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Received: 19 August 2025

Accepted: 14 November 2025

Published online: 08 December 2025

Cite this article as: Baky M.H., Kabbash E.M., Serag A. *et al.* Unveiling crucifer metabolomes *via* UPLC-HRMS/MS and chemometric analysis of edible and non-edible varieties. *Sci Rep* (2025). <https://doi.org/10.1038/s41598-025-29178-w>

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Unveiling Crucifer Metabolomes via UPLC-HRMS/MS and Chemometric Analysis of Edible and Non-Edible Varieties

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Abstract

Cruciferous green vegetables are considered as one of the most important consumed vegetables world-wide owing to their rich nutritive value, characteristic taste as well as health benefits. Cruciferous vegetables are rich in a myriad of bioactive phytochemicals including phenolic compounds and glucosinolates (GLS). The current work aims to assess heterogeneity in secondary metabolites profile among six cruciferous green leafy vegetables including *Brassica oleracea* (cabbage), *B. oleracea* var. *Italica* (broccoli), *B. oleracea* var. *oleracea* (cauliflower), *B. rapa* (turnip), *Raphanus sativus* L. (radish), and *Nasturtium officinale* (watercress) using UHPLC-HRMS/MS coupled with chemometric analysis. A total of 149 metabolites were identified belonging to different phytochemical classes including flavonoids, phenolic acids, steroids, anthocyanins, fatty acids/acyl esters, and GLS. Flavonoids were detected as the most abundant class in cruciferous vegetables represented by 48 peaks, especially Broccoli, cabbage and cauliflower. Radish exhibited unique flavonoid profiles including kaempferol-*O*-pentosylhexoside-*O*-deoxyhexoside, kaempferol-*O*-hexosyl-*O*-di-deoxyhexoside, and quercetin-*O*-tri-deoxyhexoside, along with indole-derived GLS known as methoxyspirobrassinin. Distinct phenolic acid profile was observed where di-sinapoyl-*O*-hexoside was detected only in turnip and broccoli, p-coumaroyl-malic acid was enriched in turnip only, and feruloyl malate and coumaroyl malate were enriched in turnip and radish. Unsupervised PCA analysis revealed distinct variation of turnip from other cruciferous samples, while OPLS-DA distinguished non-edible broccoli from edible cabbage, showing enrichment of neoglucobrassicin and malic acid in broccoli and higher flavonoid and anthocyanin levels in cabbage. These findings highlight the metabolic diversity of cruciferous leaves and support

the valorization of underutilized species as potential functional food resources.

Key words

Cruciferous vegetables; glucosinolates; phenolic compounds; UHPLC-HRMS/MS; multivariate data analysis

1. Introduction

A central question for consumers when selecting plant-based foods is whether a given plant part is commonly consumed or less frequently utilized. Recently, functional foods derived from natural sources catch the interest of both consumers and nutritional specialists as it plays a pivotal role in human diet not only because they are palatable but also due to their richness in several phytochemicals of both nutritional and health-promoting properties¹. Such a revolution in functional foods manufacturing by role led to progress in quality assessment tools to ensure quality and identify new nutraceuticals. Green leafy vegetables are widely consumed worldwide either fresh or after processing or added to food recipes owing to their special taste and aroma, as well as their several health benefits ². Brassicaceae, also known as Cruciferae, is a well-known medium-sized flowering plant family that comprises *ca.* 360 genera, including 3709 species distributed worldwide ³. Brassicaceae includes a wide variety of edible vegetables used widely in food owing to their nutritional value as being rich in vitamins (A, B1, B2, B6, C, E, and K) and minerals such as magnesium, iron, and calcium ³. The most common vegetables in Brassicaceae include cabbage, cauliflower, broccoli, turnip, radish, watercress, and rocket ⁴. Owing to their richness in a myriad of phyto,

macro/micronutrients i.e., vitamins, carotenoids, dietary fibers, amino acids, minerals, glucosinolates (GLS), and phenolic compounds, brassica vegetables possess both nutritional and potential health value ⁵. Brassica vegetables encompass a large class of biologically active secondary metabolites including GLS and other sulfur containing compounds ^{6,7}. GLS (β -thioglucoside-*N*-hydroxysulfates) structure is composed of a β -D-glycopyranose moiety attached by sulfur-linkage to a hydroximinosulfate ester with another amino group ⁸. GLS possess several biological activities in addition to their culinary uses attributed to their unique flavor and taste ⁹. Owing to their richness in GLS, cruciferous vegetables are attributed several positive effects including reducing the risk of cardiovascular disease, anticancer effect against lung, stomach, bladder, and colorectal ¹⁰. Despite their nutritional richness, not all Brassicaceae leaves are equally consumed. For instance, cabbage, radish, and watercress leaves are commonly eaten, while the leaves of broccoli, cauliflower, and turnip are frequently discarded as by-products ⁷. This disparity is largely related to sensory attributes (bitterness, fibrous texture), cultural dietary preferences, and, in some cases, higher levels of antinutritional compounds, despite the fact that these leaves remain edible and potentially valuable sources of nutrients and bioactives ².

Cabbage (*Brassica oleracea*) is one of the most consumed edible cruciferous leafy vegetable worldwide owing to its special taste and richness in antioxidant phytochemicals ¹¹. Broccoli (*Brassica oleracea* var. *italica*) is likewise nutritionally important vegetable for its richness in vitamins A, B2, and C, minerals, in addition to phytonutrients such as polyphenols, β -carotene and α -tocopherol, and isothiocyanates ¹². Cauliflower (*Brassica oleracea* var. *oleracea*) is rich of antioxidants phytonutrients such as GLS, ascorbic acid and polyphenols ¹³. Turnip (*Brassica rapa*) is a widely consumed brassica rich in GLS, flavonoids, indoles, phenolics, and essential oil ¹⁴. Radish (*Raphanus sativus* L.) leafy part is typically consumed green to encompass several chemicals including

vitamins, carbohydrates, minerals, flavonoids, and GLS¹⁵. Watercress (*Nasturtium officinale*) is an important member of brassica vegetables rich in phytochemicals specially GLS¹⁶.

Ensuring the quality of plant-based functional foods, including chemical attributes, is important to assess their food or nutraceutical value based on chemical composition¹⁷. Recently, metabolomics represents an important platform used to characterize the quality of food-stuff using different spectroscopic techniques such as mass spectrometry based chromatographic techniques¹⁸. Ultra-high performance liquid chromatography coupled with high resolution mass analyzer (UPLC-MS/MS) is a platform well suited for profiling secondary metabolites. We have previously reported on the application of MS-based metabolomics in aroma and nutrients profiling in cruciferous leafy vegetables *via* gas chromatography mass spectrometry (GC-MS) in context to edible and non-edible plants⁷. Compared to GC-MS that targets aroma compounds, UPLC-MS/MS technique is more suited for large molecular weight polar metabolites that account for health benefits^{19,20}. Distribution of secondary metabolites among leafy green crucifers in the context of edible or nonedible has yet to be reported, especially targeting its GLS composition. Further application of multivariate data analysis including unsupervised principal component analysis (PCA) and supervised orthogonal projection to latent structures discriminant analysis (OPLS-DA) were employed for samples' classification. Compared with PCA, OPLS-DA has a greater capacity for marker recognition and discrimination among samples by supplying the most pertinent variables for the distinction among two class groups².

To date, the distribution of secondary metabolites among cruciferous leafy vegetables has not been systematically reported in relation to consumption practices, particularly regarding GLSs and phenolic compounds. Here, we employed UHPLC-HRMS/MS combined with multivariate data analysis,

including PCA and supervised OPLS-DA, to assess metabolic heterogeneity among six cruciferous leafy vegetables three commonly consumed (cabbage, radish, watercress) and three less commonly consumed (broccoli, cauliflower, turnip) collected from the same origin. The main goal of this study was to investigate the heterogeneity of secondary metabolites in these six cruciferous leafy vegetables, with emphasis on GLSs and phenolic compounds, and to highlight the nutritional and potential health relevance of underutilized leafy parts. Such comprehensive profiling may support the valorization of agricultural by-products and contribute to their future use in functional foods and nutraceutical development.

2. Results & Discussion

2.1. Metabolites identification in Cruciferous leaves *via* UPLC-MS/MS

UPLC-MS/MS analysis led to the annotation of 149 metabolites in the examined cruciferous vegetables based on accurate mass, fragmentation patterns, comparison with literature, and in-house database searching. The derived base peak chromatograms for different extracts showed both qualitative and quantitative differences among the examined crucifers. Analysis was carried out in both negative (**Fig. 1**) and positive (Suppl. **Fig. S1**) ionization modes, providing greater coverage of the crucifer metabolome belonging to different classes including glucosinolates (GLS) (15 Peaks), phenolic acids (16 peaks), flavonoids (42 peaks), anthocyanidins (9 peaks), amino acids (10 peaks), fatty acids/fatty acyl glycosides (20 peaks), sulfolipids (3 peaks), and sterols (4 peaks), in addition to a number of identified metabolites belonging to different classes (**Fig. 2**). Due to their sulfate glucose core, Brassicaceae-specific glucosinolates ionize more readily in negative electrospray ionization mode (ESI) and can be better detected²⁰. Also, secondary metabolites such as phenylpropanoids and flavonoids can be more readily detected in negative mode due to their relatively acidic nature. In contrast, metabolites showed improved detection

in positive mode including polyamines, alkaloids, anthocyanins, and sterols, warranting the value of employing both modes in detection. Metabolite identification was based on their molecular formula, elution order, and main fragment ions in comparison to previously reported data (**Table 1**) and is detailed in the next subsections for each metabolite class.

