic pathways are interconnected.

While many important plant natural products have complicated structures with numerous chiral centers, making chemical synthesis both challenging and economically unviable, some have simple structures and are easy to synthesize chemically. Therefore, these chemicals are frequently created using natural harvest, semi-synthesis, heterologous manufacture, or plant cell culture approaches to utilize native biological processes [6]. Alternatively, metabolic engineering could be employed in these biological systems to control flux via both primary and secondary metabolic pathways, directing carbon flow toward desired products. However, rather than targeting gene(s) for a specific industrially important phytochemical, enrichment of an entire biosynthetic pathway or co-occurring biosynthetic pathways are smarter strategies for producing economically important related phytochemicals [8]. For this purpose, co-occurring plant biosynthetic pathways attributed to the production of specific metabolites responsible for particular bioactivities need to be identified first.

Since bioactivities are dependent on the phytochemical profiles, whereas the phytochemical profiles are dictated by enrichments of plant metabolic pathways, in the present study, we propose a reductionist and reverse-association approach to associate the bioactivities with the metabolic pathway enrichments to identify putative phytometabolic pathways that could be potentially targeted through metabolic engineering. Gas chromatography-mass spectrometry (GC-MS)-based untargeted metabolomics was employed to identify volatile aroma compounds of the spice fruits of *Coriandrum sativum* L., *Trachyspermum ammi* (L.) Sprague ex Turrill, *Cuminum cyminum* L., and *Foeniculum vulgare* Mill. The choice of spice was based on the common plant family Apiaceae which is known to contain the healthiest fatty acid profile among all other spice families [9], for possessing potent antioxidant activities [10], and for its extensive use in traditional medicine with industry-relevant utilities [11]. Additionally, since phytochemical composition in different parts of the same plant may differ significantly, that likely contributes to the differential bioactivities of medicinal plants [12–14], we have utilized same plant part (*i.e.*, whole dried fruit composed of pericarp and the seed) to achieve homogeneity in the metabolomics data. Using similar approaches, previously, the metabolomic basis of preference for *Aloe vera* over closely-related other *Aloe* spp. for nutritional, therapeutic, and cosmetic purposes was identified [15]. In the present study, a panel of antioxidant and free-radical scavenging assays were performed to evaluate the bioactivities of the spice materials that served as correlative indicators of the phytometabolic pathway enrichments. Collectively, multiple clusters of enriched co-occurring metabolic pathways were identified, which could be potentially targeted through metabolic engineering approaches for the improvement of the overall antioxidant activities of spices belonging to the Apiaceae family.

2. Material and methods

2.1. Sample preparations

Spice materials were procured from commercial vendors, authenticated, and voucher specimen samples were preserved with accession no. MSC/BTD/22/CS (*C. sativum*), MSC/BTD/22/TA (*T. ammi*), MSC/BTD/22/CC (*C. cyminum*), and MSC/BTD/22/FV (*F. vulgare*). Methanolic extracts of the dried spice materials were prepared for antioxidant and free radical scavenging assays, as described previously [16]. For GC-MS analysis, the lyophilized spice extract (1 mg) was mixed with 1 mL *n*-hexane. Then, the mixture was incubated for 2-d at 4 °C with continuous shaking and centrifuged at 15,000 rpm for 20 min at 25 °C. The supernatant (200 μL) was dried under N_2 and then mixed with 20 μL of N, O-bis (trimethylsilyl) trifluoroacetamide + trimethylchlorosilane (99:1 v/v) mixture and incubated for 60 min at 25 °C with occasional vortex, and then sealed in autosampler vials with polytetrafluoroethylene cap using N_2 flushing.

2.2. Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS was performed following our prior standardized method for phytochemical analysis [14,17–19]. In brief, pooled extracts ($n = 3$) were analyzed using Thermo Scientific Trace 1300 gas chromatography instrument attached to Thermo Scientific ISQ QD single quadrupole mass spectrophotometer. The GC was equipped with TG-5MS column (30 m × 0.25 mm X 0.25 μm). The injector temperature was set at 250 °C, and the initial temperature of the program was set at 60 °C (solvent delay 5 min) with a hold of 4 min, followed by a ramp of 5 °C–290 °C with a hold of 10 min (60-min program). Derivatized samples (1 μL) were injected in a splitless

mode (split flow 50 mL/min) with a splitless time of 0.80 min, using a Thermo Scientific AI-1310 auto-sampler with a constant flow of helium gas (1 mL/min). MS transfer line temperature was set at 290 °C with an ion source temperature of 230 °C (electron ionization). The samples were analyzed at electron energy 70 eV (vacuum pressure: 2.21e-0.5 Torr), and the mass analyzer range was set to 50–650 amu. GC-MS data were analyzed using Thermo Xcalibur software version 2.2. MS data were analyzed using Automated Mass Spectral Deconvolution and Identification System (AMDIS) version 2.70. The major and essential compounds were identified by mass fragmentation patterns (m/z) of the reference parent compound (molecular peak and base peak) using MS Interpreter version 2.0 and by matching with the reference database of the National Institute Standard and Technology (NIST) with a MS Library V2011.

2.3. Free-radical scavenging assays

Based on the intracellular free-radical forming mechanisms, a total of 7 different free radical scavenging assays (i.e., hydroxyl radical, $OH^{\bullet}$; superoxide radical, $O_2^{\bullet-}$; singlet oxygen, $^1O_2$; hypochlorous acid, HOCl; hydrogen peroxide, H_2O_2; nitric oxide, NO; Peroxynitrite, $OONO^-$) were selected to assess the overall antioxidant activities of the spice samples [20]. The reducing potential of the spices as a surrogate indicator of the overall antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Since transition metals can accelerate intracellular free-radical formation cascade by potentiating the Fenton reaction, iron-chelation activity was measured, and to evaluate the potentials of the spices to limit free-radical mediated peroxidation of cellular lipid, lipid peroxidation assay was performed using chicken brain sample obtained from a local slaughterhouse. All assays were performed against appropriate standards as per previously standardized methods that were adapted to a reduced volume suitable for microplates [21–25]. The range of the highest dose of sample for each assay was based on the linear response range for respective standard compounds in the final volume of the reaction mixture.

2.4. Enrichment and pathway analysis

MetaboAnalyst V5 was used to analyze the biochemical pathway enrichment using the metabolite abundance data sets obtained from the GC-MS analysis [26]. Enrichment analysis was performed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) and was used to investigate how groups of functionally related metabolites are significantly enriched that would potentially eliminate requirements of preselect compounds based on arbitrary cut-off thresholds [27]. Identified metabolites were mapped against PubChem and KEGG identifiers. Secondary metabolite pathway maps were created using interactive pathway explorer (iPATH) version 3 based on the biosynthesis of secondary metabolites global pathway mapped against KEGG background [28].