2.1.1. Phenolic acids and their glycosides

The negative ionization mode led to the detection of 16 phenolic acids either free or conjugated with sugars and/or organic acids. The major phenolic acids detected in cruciferous leafy vegetables included quinic acid (peak 9) [(M-H)⁻ m/z 191.0566 (C₇H₁₁O₆)⁻], cinnamic acid (peak 28) [(M-H)⁻ m/z 147.0456 (C₉H₇O₂)⁻], and ferulic acid (peak 102) [(M-H)⁻ m/z 193.0506 (C₁₀H₉O₄)⁻]. Phenolic acid glycosides were also detected in gentisic acid-*O*-hexoside (peak 32) [(M-H)⁻ m/z 315.0737 (C₁₃H₁₅O₉)⁻], vanillic acid-*O*-hexoside (peak 33) [(M-H)⁻ m/z 329.0884 (C₁₄H₁₇O₉)⁻], feruloyl-*O*-hexoside (peak 61) [(M-H)⁻ m/z 355.1042 (C₁₆H₁₉O₉)⁻], and caffeoyl-*O*-hexoside (peak 109) [(M-H)⁻ m/z 341.0915 (C₁₅H₁₇O₉)⁻] with the main fragment ion peaks at m/z 153.0194, m/z 167.0351, m/z 193.0520, m/z 223.0560 and 180.9824, respectively, corresponding to the deprotonated phenolic acid aglycone. Sinapic acid and sinapoyl esters are commonly reported in Brassicaceae family ^{21,22}, and to account for its leafy taste. Several sinapic acid glycosidic conjugates were annotated including sinapoyl-*O*-hexoside (peak 69) [(M-H)⁻ m/z 385.1133 (C₁₇H₂₁O₁₀)⁻] with fragment ion at m/z 223.0560 [M-H-162]⁻ of deprotonated sinapic acid, , and peak 104 for sinapoyl-*O*-malate [(M-H)⁻ m/z 339.0741 (C₁₅H₁₅O₉)⁻] that was detected only in turnip with fragment ion peak at m/z 223.0618 of deprotonated sinapic acid. Malate esters were likewise detected in phenolic acids including *p*-coumaroyl-malic acid (peak 101) [(M-H)⁻ m/z 279.0526 (C₁₃H₁₁O₇)⁻] found exclusively in turnip, and feruloyl-*O*-malate (peak 105) [(M-H)⁻ m/z 339.0741 (C₁₅H₁₅O₉)⁻], based on the main loss of 116 amu corresponding to malic acid moiety. The examined 6 Brassicaceae samples showed qualitative

differences in phenolic acid esters, in which feruloyl malate [(M-H)⁻ *m/z* 339.0741 (C₁₅H₁₅O₉)⁻] and coumaroyl malate [(M-H)⁻ *m/z* 339.0741 (C₁₅H₁₅O₉)⁻] were most prominent in turnip and radish.

Organic acids such as gluconic acid (peak 2) [(M-H)⁻ *m/z* 195.0507 (C₆H₁₁O₇)⁻], malic acid (peak 10) [(M-H)⁻ *m/z* 133.0331 (C₄H₅O₅)⁻] and citric acid (peak 13) [(M-H)⁻ *m/z* 191.0186 (C₆H₇O₇)⁻] were also detected, in which gluconic acid was the most abundant acid in 3 brassica taxa including the non-edible leaves, while present at low levels in turnip, radish, and watercress. Organic acids, including malic and citric acids, play a pivotal role in food safety acting as acidulant preservative ²³.

2.1.2. Flavonoid glycosides

A total of 42 flavonoids were annotated, of which 28 were flavonoid glycosides versus 14 acylated flavonoid glycosides. The identified flavonoids were mainly glycosylated derivatives of three flavonols viz., kaempferol, quercetin, and isorhamnetin, while only one apigenin conjugate was detected in turnip. The sugar moieties found in cruciferous vegetables typically occur as mono-, di-, tri-, tetra-, and pentaglycosides ²⁴, they are also commonly found acylated by different hydroxycinnamic acids ²⁵, to further improve their absorption and cellular target interaction with myriad of health benefits including antimicrobial, antiparasitic, anti-inflammatory, anti-nociceptive, analgesic, and anti-complementary ²⁶. In MS/MS analysis, the nature of sugars and acyl groups could be revealed from elimination of the sugar residues, that are 162 amu for hexose (C₆H₁₀O₅), 146 amu for deoxyhexose (C₆H₁₀O₄) and 132 for pentose (C₅H₈O₄), while acyl groups showed the losses of 176 amu for feruloyl, 162 amu for caffeoyl, 146 amu for *p*-coumaroyl, 206 for sinapoyl, 192 amu for methoxycaffeoyl and 116 for malic acid moieties. Analysis of crucifers revealed that the highly glycosylated flavonoids, including tetra- and penta-hexosides are more characteristic of the four *Brassica* taxa, albeit not detected in radish and watercress. In detail, highly glycosylated non-acylated flavonoids were

found in negative mode as tetra-glycosides of quercetin, kaempferol, and isorhamnetin in peaks 40 with m/z 933.2563, peak 41 with m/z 933.2478, peak 99 with m/z 933.1791, and peak 66 with m/z 887.2451 ($C_{38}H_{47}O_{24}$). MS² Fragment ions further distinguished between structural isomers in peaks 39, 45 and 99. For example in case of peak 40, fragment ion at m/z 771 [M-H-162 ($C_6H_{10}O_5$)]⁻, m/z 609 [M-H-162 ($C_6H_{10}O_5$)-162 ($C_6H_{10}O_5$)]⁻, m/z 489 [M-H-162 ($C_6H_{10}O_5$)-162 ($C_6H_{10}O_5$)-120]⁻ and aglycon fragment ion of kaempferol at m/z 285 [M-H-162 ($C_6H_{10}O_5$)-162 ($C_6H_{10}O_5$)-324 ($C_{12}H_{20}O_{10}$)]⁻ was assigned as kaempferol-*O*-dihexoside-di-*O*-hexoside. While MS² fragment ions for peak 41 at m/z 771 [M-H-162 ($C_6H_{10}O_5$)]⁻, m/z 447 [M-H-162 ($C_6H_{10}O_5$)-324 ($C_{12}H_{20}O_{10}$)]⁻ and m/z 301 [M-H-162 ($C_6H_{10}O_5$)-324 ($C_{12}H_{20}O_{10}$)-146 ($C_6H_{10}O_4$)]⁻ was identified as quercetin-*O*-dihexoside-*O*-hexosyl-deoxyhexoside. Peak 99 MS² fragment ions at m/z 771 [M-H-162 ($C_6H_{10}O_5$)]⁻, m/z 639 [M-H-162 ($C_6H_{10}O_5$)-132 ($C_5H_8O_4$)]⁻, m/z 477 [M-H-162 ($C_6H_{10}O_5$)-132 ($C_5H_8O_4$)-162 ($C_6H_{10}O_5$)]⁻ and m/z 315 relative to isorhamnetin post loss of another hexose moiety was identified as isorhamnetin-*O*-pentosyl-tri-*O*-hexoside (Suppl. **Fig. S1**).

Peak 66 showed MS² fragment ions at m/z 755 [M-H-132 ($C_5H_8O_4$)]⁻ due to loss of pentose moiety, m/z 593 [M-H-132 ($C_5H_8O_4$)-162 ($C_6H_{10}O_5$)]⁻, and m/z 431 [M-H-132 ($C_5H_8O_4$)-162 ($C_6H_{10}O_5$)-162 ($C_6H_{10}O_5$)]⁻ assigned it as kaempferol-*O*-pentosyldeoxyhexoside-di-*O*-hexoside.

A total of 14 flavonol glycosides were acylated with hydroxycinnamic acids i.e., ferulic, caffeic, sinapic, and coumaric acid. They were detected most prominent in the 4 cruciferous viz., cabbage, broccoli, cauliflower, and turnip. For example, peak 36 [(M-H)⁻ m/z 1095.2897 ($C_{48}H_{55}O_{29}$)]⁻ showed MS² fragment ions at m/z 933.2426 [M-H-162 ($C_9H_6O_3$)]⁻ due to the loss of caffeic acid moiety, m/z 787.1872 [M-H-162 ($C_9H_6O_3$)-146 ($C_6H_8O_4$)]⁻ relative to the loss of deoxyhexose sugar and m/z 463.0463 [M-H-162 ($C_9H_6O_3$)-146 ($C_6H_8O_4$)-324 ($C_{12}H_{20}O_{10}$)]⁻ due to the successive sugar loss ultimately yielding aglycon fragment ion at m/z 301 annotated as quercetin-*O*-caffeoyldeoxyhexosyl-*O*-tri-hexoside (Suppl. **Fig. S2**). Peak 80 [(M-H)⁻ m/z

725.1912 ($C_{32}H_{37}O_{19}^-$) showed fragment ions at m/z 593.1489 [M-H-132 ($C_5H_8O_4$)]⁻ due to the loss of pentose moiety, m/z 417.0817 [M-H-132 ($C_5H_8O_4$)-176 ($C_{10}H_8O_3$)]⁻ for the loss of acyl and pentose moieties, identified as kaempferol- *O*-acyl-*O*-di-pentoside (Suppl. **Fig. S3**). A number of identified flavonoids were detected only on radish this include 3 non-acylated flavonoid glycosides as in peak 75 [(M-H)⁻ m/z 725.1937($C_{32}H_{27}O_{19}^-$)], peak 92 [(M-H)⁻ m/z 739.2100 ($C_{33}H_{39}O_{19}^-$)], and peak 93 [(M-H)⁻ m/z 739.2052 ($C_{33}H_{39}O_{19}^-$)], in addition to 3 acylated flavonoid glycosides as in peaks 67 [(M-H)⁻ m/z 741.1876 ($C_{35}H_{33}O_{18}^-$)], peak 80 [(M-H)⁻ m/z 725.1912 ($C_{32}H_{37}O_{19}^-$)], peak 90 [(M-H)⁻ m/z 739.2076 ($C_{33}H_{39}O_{19}^-$)] Thus radish may be considered as a prospective extract with potential effects such as antimicrobial, antiparasitic, anti-inflammatory, and analgesic effects ²⁷. A proanthocyanidin was identified in peak 128 [(M-H)⁻ m/z 881.4929($C_{45}H_{37}O_{19}^-$)] with fragment ions at m/z 593 [M-H-288]⁻ and m/z 305 [M-H-288-288]⁻ due to successive losses of catechin units with quinone methide (QM) fragmentation and appearance of gallocatechin fragment ion peak at m/z 305 identified as gallocatechin-(epi)catechin-(epi)catechin detected in all extracts except cauliflower and cabbage ²⁸ (Suppl. **Fig. S8**).

2.1.3. Anthocyanins

Anthocyanins are widely distributed as colored pigments in different fruits and vegetables ²⁹. In crucifers, anthocyanins are highly conjugated with different sugars and/or acyl groups to improve their stability ³⁰. Due to their existence in a positively charged cationic form, they are more sensitive to be detected in positive ionization mode for such flavonoid subclass ²⁹ and confirmed in this study. Nine anthocyanins were detected belonging to either cyanidin or delphinidin aglycones. For example, peak 39 [(M+H)⁺ m/z 935.2672 ($C_{39}H_{51}O_{26}^+$)]⁺ showed MS/MS ions at m/z 773 [M+H-162]⁺, m/z 611[M+H-2x 162 amu]⁺, and finally m/z 287 for its aglycone post losses of hexose moieties assigned as cyanidin-*O*-tetra-hexoside-identified in broccoli

and cabbage (Suppl. **Fig. S7**). Peak 62 [(M+H)⁺ m/z 773.2213 (C₃₃H₄₁O₂₁)⁺] is a delphinidin derivative showed MS/MS ions at m/z 611 [M+H-162]⁺, m/z 449 [M+H-2x 162 amu]⁺, and finally m/z 303 for delphinidin aglycone post losses of deoxyhexose moiety identified as delphinidin-*O*-deoxyhexoside-di-*O*-hexoside detected in radish and watercress. Among the examined crucifers, anthocyanins were detected in radish followed by turnip, in contrast no anthocyanins were detected in cauliflower. A thorough examination for radish and turnip leaves will be of interest owing to the anti-mutation, liver protection, and inhibiting the metastasis of tumor cells properties of such metabolites ³¹.

2.1.4. Glucosinolates (GLS)

GLS were detected in the negative ion mode due to the sulfate moiety showing characteristic fragment ions at m/z 275, 259, 241, 195, and 97 ³². Fragment ion at m/z 259 corresponded to sulfated hexose moiety, while m/z 195 was attributed to the thio-glucose moiety. A total of 15 GLS were identified to be more prevalent in non-edible crucifers than edible ones (**Fig. 3**). This includes peak 14 [(M-H)⁻ m/z 422.0228 (C₁₁H₁₉NO₁₀S₃)⁻] identified as glucoiberin and detected in cabbage and turnip only. Characteristic MS² fragment ions at m/z 358.0237 [M-H-64 (CH₃SO)]⁻ indicative of a loss of methyl sulfoxide, m/z 195.0123 of thio-glucose residue and m/z 96.6577 of the sulfate moiety led to its assignment as glucoiberin ³³ (Suppl. **Fig. S4**), first time to be reported in turnip. Another GLS found only in turnip assigned as gluconapinin peak 15 [(M-H)⁻ m/z 372.0422 (C₁₁H₁₈NO₉S₂)⁻] with MS² product ions at m/z 292.087 [M-H-80 (SO₃)]⁻ due to the neutral loss of 80 amu (SO₃), and m/z 195.057 of thio-glucose moiety (Suppl. **Fig. S5**), following other previously reported studies ³⁴. Peak 16 [(M-H)⁻ m/z 436.0426 (C₁₂H₂₂NO₁₀S₃)⁻] is a prominent GLS identified in broccoli, cauliflower, watercress and radish, exhibiting the main fragment ion peak at m/z 195.0421 of thio-glucose residue and identified as glucoraphanin previously reported in *Brassica* species ³⁵ (Suppl. **Fig. S6**).

Several GLS were detected exclusively in radish and warranting for future studies on vegetables targeting its GLS profile including peak 17 [(M-H)⁻ *m/z* 434.0255 (C₁₂H₂₀NO₁₀S₃)⁻], peak 64 [(M-H)⁻ *m/z* 508.0958 (C₁₆H₃₀N₂O₁₁S₃)⁻], and peak 76[(M-H)⁻ *m/z* 506.1129 (C₁₇H₃₂NO₁₀S₃)⁻] identified as glucoraphenin, (methylsulfonyl)octylglucosinolate, and glucoarabin (methylsulfinyl)nonylglucosinolate. These are aliphatic GLS detected previously in cabbage ¹⁸ and first time reported in radish. In contrast to aliphatic GLS that showed diversity in composition among examined crucifers, indole GLS were detected in all examined crucifers. A major indole-derived GLS detected in all examined Brassicaceae, though most prominent in radish was methoxyspirobrassin in peak 125 [M-H]⁻ *m/z* 279.1610(C₁₂H₂₇N₂ OS₂)⁻]. Although some of the identified GLS were first reported in radish and turnip, non-edible broccoli and cauliflower showed to have more identified GLS. This raises interest in the valorization of such by-products for further analysis. Although certain GLS were newly reported in radish and turnip, non-edible species such as broccoli and cauliflower exhibited higher GLS diversity and abundance, suggesting potential valorization of these agricultural by-products as rich sources of bioactive GLS. This observation aligns with growing interest in the recovery of functional compounds from cruciferous waste streams for nutraceutical and pharmaceutical applications.

2.1.5. Fatty acids and fatty acyl esters

A total of 20 fatty acids were detected in examined crucifers, exhibiting relatively minor variation among examined taxa compared to secondary metabolites such as GLS and flavonoids. Major prominent peaks identified in all samples included peaks 124, 127, 137, and 130 of which several are newly reported in crucifers belonging to hydroxylated fatty acids. Peak 124 [M-H]⁻ *m/z* 327.2721 (C₁₈H₃₁O₅)⁻] showed fragment ion peaks at *m/z* 309.2078 [M-H-18]⁻ and *m/z* 291.1971 [M-H-18-18]⁻ due to successive losses of water molecules, identified as trihydroxyoctadecadienoic acid most

prominent in broccoli, radish, and turnip. Peak 127 [(M-H)⁻ m/z 307 (C₁₈H₂₇O₄)⁻] showed fragment ion peaks at m/z 289.1532[M-H-18]⁻ and m/z 235.1342 [M-H-18-18]⁻ due to successive losses of water identified as hydroxy-oxo-octadecatrienoic acid being most prominent in broccoli and cabbage. Likewise, peak 137 [M-H]⁻ m/z 295.2276 (C₁₈H₃₁O₃)⁻] with MS² fragments at m/z 277.1036 [M-H-18]⁻ due to loss of water and m/z 195 corresponding to OH-CH (CH₂)₈COO⁻ fragment identified as hydroxylinoleic acid. Two A glycosylated oxylipid peak 145 [M-H]⁻ m/z 417.2856 (C₂₂H₄₁O₇)⁻] are detected in all examined cruciferous leaves except for cabbage and is first time to be reported in Brassicaceae. Identification was based on main fragment ion corresponding to the loss of hexose moiety with MS² fragment at m/z 255.232 as in case of peak 130 [M-H]⁻ m/z 721 (C₃₄H₅₇O₁₆)⁻. Because fatty acids and their derivatives contribute not only to the nutritional lipid profile of cruciferous vegetables but also to their bioactivity and health-promoting effects, their identification provides an important link between metabolomic profiling and potential functional food applications.