2.5. Statistical analysis

All quantitative data are reported as the mean ± SD of three measurements. Statistical analysis was performed by paired ANOVA using GraphPad V8. $P < 0.05$ was considered significant. The percentage of inhibition/scavenging was calculated by the formula $\frac{X0-X1}{X0}X100$, where X0 was the absorbance of the control and X1 was the absorbance in the presence of the samples and standard. The half maximal inhibitory concentration (IC_{50}) values were calculated by formula $Y = \frac{A1}{X+A1}X100$, where $A1 = IC_{50}$, Y = response (Y = 100% when X = 0), X = inhibitory concentration. The enrichment ratio for metabolites and pathways was calculated based on observed hit/expected hit values. Holm-Bonferroni correction for *P*-value and false discovery rate (FDR) was calculated for all entries of enrichment analysis. Pathway analysis was performed using Fisher's Exact Test. Multivariate analysis was performed using a

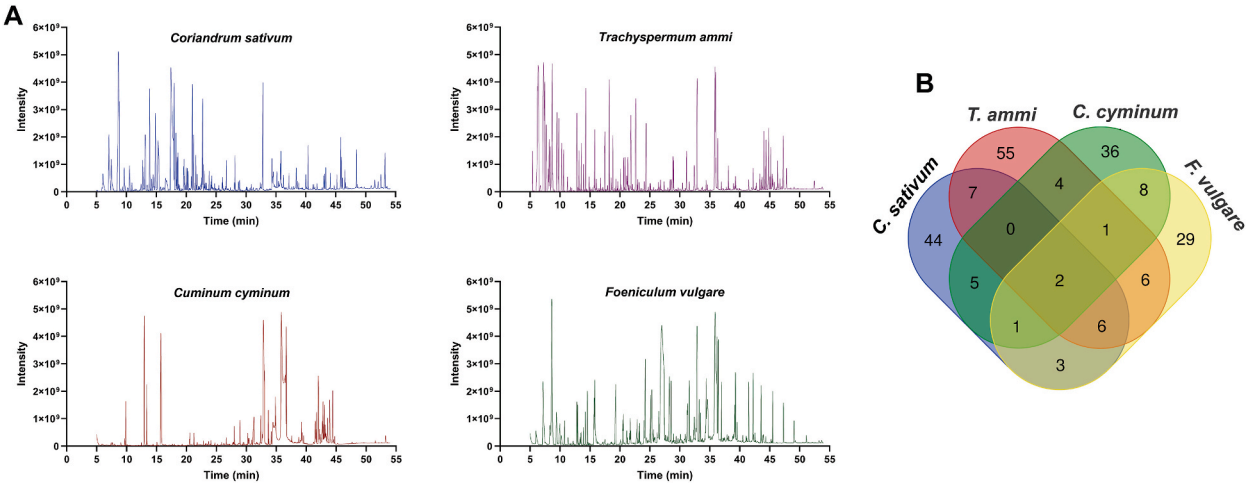


Fig. 1. (A) Gas chromatograms of test materials. **(B)** Vein diagram demonstrating number of metabolites identified in each sample and the number of common metabolites identified. Data corresponds to Supplementary Tables 1–4.

dimension reduction tool using SPSS V23. KMO and Bartley's test were performed to study the underlying dimensions of the variables.

3. Results

3.1. Phytochemical identification

A total of 68, 81, 58, and 56 phytochemicals were identified in *C. sativum*, *T. ammi*, *C. cyminum*, and *F. vulgare*, respectively (Fig. 1A and Tables S1–S4). The most predominant phytochemicals were linalool (14.61%), α-citral (6.88%), hexadecanoic acid (6.18%), (+)-alpha-pinene (5.73%), and linalyl anthranilate (5.38%) in *C. sativum*; sabinene (12.98%), hexadecanoic acid (6.59%), D-limonene (6.10%), elemicin (95.84%), and linoleic acid (5.37%) in *T. ammi*; glycerol (24.14%), diethylene glycol (16.36%), stearic acid (12.14%), oleic acid (9.31%), and (S,S)-butane-2,3-diol (4.87%) in *C. cyminum*; and D-limonene (17.74%), linoleic acid (6.44%), beta-phellandrene (5.97%), stearic acid (5.53%), and apiol (5.27%) in *F. vulgare*. Several compounds were commonly detected in spice samples (Fig. 1B). For instance, 7 common compounds between *C. sativum* and *T. ammi*, 5 between *C. sativum* and *C. cyminum*, 3 between *C. sativum* and *F. vulgare*; 4 common compounds between *T. ammi* and *C. cyminum*, 6 between *T. ammi* and *F. vulgare*, and 8 common compounds were identified between *C. cyminum* and *F. vulgare*. Gamma-terpinene and stearic acid were the common metabolites identified in all the spice samples. Linalool (14.61%), sabinene (12.97%), glycerol (24.14%), and D-limonene (17.44%) were the most abundant phytochemicals identified in *C. sativum*, *T. ammi*, *C. cyminum*, and *F. vulgare*, respectively.

3.2. Compound class enrichment

Chemical enrichment at various chemical classes was performed by MetaboAnalyst using the GC-MS data (Fig. 2A–D and Supplementary Tables S5–S16). Prenol lipids were the most significantly enriched chemical super-class for *C. sativum* and *T. ammi*,

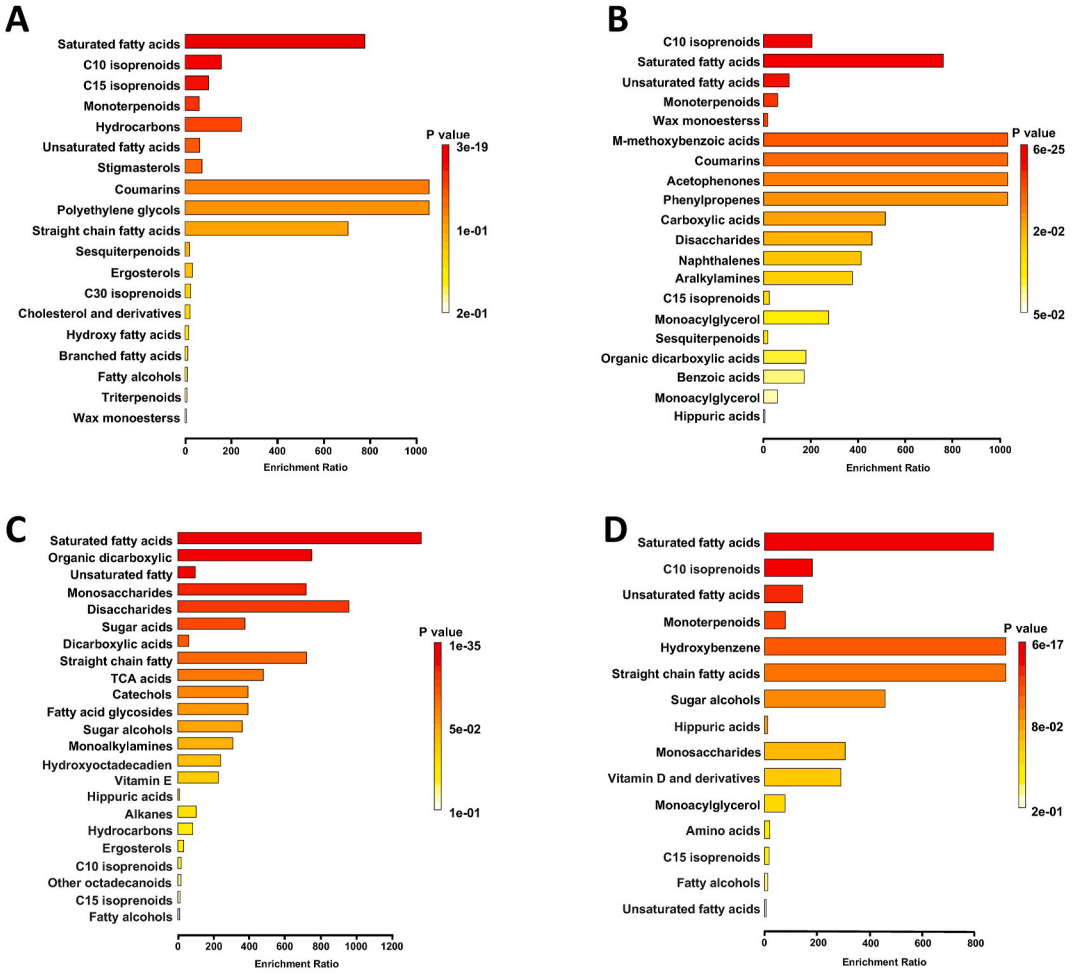


Fig. 2. Metabolite enrichment analysis of **(A)** *C. sativum*, **(B)** *T. ammi*, **(C)** *C. cyminum*, and **(D)** *F. vulgare* at sub-class of chemicals. Enrichment of super-class and main-class of chemicals are represented in Supplementary Tables 5–16.

whereas *C. cyminum*, and *F. vulgare* have an enrichment of fatty acyls. In *C. sativum* and *T. ammi*, isoprenoids were the highly enriched chemical main class, while fatty acids and conjugates were highly enriched in *C. cyminum* and *F. vulgare*. Apart from C10 isoprenoids in *T. ammi*, all other spices have the most significant enrichment of saturated fatty acids for the chemical sub-class. In the case of enrichment ratio, prenol lipid, organic nitrogen compounds, homogeneous non-metal compounds, and carbohydrates as part of the super-class, and dialkyl ethers, acetophenones and Phenylpropenes, disaccharides, and monosaccharides among main-class were highly enriched in *C. sativum*, *T. ammi*, *C. cyminum*, and *F. vulgare*, respectively. Coumarins and polyethylene glycols in *C. sativum; m*-methoxybenzoic acids, coumarins, acetophenones, phenylpropenes in *T. ammi*; saturated fatty acids in *C. cyminum*; hydroxybenzene and straight chain fatty acids in *F. vulgare* were the phytochemical sub-class with highest enrichment ratio.