2.1.6. Sulfolipids

Sulfoquinovosyl monoacylglycerols are glycolipids (SQMGs) composed of a diacylglycerol esterified with fatty acids and a sulfoquinovose moiety ³⁶. Three sulfolipids were identified in extracts that are first time to be reported in Brassica showing improved detection in negative mode and suggestive the sulphur is incorporated in lypophilic metabolites in crucifers. MS/MS spectrum of peak 142 [M-H]⁻ m/z 791.4956 (C₄₁H₇₅O₁₂S)⁻ showed fragment ions at m/z 537 corresponds to loss of (C16:0) fatty acid and m/z 249 corresponds to the deprotonated sulfoquinovose identified as SQDG (16:1/16:0) and was detected only in non-edible broccoli and cauliflower. Indeed, broccoli, cauliflower and turnip were the most rich in SQMGs exemplified by SQDG (18:3/16:0) in peak 143[(M-H)⁻ m/z 817.424 (C₄₃H₇₇O₁₂S)⁻] showing fragment ions at m/z 539, m/z 561 corresponding to [M-RCOOH]⁻ where RCOOH are the C18:3 and C16:0 fatty acids,

respectively, and a fragment ion peak at m/z 297.2425 [539–243 (C₆H₁₁O₈S)]⁻, due to the loss of sulfoquinovosyl anion (Suppl. **Fig. S9**). In contrast, peak 144 [(M-H)⁻ m/z 819.436 (C₄₃H₇₉O₁₂S⁻)] showed fragment ions at m/z 566.3446, m/z 540.3283 corresponding to [M-RCOOH-H]⁻ where RCOOH are the C18:1 and C16:1 fatty acid, respectively, identified as SQDG (18:1/16:1) was detected in all most extracts. Within examined extracts, broccoli and cauliflower appeared most rich in sulfolipids, and yet to be tested for their role in health effects or to be targeted for isolation from these sources ³⁷.

2.1.8. Nitrogenous compounds

A total of 6 nitrogenous compounds were detected exclusively in cauliflower and radish including peak 56 [(M-H)⁻ m/z 452.1662 (C₁₄H₃₀NO₁₅⁻)] with MS² fragments at m/z 362, 184 and 179, peak 58 [(M-H)⁻ m/z 372.1271 (C₁₆H₂₂NO₉⁻)] and peak 73 [(M-H)⁻ m/z 210.0787 (C₁₀H₁₂NO₄⁻)] with 162 amu mass differences, and annotated as methoxytyrosin-*O*-hexoside and methoxytyrosin, respectively, posing cauliflower and radish as potential sources of these amino acid conjugates. Although the presence of methoxylated derivatives of tyrosine and related amino acids is previously reported in Brassica ³⁸, methoxytyrosin-*O*-hexoside and methoxytyrosin are first time to be identified in cauliflower and radish.

2.2. Multivariate data analysis of cruciferous leaves UPLC-MS/MS metabolite dataset

Unsupervised principal component analysis (PCA) model was employed to assess metabolite heterogeneity among examined crucifer leaves. Further, supervised orthogonal projection to latent structures discriminant analysis (OPLS-DA) was employed for classification between edible and non-edible cruciferous, alongside identification of markers.

2.2.1. Unsupervised PCA analysis of crucifer leaves

A PCA model (**Fig. 4**) was performed for both positive and negative metabolite datasets, resulting in two independent PCs with $R^2= 0.799$ and $Q^2= 0.607$, and $R^2= 0.717$ and $Q^2= 0.499$, respectively. The PCA score plot of the negative mode dataset (**Fig. 4A**) revealed clear differentiation of turnip on the left side of PC1, indicating its distinct composition compared to other cruciferous situated in five separate clusters consisting of radish, watercress, cabbage, broccoli, and cauliflower along PC2. Metabolites that influenced segregation were revealed from the corresponding loading plot (**Fig. 4B**) indicating that some polyphenols such as ferulic acid derivatives (peak106), rhamnetin-*O*-hexoside (peak113), as well as trihydroxyoctadecadienoic acid (peak124) were enriched in turnip, turnip, and posing as a rich source of polyphenols in crucifers, yet to be tested using proximate assays for phenolics. In contrast, the presence of gluconic acid accounted for cabbage, broccoli, and cauliflower separate clustering on the positive side of PC1. Gluconic acid was detected at relatively much higher levels in these crucifers compared to turnip, though of less taxonomical value as primary metabolite.

Similarly, PCA score plot for positive mode (**Fig. 4C**) confirmed the distinct turnip composition, being positioned far to the left of PC1. The close segregation of broccoli and cabbage on the positive left side implied similar metabolite composition and suggests that nonedible broccoli leaves share similarities with edible cabbage. Analysis of the corresponding loading plot (**Fig. 4D**) revealed richness in cyanidin-*O*-dihexoside-*O*-dihexoside and phenylalanine-*O*-hexoside in turnip. In contrast, the segregation of broccoli and cabbage was influenced by their richness in indole-acetic acid (peak 122). Additionally, loading plot revealed acyl carnitines exemplified by glutaroyl-carnitine (peak 21), fructosyl-valine (peak18), and methylmalonylcarnitine peak 12 in radish, cauliflower, and watercress posing them as potential source of acylcarnitines, especially considering

their anti-obesity effects as they play a pivotal role in energy production and metabolism of fatty acids ³⁹.

2.2.2. Supervised OPLS-DA modelling of crucifer leaves

Compared to unsupervised PCA analysis, OPLS-DA allows for the maximum separation between groups and can identify key metabolites that contribute to the differentiation in a supervised pairwise manner. The first OPLS-DA model was developed to confirm turnip marker metabolites that showed distinct separation from other cruciferous vegetables in PCA analysis (**Fig. 4**). OPLS-DA Models were constructed for positive (**Fig. S10A**) and negative (**Fig. S10C**) ion mode dataset revealed distinct separation of turnip from other crucifers along the predictive component. The models showed high predictive capability with $Q^2 = 0.98$, $R^2Y = 0.99$ and $Q^2 = 0.99$, $R^2Y = 0.99$, for positive and negative datasets, respectively. Moreover, permutation tests confirmed the robustness of the models with negative Q^2 intercept values and further validated the significance of the observed separation between turnip and other cruciferous vegetables. CV-ANOVA tests with p values less than 0.05 indicated model significance, further supporting the observed separation between turnip and other cruciferous vegetables. Metabolites that influence such patterns can be verified from the respective loading S-plots of negative (**Fig. S10B**) and positive (**Fig. S10D**) datasets. Interestingly, mostly polyphenols viz. ferulic acid, and rhamnetin-O-hexoside and cyanidin-O-dihexoside-O-dihexoside, that appeared to be significantly higher in turnip compared to other crucifer vegetables, and in agreement with PCA results. In addition, turnip was also enriched in primary metabolites viz. tri-hydroxyoctadecadienoic acid and phenylalanine-O-hexoside, which further contribute to its unique metabolite composition compared to other cruciferous vegetables.

The second OPLS-DA model aimed to analyze metabolite composition of cabbage as an edible cruciferous vegetable and compared with other vegetables. The OPLS-DA analysis (**Fig. S11**) revealed clear differentiation

of cabbage and other cruciferous vegetables, indicating that cabbage has a distinct flavonoid composition. Of these flavonoids, quercetin-*O*-deoxyhexosyl-*O*-hexoside, quercetin-dihexoside-*O*-deoxyhexoside were enriched in cabbage compared to other crucifers/quercetin has potential antioxidant, anti-inflammatory, in addition to anti-cancer properties⁴⁰. Other metabolites belonging to fatty acids i.e stearidonic acid and hydroxylinolenic acid were found enriched in cabbage compared to other cruciferous vegetables. Another OPLS-DA (**Fig. S12**) was employed to model broccoli against other edible crucifers, showing clear separation of broccoli along the predictive component. Key markers for broccoli included quinic acid, neoglucobrassicin, and indole acetic acid.

A final OPLS-DA comparison was adopted to model cabbage as an example of edible cruciferous vegetable in one class against broccoli as non-edible crucifer in another class (**Fig. S13**). Clear separation was observed attributed to quercetin-*O*-deoxyhexosyl-*O*-hexoside, quercetin-dihexoside-*O*-deoxyhexoside, and gluconic acid richness in cabbage.