3.3. Identification of co-regulated phytochemicals and pathways

To decipher the specific phytochemicals that are under common biosynthetic mechanisms in the Apiaceae family spices, PCA analysis based on identified phytochemicals at main- and sub-classes were performed (Fig. 3A and B). Data indicated clear segregation of phytochemical co-occurrence clusters from the higher to lower phytochemical classes. For instance, two predominant clusters viz. cluster one consisting of naphthalenes, amines, carboxylic acids, fatty esters, monoradylglycerols, benzoic acids, and acetophenones; and cluster two consisting of disaccharides, benzamides, organic dicarboxylic acids, fatty acids and conjugates, octadecanoids, fatty acyl glycosides, fatty amines, benzenediols, and monosaccharides, were detected in the phytochemical super-class (Fig. 3A). Three clusters at the main class viz. hydrocarbons, cholesterol and derivatives, hydroxy fatty acids, branched fatty acids, C15 and C30 isoprenoids in cluster 1; straight chain fatty acids, fatty alcohols, and ergosterols in cluster 2; and saturated fatty acids, alkenes, sugar alcohols, dicarboxylic acids, catechols, organic dicarboxylic acids, and disaccharides, hippuric acids in cluster 3 were identified. In contrast to phytochemical classes, biosynthesis of which remains under the control of metabolic pathways, PCA of the metabolic pathways revealed multiple co-occurring pathways (Fig. 3C), viz., monoterpenoid biosynthesis, brassinosteroid biosynthesis, phenylalanine metabolism, nitrogen metabolism and arachidonic metabolism in cluster 1; butanoate metabolism, N-glycan biosynthesis, steroid biosynthesis, and biosynthesis of unsaturated fatty acids in cluster 2; fatty acid biosynthesis, pyruvate metabolism, terpenoid backbone biosynthesis, sulfur metabolism, alanine-aspartate-glutamate metabolism, arginine metabolism, alpha linoleic acid metabolism, and TCA cycle in cluster 3; glycolysis/gluconeogenesis, glycerolipid metabolism, tyrosine metabolism, and galactose metabolism in cluster 4; linoleic acid metabolism, fatty acid elongation, and fatty acid degradation in cluster 5; and aminoacyl tRNA biosynthesis, Isoquinoline alkaloid biosynthesis, propanoate metabolism, cutin-suberin-wax biosynthesis, and phenylalanine-tyroside-tryptophan biosynthesis in cluster 6.

3.4. Pathway enrichment

Biochemical pathway enrichment map of spices was created by MetaboAnalyst using the GC-MS data. The top five pathway impact was observed in *C. sativum* for linoleic acid metabolism, cutin, suberin and wax biosynthesis, alpha-linolenic acid metabolism, steroid biosynthesis, and sesquiterpenoid and triterpenoid biosynthesis (Fig. 4A). Linoleic acid metabolism, phenylalanine metabolism, lysine degradation, alpha-linolenic acid metabolism, and cutin, suberin and wax biosynthesis were impacted in *T. ammi* (Fig. 2B); cutin, suberin and wax biosynthesis, lysine degradation, galactose metabolism, alpha-linolenic acid metabolism, and tyrosine metabolism in *C. cyminum* (Fig. 2C); and linoleic acid metabolism, isoquinoline alkaloid biosynthesis, cutin, suberin and wax biosynthesis, tyrosine metabolism, and alpha-linolenic acid metabolism in *F. vulgare* (Fig. 2D) was the top five impacted biochemical pathways. Among all identified pathways, biosynthesis of unsaturated fatty acids was most significantly impacted in all the spice samples. The list of all metabolic pathway enrichments for each sample is provided in Supplementary Tables S17–S20, and identified pathways mapped against plant secondary metabolite biosynthesis network, and whole plant metabolic pathway network are represented in Supplementary Figs. S1–S5.

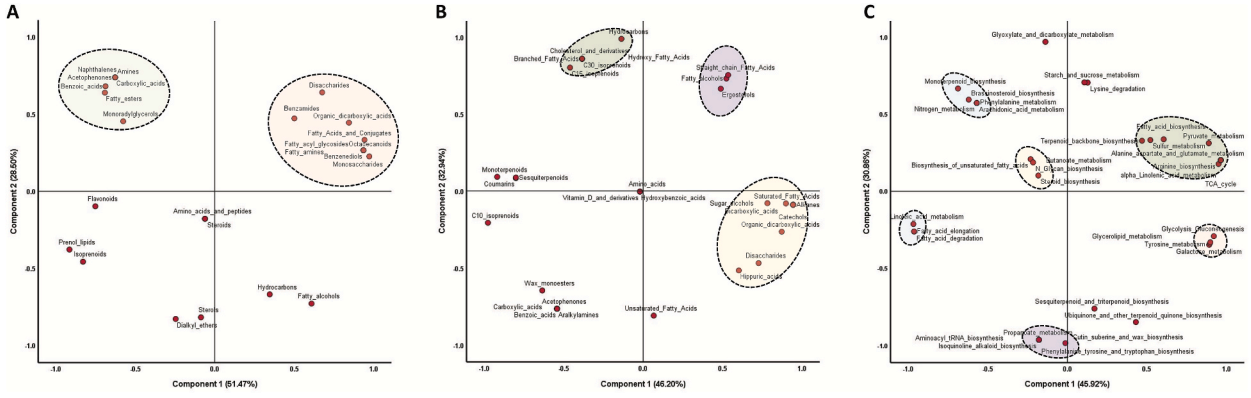


Fig. 3. Principle component analysis demonstrating clustering of metabolites of main-class **(A)** and sub-class **(B)**, clustering of closely-associated enriched pathways **(C)**.