2.3. Marker metabolites quantification and Statistical analysis

The one-way ANOVA results revealed highly significant differences (FDR-adjusted $p < 0.001$) among cruciferous species for all identified marker metabolites (**Table 2**), confirming strong metabolic differentiation within the dataset. The abundance of top 10 significant metabolites based on peak area was listed in **Table S1**. Phenolic acids and flavonoid glycosides are the major contributors to interspecies metabolic variability within cruciferous vegetables. The boxplots of marker metabolites detected in the negative ionization mode (**Figure S14**) clearly demonstrate distinct species-dependent variations in metabolite accumulation among cruciferous vegetables. Turnip and watercress were enriched in quercetin-, rhamnetin-, and kaempferol-glycosides, while broccoli and cabbage exhibited higher levels of organic acids such as gluconic and malic acids. Lipid-derived

metabolites such as trihydroxyoctadecadienoic acid and palmitoleic-linolenic-*O*-hexoside were more prominent in broccoli and cauliflower. In the positive ionization mode (**Table 3**), all detected marker metabolites displayed highly significant interspecies differences ($p < 0.001$, FDR-adjusted $p < 0.001$), with large effect sizes ($\eta^2 > 0.92$), indicating strong metabolic discrimination among cruciferous vegetables. The abundance of top 10 significant metabolites based on peak areas was listed in **Table S2**. The most discriminative metabolites were the anthocyanin derivatives delphinidin-*O*-deoxyhexoside and cyanidin-*O*-tetra-hexoside. Amino acid derivatives such as fructosyl-valine, hexose phenylalanine, and indole-acetic acid also showed strong species-dependent differences ($\eta^2 = 0.985$ - 0.990). Lipid-related compounds, including monolinolenin and stearidonic acid, exhibited slightly lower but still substantial η^2 values (0.974 - 0.981). Boxplots of the metabolites detected in the positive ionization mode (**Figure S15**) reveal clear interspecies differences in metabolite abundance, further emphasizing the metabolic diversity within cruciferous vegetables.

3. Conclusion

Secondary metabolites heterogeneity in 6 cruciferous leafy vegetables in the context of being edible and none-edible is introduced herein through UPLC-MS/MS-based metabolomics coupled with multivariate data analyses. UPLC-MS/MS analysis led to the detection of 149 peaks, including mostly phenolic acids, flavonoids, fatty acids/acyl esters, steroids, anthocyanins, and glucosinolates. Several identified metabolites are newly reported in crucifers belonging to hydroxylated fatty acids, oxylipid, sulfolipids, and tyrosine derivatives, besides a number of detected GLS for the first time in turnip include glucoiberin, and radish including (glucoraphenin, (methylsulfonyl)octylglucosinolate, and glucoarabin (methylsulfinyl)nonylglucosinolate)). Nonedible broccoli and cauliflower showed exclusively a number of identified compounds belonging to GLS, flavonoids and sulfolipids, posing them to be tested for their role in health effects or to be targeted for isolation from these sources. Turnip is shown to

have a more characteristic profile based on the identified metabolites belonging to phenolic acids, sinapoyl derivatives, and anthocyanins. Multivariate data analysis revealed that turnip was the most distinct among all examined leaves. ferulic acid derivatives, and rhamnetin-*O*-hexoside, alongside tri-hydroxyoctadecadienoic acid were key metabolites helped in discrimination of turnip from other samples. Quercetin-*O*-deoxyhexosyl-*O*-hexoside, quercetin hexoside deoxyhexoside-*O*-hexoside, gluconic acid, hydroxylinolenic acid, and palmitoleic-linolenic-*O*-hexoside were enriched in cabbage than other cruciferous leaves. Moreover, quinic acid, and neoglucobrassicin were enriched in broccoli as nonedible cruciferous vegetable while, quercetin-*O*-deoxyhexosyl-*O*-hexoside, quercetin-dihexoside-*O*-deoxyhexoside, and gluconic acid were enriched in edible cabbage leaves. A limitation of this study is the relatively small sample size, with only three biological replicates analyzed per variety. However, the consistent clustering and clear separation observed in the multivariate analyses (PCA and OPLS-DA) support the robustness and reproducibility of the detected metabolic patterns and have yet to be confirmed by analyzing specimens from other resources. Our study provides complementary phytochemical evidence that supports the functional components of cruciferous leaves to corroborate their utilization as functional foods or further in nutraceuticals.

4. Materials and Methods

4.1. Plant Material

Six cruciferous leaves *viz.* *Brassica oleracea* (cabbage), *B. oleracea* var. *Italica* (broccoli), *B. oleracea* var. *oleracea* (cauliflower), *B. rapa* (turnip), *Raphanussativus L.* (radish), and *Nasturtium officinale* (watercress) were collected from local farm in Qualuob, El-Qualuobia governorate (30.2220° N, 31.3084° E), Egypt, during October and November 2019. The botanical identification of the plants was confirmed by Prof. Dr. Rim Hamdy, Botany Department, Faculty of Science, Cairo University, Egypt. Fresh leaves were lyophilized and kept in airtight containers at -80 °C till preparation for

UPLC-MS/MS analysis. Metabolite extraction and LC-MS analysis were conducted in 2020. Three biological replicates were analyzed for each sample. A voucher specimen was deposited at the College of Pharmacy Herbarium, Cairo University, Cairo, Egypt.

4.2. Chemicals and reagents

Acetonitrile and formic acid (LC-MS grade) were obtained from J. T. Baker (The Netherlands); Milli-Q water was used for UHPLC analysis. Chromobond C18 (500 mg, 3 mL) cartridge was purchased from Macherey-Nagel (Düren, Germany). All other chemicals and standards were purchased from Sigma-Aldrich (St Louis, MO, USA).

4.3. Preparation of leaf extracts for UHPLC-MS analysis

The extraction of the cruciferous leaves was performed as previously described ⁴¹. Briefly, freeze dried leaves were ground with a pestle in a mortar, with 2 g of each leaf freeze dried powder homogenized with 5mL 100% MeOH containing 10 mg mL⁻¹ umbelliferone (an internal standard used for retention time alignment of UHPLC-MS features) using a Turrax mixer (11 000 rpm) for 20 sec periods with recession time of 1 min. Extracts were then vortexed vigorously and centrifuged at 3000 g for 30 min to remove plant debris. For solid phase extraction, 500 µL were aliquoted and loaded on a (500 mg) C18 cartridge, which was preconditioned with methanol and water. Samples were then eluted using 3 µL 70% MeOH and 3 µL 100% MeOH, and eluents were evaporated to dryness under a gentle nitrogen stream. The obtained dry residue was re-suspended in 500 µL 100% methanol for further UHPLC-MS analysis.

4.4. High-resolution UPLC-MS/MS analysis

The analytical conditions for high-resolution UHPLC-MS/MS were employed as previously described ⁴¹. The UHPLC analysis was performed on an Acquity UHPLC System (Waters) equipped with an HSS T3 column (100-1.0 mm, particle size 1.8 µm; Waters). The analysis was carried out by applying the following binary gradient at a flow rate of 150 mL min⁻¹: 0-1 min, isocratic 95% A (water/formic acid, 99.9/0.1 [v/v]), 5% B

(acetonitrile/formic acid, 99.9/0.1 [v/v]); 1–16 min, linear from 5 to 95% B; 16–18 min, isocratic 95% B; and 18–20 min, isocratic 5% B. The injection volume was 3.1 mL (full loop injection). Eluted compounds were detected from m/z 90 to 1000 using a MicroTOF-Q hybrid quadrupole time-of flight mass spectrometer (Bruker Daltonics) equipped with an Apollo- II electrospray ion source in negative and positive (deviating values in brackets) ion modes using the following instrument settings: nebulizer gas, nitrogen, 1.4 (1.6 bar); dry gas, nitrogen, 6.1 min⁻¹, 190 °C; capillary, 5000 V (+4000 V); end plate offset, 500 V; funnel 1 RF, 200 Vpp; funnel 2 RF, 200 Vpp; in-source CID energy, 0 V; hexapole RF, 100 Vpp; quadrupole ion energy, 5 eV (3 eV); collision gas, argon; collision energy, 7 eV (3 eV); collision RF, stepping 150/350 Vpp (200/300Vpp), (timing 50/50); transfer time, 58.3 ms; prepulse storage, 5 ms; pulser frequency, 10 kHz; and spectra rate, 3 Hz. Internal mass calibration of each analysis was performed by infusion of 20 mL 10 mM lithium formate in isopropanol: water, 1 : 1 (v/v), at a gradient time of 18 min using a diverter valve. For Auto-MS/MS analysis, precursor ions were selected in Q1 with an isolation width of 3–10 Da and fragmented at collision energies of 15–70 eV using argon as a collision gas. Product ions detection was performed using the same settings as above, but with funnel 2 RF 300 Vpp in negative mode. Metabolites were characterized by their retention times relative to external standards, accurate MS, and the domino MS/MS spectra in comparison to our in-house database, phytochemical dictionary of natural products database, and reference literature. Levels of annotation were reported according to the guidelines of the metabolomics society compound identification work group at their annual meeting (Brisbane, Australia) ⁴². Pattern isotopic similarity is already used in formula prediction by Bruker compass program. Metabolite annotation was accepted only when isotopic pattern fit exceeded 85%, and the observed mass error was below 10 ppm; few signals with mass deviation > 10 ppm were manually re-evaluated and excluded when confidence was low. Each crucifer species was represented by three independent biological

replicates, which were extracted and analyzed separately to ensure reproducibility and robust statistical comparison.