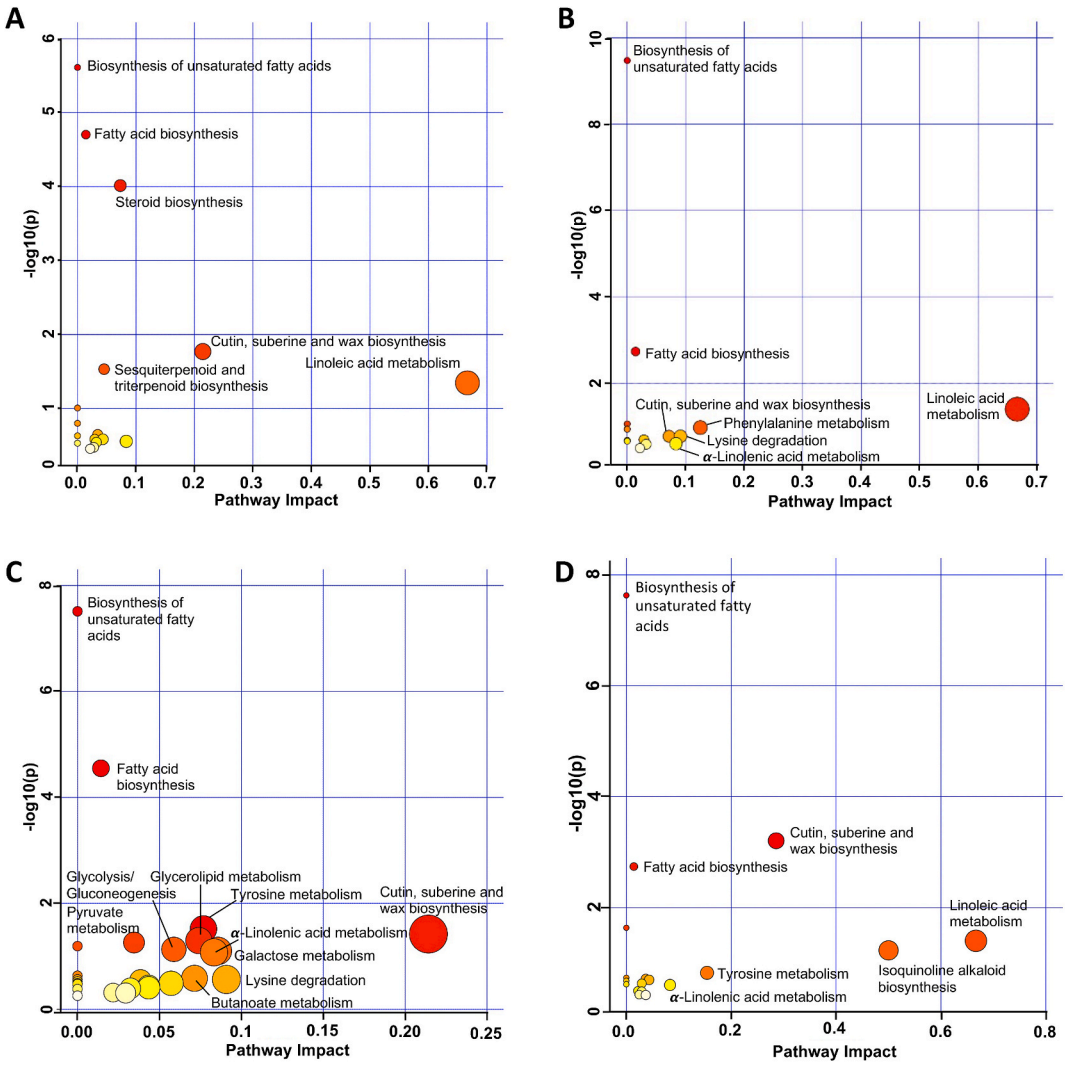


Fig. 4. Metabolic pathway enrichment **(A)** *C. sativum*, **(B)** *T. ammi*, **(C)** *C. cyminum*, and **(D)** *F. vulgare*. Data corresponds to Supplementary Tables 17–20.

3.5. Antioxidant effects

The antioxidant potentials of spices were evaluated using various *in vitro* free-radical scavenging assays and compared against suitable standards. Collectively, data indicated a dose-dependent free-radical scavenging effect of all spices and standards (Fig. 5A–J). No trend of reaction saturation towards the highest concentration was observed for any of the assays. Based on IC_{50} values (Supplementary Table S21), *C. sativum* demonstrated comparatively most superior activities for all the assays except for $OH^{\bullet}$ scavenging, where *T. ammi* showed superior bioactivities, and DPPH and HOCl scavenging where *C. cyminum* showed superior bioactivities.

3.6. Effects of phytochemical classes and corresponding pathway enrichments on the antioxidant activities

Multivariate analysis was performed to decipher the association between different classes of phytochemicals in the four selected spices of the Apiaceae family and the antioxidant activities with various classes of phytochemicals and their corresponding metabolic pathways. Two major clusters were identified for the antioxidant activity and chemical-class association viz. in cluster one (Fig. 6A) consisting of NO, lipid peroxidation, HOCl, H_2O_2, and $OONO^-$ were related to unsaturated fatty acids, acetophenones, carboxylic acids, benzoic acids, alkylamines, and wax monoesters; whereas in cluster two, $O_2^{\bullet-}$, Fe^{2+} and $HO^{\bullet}$ was associated with hippuric acids, disaccharides, organic dicarboxylic acids, sugar alcohols, catechols, alkanes, saturated fatty acids, fatty acid glycosides, and dicarboxylic acids. Linear correlation analysis revealed significant associations between several antioxidant activities with specific chemical classes (Fig. 6B). $OH^{\bullet}$, $OONO^-$ and $^1O_2$ scavenging activity were closely associated with the chemical classes, whereas coumarins, ergosterols, sesquiterpenoids, and unsaturated fatty acids were closely correlated with antioxidant activities. When the results of the

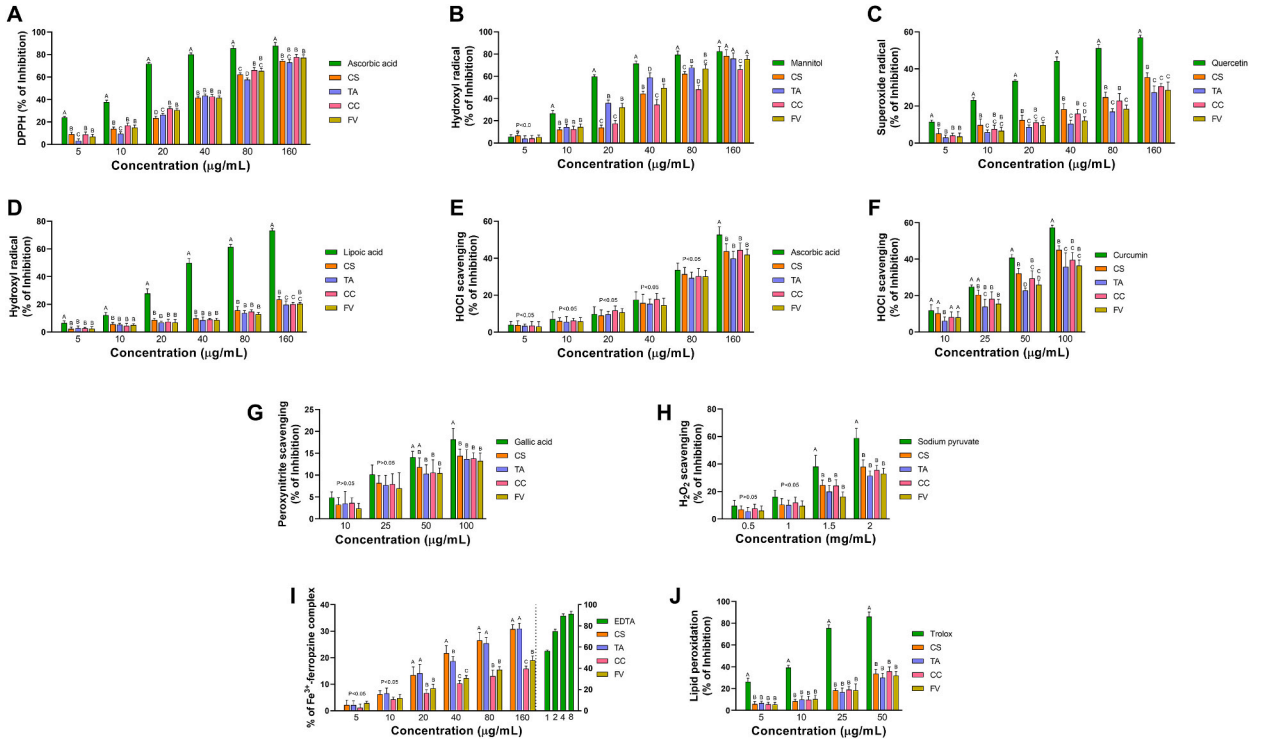


Fig. 5. Antioxidant and free-radical scavenging activities pertaining to **(A)** DPPH assay; **(B)** Hydroxyl radical scavenging; **(C)** Superoxide radical scavenging; **(D)** Singlet oxygen scavenging; **(E)** Hypochlorous acid scavenging; **(F)** Nitric oxide scavenging; **(G)** Peroxynitrite scavenging; **(H)** Hydrogen peroxide scavenging; **(I)** Iron chelation; and **(J)** Lipid peroxidation inhibitory activities. IC_{50} values for each assay is provided in Supplementary Table 21.