4.5. MS data processing for multivariate data analysis

Features extraction of the examined cruciferous leaves MS files was performed using XCMS package under R 2.9.2 environment. The script employed for peak detection, alignment, and grouping of the extracted ion chromatograms (**Suppl. Code S1**) follows the exact steps described in ⁴¹. Normalization and Pareto scaling of the features prior to modelling were performed prior to multivariate models *i.e.*, PCA and OPLS-DA using SIMCA-P Version 13.0 (Umetrics, Umea, Sweden).

Data availability statements

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Authorship contribution

E. M. K.: Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **M. H. B.:** Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **A. S.:** Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **S. D.:** Methodology, Review & editing. **M. A. F.:** Conceptualization, Methodology, Investigation, Writing - review & editing.

Acknowledgements

Dr. Mohamed Farag acknowledges the Alexander von Humboldt foundation, Germany.

Declarations

Plant ethics

The botanical identification of the plants was confirmed by Prof. Dr. Rim Hamdy, Botany Department, Faculty of Science, Cairo University, Egypt. The methods in plant collection and experimentation were carried out in accordance with the guidelines prescribed by the American Society of Plant Taxonomists and adopted by the institutional research committee.w

Competing interests

The authors declare that they have no competing interests.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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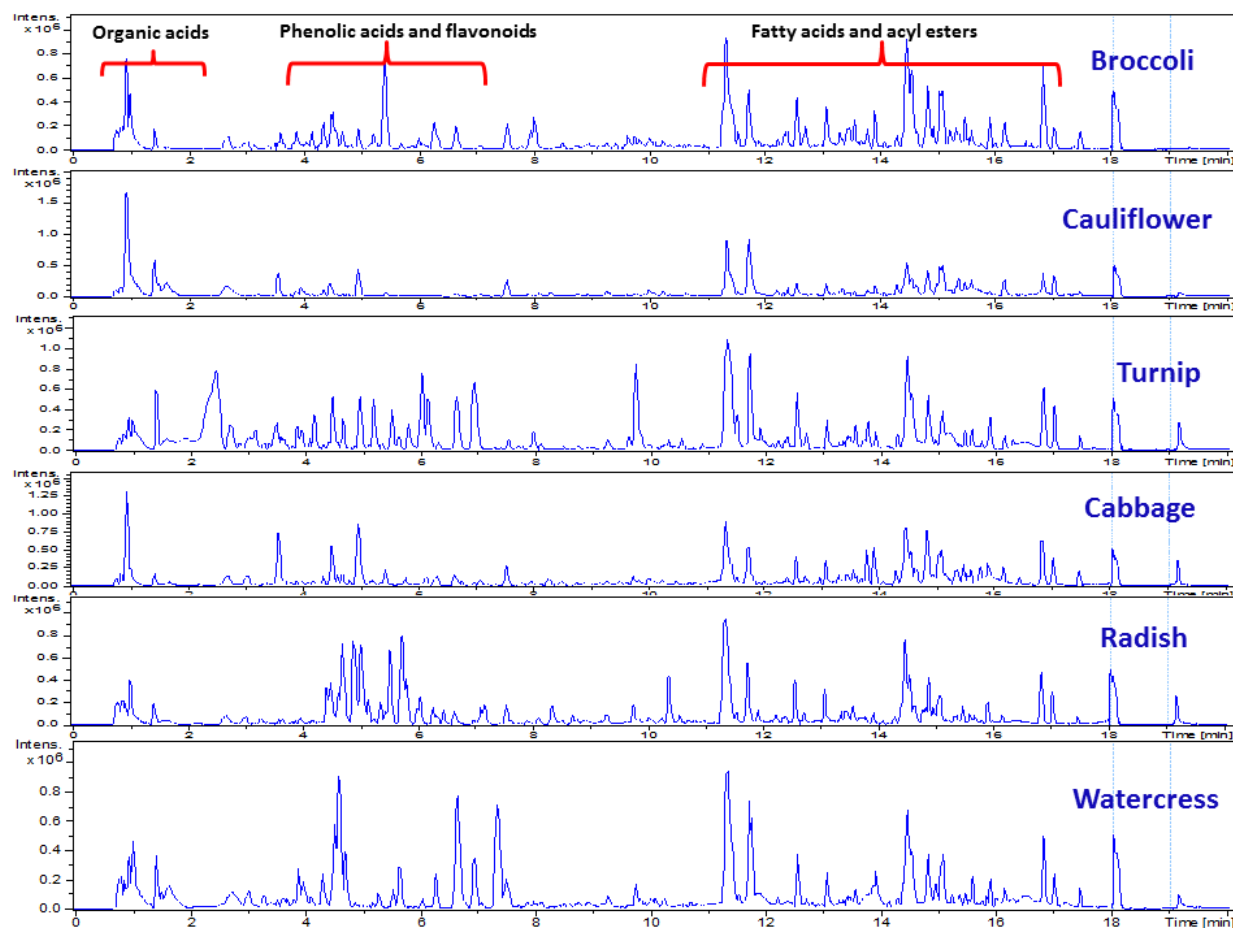


Fig. 1. Representative UPLC-MS/MS chromatogram of cruciferous leaves methanol extracts traces analyzed in the negative ion mode

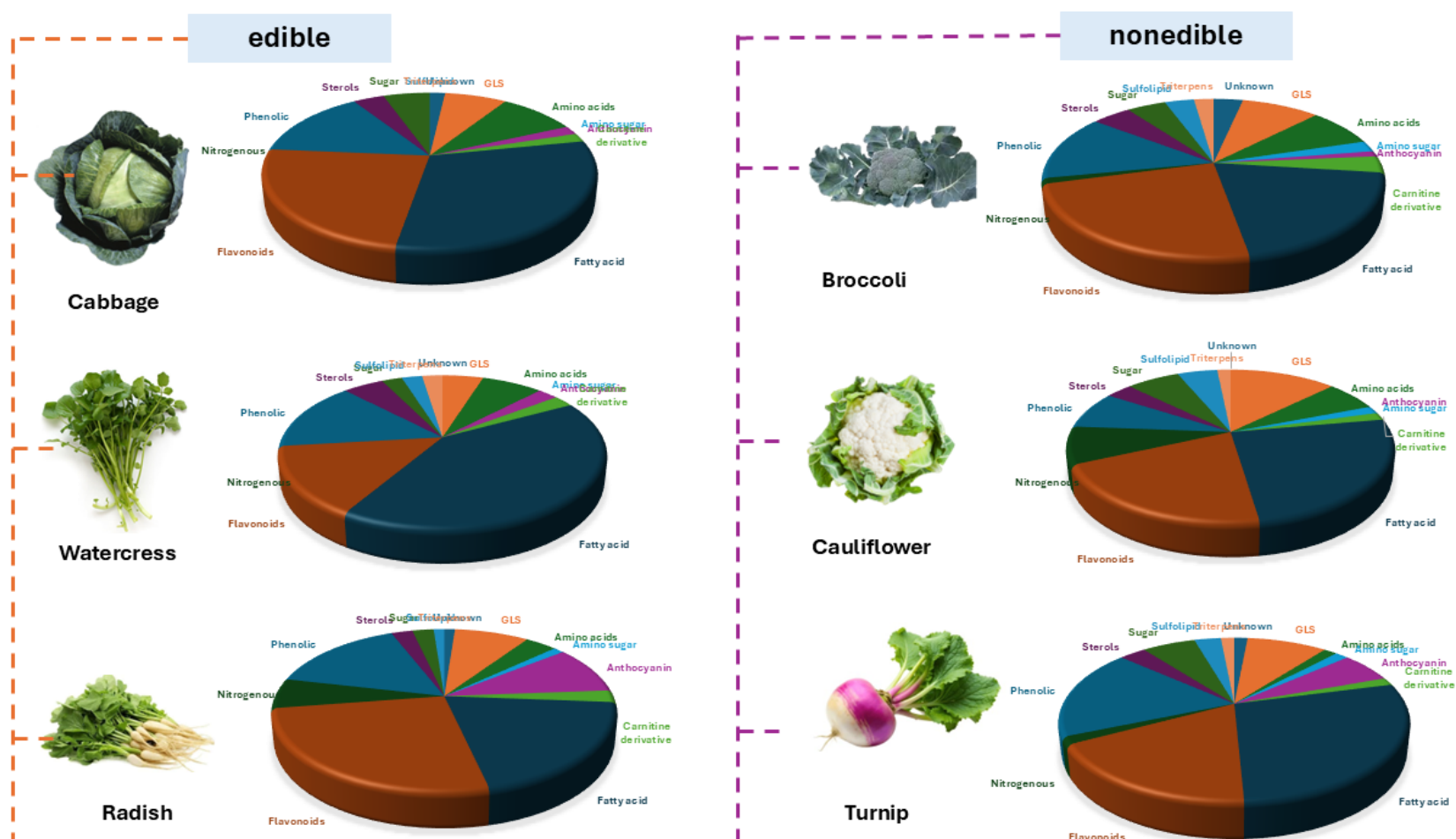
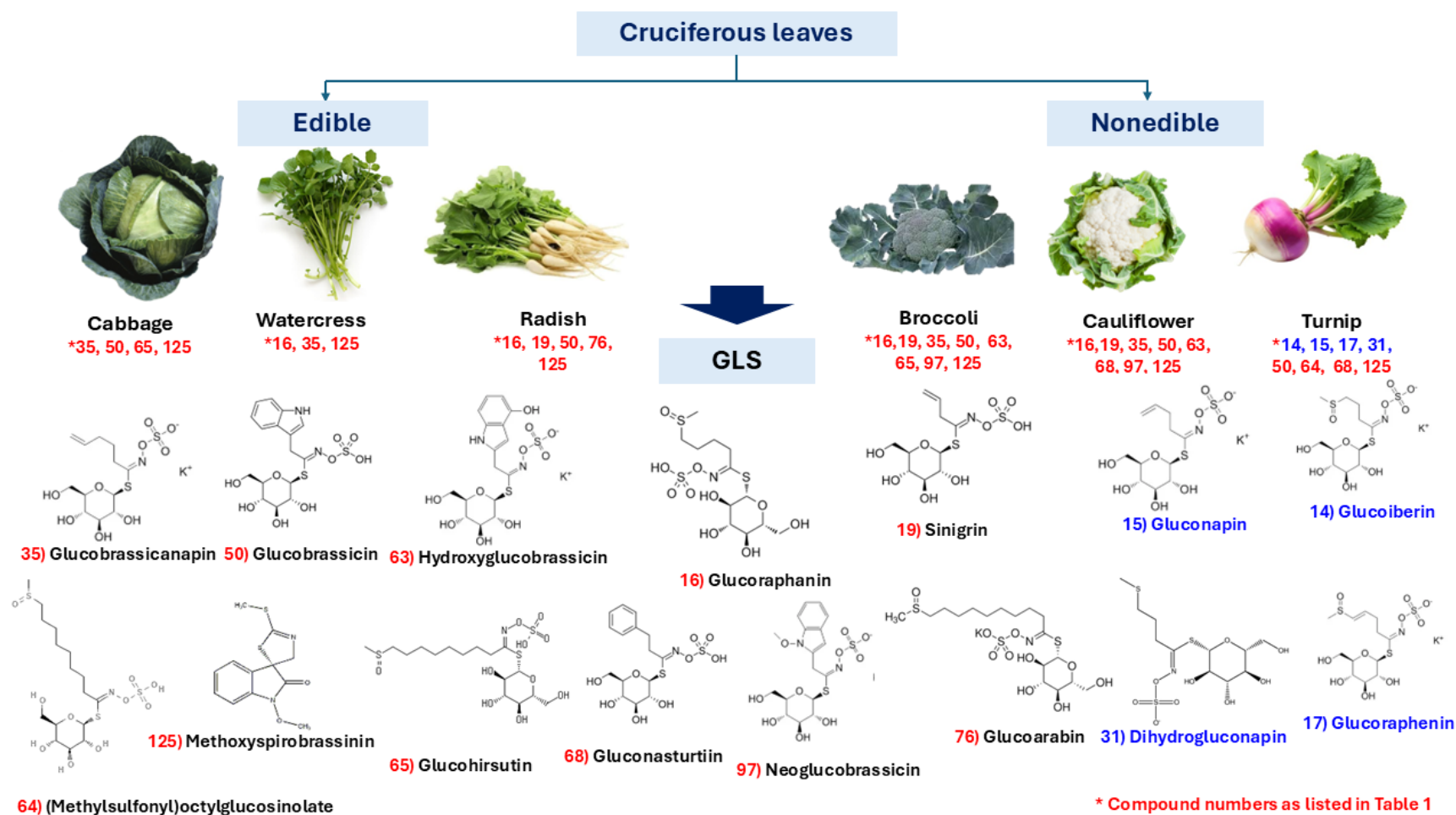