antioxidant assays were analyzed against pathway enrichments (Fig. 6C), two separate clusters were revealed, viz. cluster one consisting of HOCl, lipid peroxidation, H_2O_2, NO, $ONOO^-$, $^1O_2$ and $O_2^{\bullet-}$ clustered with isoquinoline alkaloid biosynthesis, aminoacyl tRNA biosynthesis, propanoate metabolism, phenylalanine-tyrosine-tryptophan metabolism, and ubiquinone and other terpenoid quinone biosynthesis pathways; while in cluster two, iron chelation and hydroxyl radical scavenging activities were clustered with glycerolipid metabolism, galactose metabolism, tyrosine metabolism, alpha-linoleic acid metabolism, butanoate metabolism, arginine biosynthesis, alanine-aspartate-glutamate metabolism, and glycolysis-gluconeogenesis pathway enrichments. Linear correlation analysis revealed hydroxyl radical scavenging activities to be closely associated with the metabolic pathway enrichments. In contrast, the fatty acid biosynthetic pathway was highly associated with the free-radical scavenging effects (Fig. 6D).

4. Discussion

High throughput metabolomic approaches have been employed for phytochemical fingerprinting and chemotaxonomic identification of plants. Although a plethora of polar secondary metabolites have been identified, volatile aromatic metabolites have been primarily attributed to the bioactivities of spices and are of high commercial interest [29]. Since aromatic plants are rich in volatile metabolites, GC-MS is the most preferred untargeted metabolomics approach for spices. Moreover, the usefulness and significance of GC-MS based metabolomics method to better comprehend the complexity of phytochemical mixtures, the roles that their constituents perform, and how their biosynthesis is metabolically regulated, are anticipated to be significant in terms of crop improvement through metabolic engineering strategies. In the present study, we have identified common metabolic pathways in spices belonging to the Apiaceae family that can be enriched to obtain improved aromatic properties with antioxidant activities. Choice of spices was based on the fact that *C. sativum*, *T. ammi*, *C. cyminum*, and *F. vulgare* are the most commonly utilized aromatic ingredients in Southeast Asian culinary culture [30], tremendous utility in traditional medicinal systems [31,32], and evidence-based reports of diverse pharmacological activities [33,34].

Variations in plant aromatic fingerprints are caused by a variety of factors, including differential gene expression patterns, altered enzymatic activities, and changes in plant growth parameters. For instance, homologs of the same enzyme can utilize coniferyl acetate and NADPH to synthesize eugenol in sweet basil but isoeugenol in petunia [35]. These enzymatic variations are also reported in plant-part specific such as in the case of plants belonging to the *Allium* spp., where bundle sheath and phloem cells were rich in the enzyme cysteine synthase enzyme, whereas alliinase enzyme responsible for the formation of diallylthiosulfinate (aka, Allicin) is found only in the bundle sheath cells [36]. Therefore, phytochemical profiling of spices belonging to the same family and common plant parts

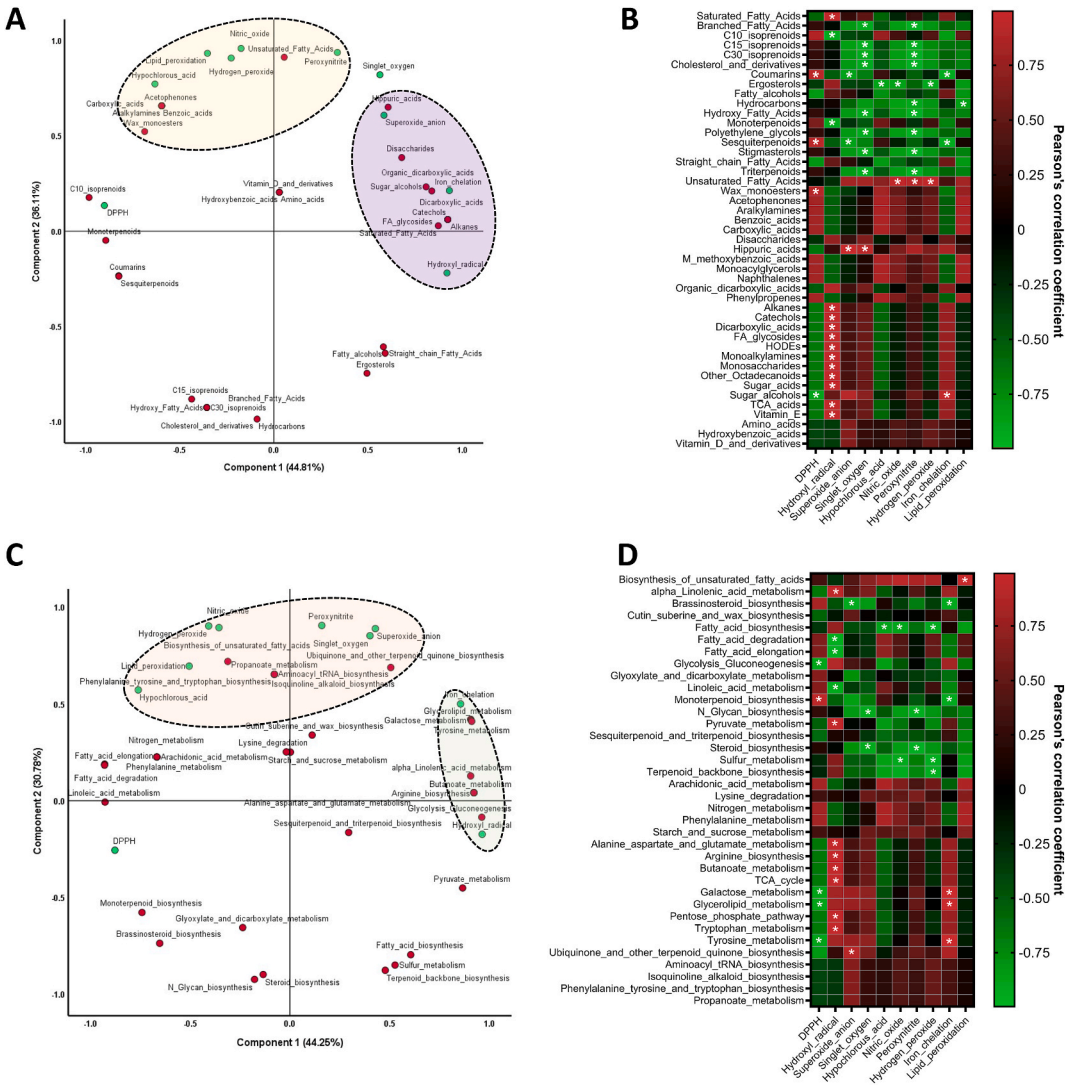


Fig. 6. Data demonstrates association between results of antioxidant assays with metabolite sub-class and pathway enrichments. Principle component analysis demonstrating close association of antioxidant activities with **(A)** metabolites and **(C)** pathways. Heatmap demonstrating linear correlation of antioxidant activities with **(B)** chemical class and **(D)** pathway enrichments.

utilized for aromatic and therapeutic purposes are better candidates for comparative metabolomic studies.

The GC-MS analysis revealed highly enriched profiles of fatty acids in all four spices. This was rather expected since, on a quantitative and qualitative scale, saturated and unsaturated fatty acids are the primary biosynthetic source for plant volatiles [37]. Spices belonging to the Apiaceae family were previously found to possess the 'healthiest fatty acid' profile, which potentially contributes to the antioxidant activities [9]. Since fatty acids serve as a natural pool of carbon source favoring the production of aroma compounds, fatty acids associated with the spice essential oil, and as commercially-important metabolites, fatty-acid metabolic engineering is considered a lucrative strategy for the improvement of spice quality [38]. Common to most plant species, enrichment of C18 unsaturated fatty acids (e.g., oleic (18:1), linoleic (18:2), α-linolenic (18:3) acids) were identified in the spice samples. Unsaturated fatty acids possess potent antioxidant effects besides, virgin olive oil rich in unsaturated fatty acids has demonstrated antioxidant benefits in patients with stable coronary heart disease [39]. Coumarin compounds rich in spices were earlier reported to possess antioxidant activities that prompted the chemical synthesis of structural analogs [40]. Also, metabolic engineering strategies for their increased production in spices have been explored [41]. A variety of sterols (e.g., campesterol, sitosterol, stigmasterol) identified in the present study are known for their health-beneficial effects in terms of lowering hyperlipidemia and reducing obesity, cardiometabolic benefits, reducing insulin resistance, and lowering inflammation, primarily through antioxidant benefits [42]. We also identified a variety of highly enriched terpenoids (aka, isoprenoids) in all four spices. Terpenoids not only represent one of the most diverse bioactive classes of phytochemicals but are also among the chief aroma compounds of spices [43]. Since a detailed discussion on the bioactivities of the identified phytochemicals is out of the scope of the current study, a table summarizing the bioactivities of the phytochemicals is

presented as Supplementary Table S22.

Based on the diversity of phytochemical classes, metabolic pathway impacts were calculated, revealing significantly impacted common pathways in the spice samples. Various fatty acid biosynthetic pathways were identified to be significantly impacted. Extensive genetic and metabolic enrichment strategies have been applied to improve the quality of capsaicin, which is a fatty acid-derived aromatic compound generated through the action of enzymes involved in fatty-acid synthesis pathway such as acyl carrier protein 1, ketoacyl-ACP synthase I, and Acyl-(acyl-carrier-protein) hydrolase, and is associated with the extent of the pungency of chili peppers [44]. This is relevant for aromatic plant species like spices since fatty acid-derived metabolites are formed ubiquitously through α-oxidation, β-oxidation and the lipoxygenase pathways [45]. In *C. sativum* and *F. vulgare*, highly enriched straight-chain fatty acid was observed, which are known to be degraded through α- and β-oxidation to produce aroma compounds [37]. Fatty acid biosynthetic pathways are the precursor for generating a variety of bioactive phytochemicals in plants. Therefore, the enrichment of fatty-acid metabolism pathways in spices could potentially generate spices with improved bioactivities and aromatic quality. In line, increased production of polyunsaturated fatty acids (e.g., arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid), including genetic strategies to improve the levels of Δ-12 modified fatty acids by transgenic expression of FAD2-like enzymes has been explored [46]. Engineering artificial hydrophobic droplets within cells that allow the accumulation and storage of lipophilic substances like terpenoids is a rapidly developing metabolic engineering method. For instance, genetic engineering strategies in *Nicotiana benthamiana* for the expression of α-bisabolol synthase with fatty acids biosynthesis regulators resulted in up to 4-times increased accumulation of bioactive sesquiterpenoids such as α-bisabolol, (E)-β-caryophyllene and α-barbatene in the lipid bodies [47]. Biosynthesis and increasing the accumulation of bioactive compounds in the lipid fraction would facilitate the efficient extraction of bioactive terpenoids from plants. In the transient *N. benthamiana* system, ectopic expression of WRINKLED1 (regulator of fatty acid biosynthesis in plastid), and a microalgal lipid droplet surface protein promotes the development of cytosolic lipid droplets. Herein, installing terpenoid biosynthetic pathways through the steering of enzymes to native and non-native compartments, as well as engineering of the pathways supplying the universal C5-building blocks for terpenoids, increase the synthesis of target terpenoids and accumulation in the lipid droplets [48].

Diverse biosynthetic pathways related to terpenoids were enriched in the spice samples. Terpenoids are universally produced from isopentenyl diphosphate and dimethylallyl diphosphate, where C10 monoterpenoids, C20 diterpenoids, and C40 teteraterpenoids are synthesized in plastids, whereas C15 sesquiterpenoids and C30 triterpenoids are formed in the cytosol [49]. The signature phytochemicals in the Apiaceae family are the diverse terpenoids coumarins and sesquiterpene lactones. These metabolites are part of the natural product class of phytochemicals known for their diverse pharmacological activities. Elicitors are frequently used to activate the secondary metabolic pathways and increase the synthesis of target terpenoids while overexpressing terpenoids biosynthesis pathway genes in homologous and ectopic plants, suppressing the expression of rival metabolic pathways, or modulating the global gene regulation pathways (e.g., key transcription factors, endogenous phytohormones, and primary metabolism), improved biosynthesis terpenoid with pharmacological properties has been achieved [50]. We also identified the enrichment of specific amino acid biosynthetic pathways in all four spices. This is relevant to the Apiaceae family since the aroma compounds and several secondary metabolites are biosynthesized from amino acids as precursors, especially phenylalanine and tyrosine [41]. Earlier studies have shown the enrichment of biosynthesis of medicinally important terpenoids, artemisinin, and peritaxel by the overproduction of amino acids [51]. Aroma compounds generated from amino acids through decarboxylation are a lucrative strategy for crop improvements. For instance, 10-fold increased biosynthesis of aroma compounds 2-phenylacetaldehyde and 2-phenylethanol were achieved by over-expression of the amino acid decarboxylase gene in tomato [52].

Using multivariate analysis, we intended to associate closely regulated plant metabolic pathways to the observed free-radical scavenging activities. In addition to generic antioxidant assay (e.g., DPPH), we tested the efficacy of the spice samples for scavenging free radicals that were generated *in vitro* using standard biochemical assays. Although pathways related to fatty acid metabolism were clustered along with the antioxidant assays, both features remained mostly inversely correlated while primary metabolic pathways (e.g., TCA cycle, pentose phosphate pathway) not directly related to fatty acid metabolisms were positively associated with the antioxidant assays. Upon pathway mapping, although enrichment of distinct yet partially overlapping metabolic pathways were identified, biosynthesis of unsaturated fatty acids, fatty acid biosynthesis, cutin-suberine-wax biosynthesis, sesquiterpenoid and triterpenoid biosynthesis, alpha-linolenic acid metabolism, fatty acid elongation, and fatty acid degradation were ranked as the most commonly enriched pathway in all the spice samples (Supplementary Figs. S1–S5). Interestingly, not all of these common pathways were co-clustered with the antioxidant activities, which likely support the fact that secondary metabolic pathways (e.g., shikimate pathway responsible for polyphenol biosynthesis) play a critical role in dictating the bioactivities by differentially affecting the biosynthesis of phytochemicals [53,54]. Nevertheless, sub-metabolic pathways related to fatty acids and terpenoid metabolism have emerged as the most predominantly enriched pathways although not predominantly associated with the antioxidant and free-radical scavenging activities. The co-enhancement of terpenoid and fatty acid metabolic pathways is supported by the evidence that terpenoid biosynthesis could be achieved by enhancing fatty acid β-oxidation to recover acetyl-CoA from lipids [55]. In relation to secondary metabolite formation, which is generally attributed to the bioactivities, this is important since terpenoids are considered the key bioactive compounds and aroma compounds in spices belonging to the Apiaceae family and can be biosynthesized by modulating the fatty acid metabolic pathways [56]. Interestingly, not only that the majority of the terpenoids identified in the spice samples were inversely correlated with the antioxidant assays, majority of the identified metabolic pathways related to terpenoids were clustered separately from the antioxidant activities. This likely indicated that although terpenoids are responsible for the aroma characteristics, phytochemicals other than terpenoids are primarily associated with the antioxidant activities. In line, common polyphenols with high abundance identified in the spice samples were mostly phytosterols which are widely recognized to have antioxidant activities, and their biosynthetic pathways are closely associated with pathways related to the fatty acid metabolism in plants [57].