Fig. 2. Pie charts representing the major classes identified in both edible and nonedible cruciferous leaves, the charts were generated based on the number of peaks identified for each class.

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Fig. 3. Identified glucosinolates in edible and nonedible cruciferous leaves, the compound in blue color were identified as markers in turnip only.

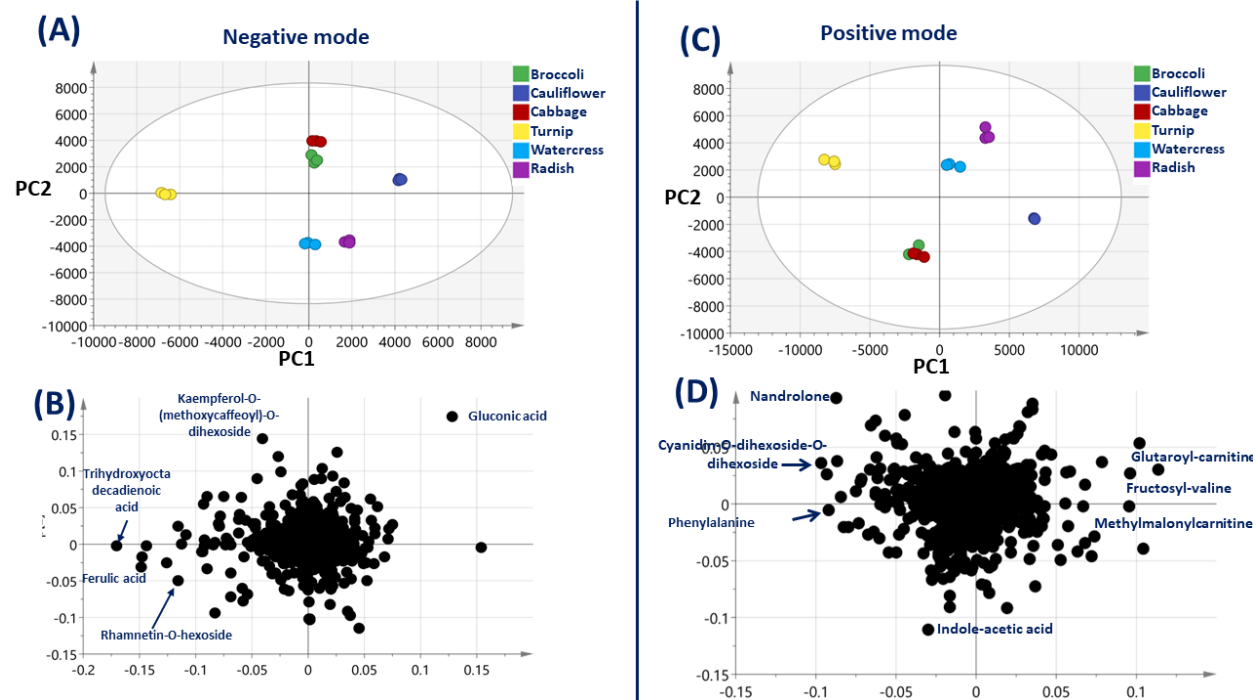


Fig. 4. Unsupervised multivariate data analyses of the studied cruciferous leaves derived from modeling secondary metabolites dataset analysed via UHPLC-HRMS/MS. **(A)** PCA score plot of positive mode with PC1 vs PC2. **(B)** Loading plot for positive mode providing variant peaks and their assignments. **(C)** PCA score plot of negative mode with PC1 vs PC2. **(D)** Loading plot for negative mode providing variant peaks and their assignments.

Table 1: Identification of secondary metabolites in cruciferous vegetables detected in UPLC-MS/MS analysis in both positive and negative mode