Finally, these observations could be influenced by certain technical and experimental limitations. For instance, since the obtained metabolic pathways were dependent on the identified phytochemicals, which in turn, were associated with the sample processing and extraction methods, we were only able to identify a fraction of the global metabolome of the spices. Methanol extraction and silylation was predicted to extract polar to mid-polar phytochemicals, whereas and GC-MS due to its limited mass-range (50–650 amu) were expected to identify low molecular weight phytochemicals. Thus, beyond the identified phytochemicals, several predominant antioxidant classes of molecules (e.g., flavonoids) [58] were sparingly identified. Therefore, a multi-solvent fractional approach coupled with both targeted and untargeted metabolomics is expected to yield better outcomes in terms of identifying a vast array of phytochemicals.

5. Conclusion

Metabolic pathway mapping of spices belonging to the family Apiaceae base on untargeted GC-MS indicate the enhancement of metabolic pathways related to fatty acid and terpenoid biosynthesis, whereas pathway association with antioxidant and free-radical assays showed inverse association with fatty acid and terpenoid biosynthesis pathways. Antioxidant potentials were rather closely associated with primary metabolism pathways which indirectly support the formation of secondary metabolites by providing carbon backbone. For spice samples of Apiaceae family, terpenoids play key role in dictating the aroma quality which is a desired characteristic for commercial exploitation. Since, secondary metabolism pathways relate to terpenoid metabolism are dependent on plant primary metabolism, improvements of primary metabolism pathways would improve both the antioxidant qualities and improved aroma qualities. However, for improved aroma qualities independent of antioxidant effects, enhancement of terpenoid and fatty acid metabolism pathways are likely sufficient. Since, the antioxidant activities of plant secondary metabolites are dependent on their abundance, whereas the concentrations of the phytochemicals depend on the enrichment of phytometabolic pathways, we demonstrate that putative common target metabolic pathways could be identified using GC-MS based untargeted metabolomics for improvement of the quality of the spices. *In vitro* studies are further required to evaluate the implication of spice quality improvement by targeting putative metabolic pathways through the proposed metabolomics-based reversed association strategy.

Data availability statement

Data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Sonia Gandhi: Data curation, Formal analysis, Investigation, Methodology. **Manas Ranjan Saha:** Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Priyankar Dey:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21392.

References

[1] D.L.T. Jessica Elizabeth, F. Gassara, A.P. Kouassi, S.K. Brar, K. Belkacemi, Spice use in food: properties and benefits, Crit. Rev. Food Sci. Nutr. 57 (2017) 1078–1088.
[2] M. Gupta, Pharmacological properties and traditional therapeutic uses of important Indian spices: a review, Int. J. Food Prop. 13 (2010) 1092–1116.
[3] T.A. Jiang, Health benefits of culinary herbs and spices, J. AOAC Int. 102 (2019) 395–411.
[4] E. Lewinsohn, F. Schalechet, J. Wilkinson, K. Matsui, Y. Tadmor, K.-H. Nam, et al., Enhanced levels of the aroma and flavor compound S-linalool by metabolic engineering of the terpenoid pathway in tomato fruits, Plant Physiol 127 (2001) 1256–1265.
[5] J. Wu, W. Chen, Y. Zhang, X. Zhang, J.M. Jin, S.Y. Tang, Metabolic engineering for improved curcumin biosynthesis in Escherichia coli, J. Agric. Food Chem. 68 (2020) 10772–10779.
[6] S.A. Wilson, S.C. Roberts, Metabolic engineering approaches for production of biochemicals in food and medicinal plants, Curr. Opin. Biotechnol. 26 (2014) 174–182.
[7] S.M. Vásquez, G.G.W. Abascal, C.E. Leal, G.A. Cardineau, S.G. Lara, Application of metabolic engineering to enhance the content of alkaloids in medicinal plants, Metab. Eng. Commun. (2022), e00194.
[8] K. Eljounaidi, B.R. Lichman, Nature's Chemists: the discovery and engineering of phytochemical biosynthesis, Front. Chem. 8 (2020) 1041.
[9] R.K. Saini, A.D. Assefa, Y.-S. Keum, Spices in the Apiaceae family represent the healthiest fatty acid profile: a systematic comparison of 34 widely used spices and herbs, Foods 10 (2021) 854.

[10] P. Thiviya, A. Gamage, D. Piumali, O. Merah, T. Madhujith, Apiaceae as an important source of antioxidants and their applications, Cosmetics 8 (2021) 111.
[11] B. Sayed-Ahmad, T. Talou, Z. Saad, A. Hijazi, O. Merah, The Apiaceae: ethnomedicinal family as source for industrial uses, Ind. Crops Prod. 109 (2017) 661–671.
[12] P. Dey, M.R. Saha, S. Roy Choudhuri, I. Sarkar, B. Halder, M. Poddar-Sarkar, et al., Oleander stem and root standardized extracts mitigate acute hyperglycaemia by limiting systemic oxidative stress response in diabetic mice, Adv Pharmacol Sci 2019 (2019).
[13] S. Dutta, A.K. Chakraborty, P. Kar, P. Dey, A. Sen, T.K. Chaudhuri, Stimulation of murine immune response by Clerodendrum infortunatum, Pharmacog. Mag. 14 (2018) 417.
[14] P. Dey, T.K. Chaudhuri, Comparative phytochemical profiling and effects of Nerium oleander extracts on the activities of murine peritoneal macrophages, Arch. Biol. Sci. 68 (2016) 515–531.
[15] P. Dey, S. Dutta, A. Chowdhury, A.P. Das, T.K. Chaudhuri, Variation in phytochemical composition reveals distinct divergence of Aloe vera (L.) Burm. F. From other aloe species: rationale behind selective preference of Aloe vera in nutritional and therapeutic use, J Evid Based Complementary Altern Med 22 (2017) 624–631.
[16] P. Dey, S. Ray, M.P. Sarkar, T.K. Chaudhuri, Chemical characterization and assessment of antioxidant potentiality of Streptocaulon sylvestre Wight, an endangered plant of sub-Himalayan plains of West Bengal and Sikkim, BMC Complement Altern. Med. 15 (2015) 1–12.
[17] P. Dey, I. Sarkar, S. Dutta, M.R. Saha, T.K. Chaudhuri, Correlative metabolomic fingerprinting and molecular docking studies of dermatological phytotherapeutics of South-Eastern Himalaya, J. Tradit. Complement. Med. 9 (2019) 243–248.
[18] P. Dey, T.K. Chaudhuri, Metabolomic fingerprinting of the volatiles in different parts of streptocaulon sylvestre, J. Herbs. Spices. Med. 23 (2017) 308–319.
[19] B.K. Tiwary, S. Dutta, P. Dey, M. Hossain, A. Kumar, S. Bihani, et al., Radical scavenging activities of Lagerstroemia speciosa (L.) Pers. petal extracts and its hepato-protection in CCl4-intoxicated mice, BMC Complement. Altern. Med. 17 (2017) 1–13.
[20] S. Dutta, P. Dey, M.R. Saha, I. Sarkar, R. Sarkar, J.A. Mardi, et al., Differential interaction with O2 and N2 free-radicals, phytochemical fingerprinting and molecular docking reveals potent antioxidant activities of three major recreational foods of the Indian subcontinent, J. Funct. Food. 39 (2017) 112–122.
[21] M.R. Saha, P. Dey, S. Begum, B. De, T.K. Chaudhuri, D.D. Sarker, et al., Effect of Acacia catechu (Lf) Willd. on oxidative stress with possible implications in alleviating selected cognitive disorders, PLoS One 11 (2016), e0150574.
[22] M.R. Saha, P. Dey, T.K. Chaudhuri, A.K. Goyal, D.D. Sarker, A. Sen, Assessment of haemolytic, cytotoxic and free radical scavenging activities of an underutilized fruit, Baccaurea ramiflora Lour.(Roxb.) Muell. Arg. Indian J Exp Biol. 54 (2016) 115–125.
[23] P. Dey, D. Chaudhuri, T.K. Chaudhuri, N. Mandal, Comparative assessment of the antioxidant activity and free radical scavenging potential of different parts of Nerium indicum, Int. J. Phytomed. 4 (2012) 54.
[24] R. Rezgui, R. Walia, J. Sharma, D. Sidhu, K. Alshagadali, S. Ray Chaudhuri, et al., Chemically defined lactobacillus plantarum cell-free metabolites demonstrate cytoprotection in HepG2 cells through nrf2-dependent mechanism, Antioxidants 12 (2023) 930.
[25] P. Dey, D. Chaudhuri, S. Tamang, T.K. Chaudhuri, N. Mandal, In vitro antioxidant and free radical scavenging potential of Clerodendrum viscosum, Int J Pharm Bio Sci 3 (2012) 454–471.
[26] J. Xia, R. Mandal, I.V. Sinelnikov, D. Broadhurst, D.S. Wishart, MetaboAnalyst 2.0—a comprehensive server for metabolomic data analysis, Nucleic Acids Res. 40 (2012) W127–W133.
[27] X. Sun, P. Dey, R.S. Bruno, J. Zhu, EGCG and catechin relative to green tea extract differentially modulate the gut microbial metabolome and liver metabolome to prevent obesity in mice fed a high-fat diet, J. Nutr. Biochem. 109 (2022), 109094.
[28] Y. Darzi, I. Letunic, P. Bork, T. Yamada, iPath3. 0: interactive pathways explorer v3, Nucleic Acids Res. 46 (2018) W510. W3.
[29] E. Christaki, E. Bonos, I. Giannenas, P. Florou-Paneri, Aromatic plants as a source of bioactive compounds, Agriculture 2 (2012) 228–243.
[30] V. Siruguri, R.V. Bhat, Assessing intake of spices by pattern of spice use, frequency of consumption and portion size of spices consumed from routinely prepared dishes in southern India, Nutr. J. 14 (2015) 7.
[31] K. Krishnaswamy, Traditional Indian spices and their health significance, Asia Pac. J. Clin. Nutr. 17 (Suppl 1) (2008) 265–268.
[32] X. Bi, J. Lim, C.J. Henry, Spices in the management of diabetes mellitus, Food Chem. 217 (2017) 281–293.
[33] N. Sharma, M.A. Tan, S.S.A. An, Mechanistic aspects of Apiaceae family spices in ameliorating alzheimer's disease, Antioxidants (2021) 10.
[34] J. Jeyabalan, F. Aqil, L. Soper, D.J. Schultz, R.C. Gupta, Potent chemopreventive/antioxidant activity detected in common spices of the Apiaceae family, Nutr. Cancer 67 (2015) 1201–1207.
[35] T. Koeduka, E. Fridman, D.R. Gang, D.G. Vassão, B.L. Jackson, C.M. Kish, et al., Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester, Proc Natl Acad Sci U S A 103 (2006) 10128–10133.
[36] M. Yamazaki, M. Sugiyama, K. Saito, Intercellular localization of cysteine synthase and alliinase in bundle sheaths of Allium plants, Plant Biotechnol. 19 (2002) 7–10.
[37] W. Schwab, R. Davidovich-Rikanati, E. Lewinsohn, Biosynthesis of plant-derived flavor compounds, Plant J. 54 (2008) 712–732.
[38] J.J. Thelen, J.B. Ohlrogge, Metabolic engineering of fatty acid biosynthesis in plants, Metab. Eng. 4 (2002) 12–21.
[39] M. Fitó, M. Cladellas, R. de la Torre, J. Martí, M. Alcántara, M. Pujadas-Bastardes, et al., Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: a randomized, crossover, controlled, clinical trial, Atherosclerosis 181 (2005) 149–158.
[40] I. Kostova, S. Bhatia, P. Grigorov, S. Balkansky, S. Parmar V, A. K Prasad, et al., Coumarins as antioxidants, Curr. Med. Chem. 18 (2011) 3929–3951.
[41] L. Huang, C.-T. Ho, Y. Wang, Biosynthetic pathways and metabolic engineering of spice flavors, Crit. Rev. Food Sci. Nutr. 61 (2021) 2047–2060.
[42] T. Vezza, F. Canet, A.M. de Marañón, C. Bañuls, M. Rocha, V.M. Víctor, Phytosterols: nutritional health players in the management of obesity and its related disorders, Antioxidants 9 (2020) 1266.
[43] A. Masyita, R.M. Sari, A.D. Astuti, B. Yasir, N.R. Rumata, T.B. Emran, et al., Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives, Food Chem. X (2022), 100217.
[44] Z.-X. Zhang, S.-N. Zhao, G.-F. Liu, Z.-M. Huang, Z.-M. Cao, S.-H. Cheng, et al., Discovery of putative capsaicin biosynthetic genes by RNA-Seq and digital gene expression analysis of pepper, Sci. Rep. 6 (2016) 1–14.
[45] W. Schwab, P. Schreier, Enzymic Formation of Flavor Volatiles from Lipids. Lipid Biotechnology, CRC Press, 2002, pp. 342–372.
[46] S.P. Singh, X.R. Zhou, Q. Liu, S. Stymne, A.G. Green, Metabolic engineering of new fatty acids in plants, Curr. Opin. Plant Biol. 8 (2005) 197–203.
[47] T.L. Delatte, G. Scaiola, J. Molenaar, K. de Sousa Farias, L. Alves Gomes Albertti, J. Busscher, et al., Engineering storage capacity for volatile sesquiterpenes in Nicotiana benthamiana leaves, Plant Biotechnol. J. 16 (2018) 1997–2006.
[48] R. Sadre, P. Kuo, J. Chen, Y. Yang, A. Banerjee, C. Benning, et al., Cytosolic lipid droplets as engineered organelles for production and accumulation of terpenoid biomaterials in leaves, Nat. Commun. 10 (2019) 853.
[49] M.E. Bergman, B. Davis, M.A. Phillips, Medically useful plant terpenoids: biosynthesis, occurrence, and mechanism of action, Molecules 24 (2019).
[50] X. Lu, K. Tang, P. Li, Plant metabolic engineering strategies for the production of pharmaceutical terpenoids, Front. Plant Sci. 7 (2016) 1647.
[51] R. Kulkarni, Metabolic engineering, Resonance 21 (2016) 233–237.
[52] D. Tieman, M. Taylor, N. Schauer, A.R. Fernie, A.D. Hanson, H.J. Klee, Tomato aromatic amino acid decarboxylases participate in synthesis of the flavor volatiles 2-phenylethanol and 2-phenylacetaldehyde, Proc Nat Acad Sci 103 (2006) 8287–8292.
[53] B.M. Twaij, M.N. Hasan, Bioactive secondary metabolites from plant sources: types, synthesis, and their therapeutic uses, Int. J. Plant Biol. 13 (2022) 4–14.
[54] R. Marchiosi, W.D. dos Santos, R.P. Constantin, R.B. de Lima, A.R. Soares, A. Finger-Teixeira, et al., Biosynthesis and metabolic actions of simple phenolic acids in plants, Phytochem. Rev. 19 (2020) 865–906.
[55] C.-C. Jin, J.-L. Zhang, H. Song, Y.-X. Cao, Boosting the biosynthesis of betulinic acid and related triterpenoids in Yarrowia lipolytica via multimodular metabolic engineering, Microb. Cell Factories 18 (2019) 1–18.

[56] X.-J. Wang, Q. Luo, T. Li, P.-H. Meng, Y.-T. Pu, J.-X. Liu, et al., Origin, evolution, breeding, and omics of Apiaceae: a family of vegetables and medicinal plants, Hortic. Res. 9 (2022).
[57] W.D. Nes, Biosynthesis of cholesterol and other sterols, Chem Rev 111 (2011) 6423–6451.
[58] L. Rubió, M.-J. Motilva, M.-P. Romero, Recent advances in biologically active compounds in herbs and spices: a review of the most effective antioxidant and anti-inflammatory active principles, Crit. Rev. Food Sci. Nutr. 53 (2013) 943–953.