N o	Rt min	Compound name	Chemical class	[M-H] ⁻ / [M+H] ⁺	Neutral Molecular formula	Neutral Molecular formula	err or	MS/MS fragmen ts	Referenc es	Broccoli	cauliflow er	Cabbage	turnip	Radish	Watercre ss
1.	0.94	Hexose	Sugar	[M-H] ⁻ 179.0569	C ₆ H ₁₂ O ₆	C ₆ H ₁₂ O ₆	- 4.2	161.0471	⁴³	+	+	+	+	+	+
2.	0.95 2	Gluconic acid	Organic acid	[M-H] ⁻ 195.0507	C ₆ H ₁₁ O ₇ ⁻	C ₆ H ₁₂ O ₇	1.5	75.0091 129.0197	²²	+	+	+	+	+	+
3.	0.96 1	Glutamine	Amino acid	[M+H] ⁺ 147.0764	C ₅ H ₁₁ N ₂ O ₃ ⁺	C ₅ H ₁₀ N ₂ O 3	- 9.3	130.0500 102.0550 85.0480	⁴⁴	+	+	+	-	+	+
4.	0.96 5	Unknown	Sugar	[M-H] ⁻ 307.1173	C ₁₂ H ₁₉ O ₉ -	C ₁₂ H ₂₀ O ₉	- 2.3	289.1063 145.0603		+	+	-	+	+	-
5.	0.96 6	Methoxygallic acid hexoside	Phenolic acid	[M+H] ⁺ 365.1053	C ₁₄ H ₂₁ O ₁₁ 1 ⁺	C ₁₄ H ₂₀ O ₁₁	8.0	203.0528 175.0192 130.0499		+	-	-	-	-	-
6.	0.96 7	Trisaccharide	Sugar	[M-H] ⁻ 539.1396	C ₂₄ H ₂₇ O ₁₄ 4 ⁻	C ₂₄ H ₂₈ O ₁₄	1.9	377.0870 215.0336 195.0515 179.0567		+	+	+	+	-	-
7.	0.96 9	Glutamic acid	Amino acid	[M-H] ⁻ 146.0456	C ₅ H ₈ NO ₄ -	C ₅ H ₉ NO ₄	2.1	102.0559	⁴⁴	+	-	+	-	+	-
8.	0.98 6	Disaccharide	Sugar	[M-H] ⁻ 377.0874	C ₁₈ H ₁₇ O ₈ -	C ₁₈ H ₁₈ O ₈	6.9	215.0338 195.0519		+	+	+	+	-	-
9.	1.02 0	Quinic acid	Phenolic acid	[M-H] ⁻ 191.0566	C ₇ H ₁₁ O ₆ ⁻	C ₇ H ₁₂ O ₆	- 2.5	173.0119 111.0008		+	+	+	+	+	+
10.	1.03 6	Malic acid	Phenolic acid	[M-H] ⁻ 133.0133	C ₄ H ₅ O ₅ ⁻	C ₄ H ₆ O ₅ ⁻	1.1	115.0031 71.0135		+	+	+	+	+	+
11.	1.04 8	Tyrosine	Amino acid	[M+H] ⁺ 182.0814	C ₉ H ₁₂ NO 3 ⁺	C ₉ H ₁₁ NO ₃	2.3	164.07 06 120.08 13		+	+	+	+	+	+
12.	1.05 1	Methylmalonylcarnitin e	Carnitine derivative	[M+H] ⁺ 262.1285	C ₁₁ H ₂₀ N O ₆ ⁺	C ₁₁ H ₁₉ NO 6	- 2.3	244.1169 174.0395		+	-	-	-	+	-

13.	1.050	Citric acid	Organic acid	$[M-H]^-$ 191.0186	$C_6H_7O_7^-$	$C_6H_8O_7$	6.6	173.0141 129.0192 111.0078		+	+	-	-	+	+
14.	1.053	Glucoiberin (3-Methylsulphinylpropyl glucosinolate)	Aliphatic Glucosinolate	$[M-H]^-$ 422.0228	$C_{11}H_{19}NO_{10}S_3^-$	$C_{11}H_{20}NO_{10}S_3$	6.3	358.0237 290.0859 195.0123 96.6577	³³	-	-	+	+	-	-
15.	1.07	Gluconapin	Aliphatic Glucosinolate	$[M-H]^-$ 372.0422	$C_{11}H_{18}NO_9S_2^-$	$C_{11}H_{19}NO_9S_2$	1.8	290.087 243.0619 195.057	⁴⁵	-	-	-	+	-	-
16.	1.10	Glucoraphanin	Aliphatic Glucosinolate	$[M-H]^-$ 436.0428	$C_{12}H_{22}NO_{10}S_3^-$	$C_{12}H_{23}NO_{10}S_3$	- 3.9	243.0636 195.0518 133.0145	⁴⁵	+	+	-	-	+	+
17.	1.434	Glucoraphenin	Aliphatic Glucosinolate	$[M-H]^-$ 434.0255 2	$C_{12}H_{20}NO_{10}S_3^-$	$C_{12}H_{21}NO_{10}S_3$	- 1.6	259.0149 243.0636 191.0198	^{46,47}	-	-	-	-	+	-
18.	1.455	Fructosyl-valine	Amino acid	280.1387	$C_{11}H_{22}NO_7^+$	$C_{11}H_{21}NO_7$	1.2	262.1282 244.1174 216.1229		+	-	-	-	-	-
19.	1.479	Sinigrin	Glucosinolate	$[M-H]^-$ 358.1987	$C_{10}H_{16}NO_9S_2^-$	$C_{10}H_{17}NO_9S_2$	- 1.3	243.0603 191.0182	⁴⁸	+	+	-	-	+	-
20.	1.537	Isoleucyl- <i>O</i> -hexoside	Amino acid	$[M-H]^-$ 292.1386 $[M+H]^+$ 294.1540	$C_{12}H_{22}NO_7^-$ $C_{12}H_{24}NO_7^+$	$C_{12}H_{23}NO_7$	5.6 2.3	202.1064 130.0867 / 132.1020		+	+	+	-	-	-
21.	1.543	O-Glutarylcarnitine	Carnitine derivative	$[M+H]^+$ 276.1432	$C_{12}H_{22}NO_6^+$	$C_{12}H_{21}NO_6$	3.7	258.1325 230.1379	⁴⁹	+	+	+	+	+	+
22.	1.727	Hydroxybutyrylcarnitine	Carnitine derivative	$[M+H]^+$ 248.1487	$C_{11}H_{22}NO_5^+$	$C_{11}H_{21}NO_5$	2.4	230.1450 212.0889	⁴⁹	+	-	-	-	-	-
23.	2.033	N-acetyl hexosamine	Amino sugar	$[M-H]^-$ 222.0619	$C_7H_{12}NO_7^-$	$C_7H_{13}NO_7$	0.3	178.0734		+	-	-	-	-	-
24.	2.038	Hexosamine	Amino sugar	$[M-H]^-$ 178.0738	$C_6H_{12}NO_5^-$	$C_6H_{13}NO_5$	- 9.7	160.06 32 142.05 26		+	-	-	-	-	-
25.	2.475	Unknown	Amino sugar	$[M-H]^-$ 424.1427	$C_{16}H_{26}NO_{12}^-$	$C_{16}H_{27}NO_{12}$	7.9	262.0924		-	+	-	+	+	-

26.	2.57	Hexose phenylalanine	Amino acid	326.1232 [M+H] ⁺ 328.1384	C ₁₅ H ₂₀ N O ₇ ⁻ C ₁₅ H ₂₂ N O ₇ ⁺	C ₁₅ H ₂₁ NO 7	3.9 5.0	164.0703 / 310.1278 166.0862		+	+	+	-	-	+
27.	2.64	Phenyl alanine	Amino acid	[M-H] ⁻ 164.0717	C ₉ H ₁₀ NO 2 ⁻	C ₉ H ₁₁ NO ₂	- 6.5	147.0463 106.0550		+	-	-	-	-	-
28.	2.72	Cinnamic acid	Phenolic acid	[M-H] ⁻ 147.0456	C ₉ H ₇ O ₂ ⁻	C ₉ H ₈ O ₂	- 2.9	103.0698		+	-	-	-	+	-
29.	2.72 3	Leucyl-Glutamine	Dipeptide	[M+H] ⁺ 260.1591	C ₁₁ H ₂₂ N 3O ₄ ⁺	C ₁₁ H ₂₁ N ₃ O ₄	3.9	242.1495 197.1277		+	-	-	+	-	-
30.	2.73 9	Protocatechuic-acid hexoside	Phenolic compound	[M-H] ⁻ 203.1393	C ₁₃ H ₁₅ O 9 ⁻	C ₁₃ H ₁₆ O ₉	- 0.3	153.0181		-	-	-	+	+	-
31.	2.96	Dihydrogluconapin (Methylpropylglucosinolate)	Glucosinolate	[M-H] ⁻ 374.0588	C ₁₁ H ₂₀ N O ₉ S ₂ ⁻	C ₁₁ H ₂₁ NO 9S ₂	- 0.8	259.1464 244.1305 164.0723	⁵⁰	-	-	-	+	-	-
32.	2.97 1	Gentisic acid- <i>O</i> -hexoside	Phenolic acid	[M-H] ⁻ 315.0737	C ₁₃ H ₁₅ O ₉ -	C ₁₃ H ₁₆ O ₉	- 5.1	153.0194		+	-	+	-	+	+
33.	3.08	Vanillic acid- <i>O</i> -hexoside	Phenolic acid	[M-H] ⁻ 329.0884	C ₁₄ H ₁₇ O ₉ -	C ₁₄ H ₁₈ O ₉	- 1.8	167.0351		+	-	-	+	+	-
34.	3.15 9	Unknown	-	[M-H] ⁻ 259.1308	C ₁₆ H ₁₉ O ₃ -	C ₁₆ H ₂₀ O ₃	5.0	241.1186 197.1298 171.1484		+	-	-	-	-	-
35.	3.45 2	Pentenylglucosinolate (Glucobrassicinapin)	Glucosinolate	[M-H] ⁻ 386.0585	C ₁₂ H ₂₀ N O ₉ S ₂ ⁻	C ₁₂ H ₂₁ NO 9S ₂	- 0.0	195.0344 96.9591	⁵⁰	+	+	+	-	-	+
36.	3.46 1	Quercetin- <i>O</i> -caffeoyldeoxyhexosyl-tri- <i>O</i> -hexoside	Flavonoid	[M-H] ⁻ 1095.289 7	C ₄₈ H ₅₅ O ₂ 9 ⁻	C ₄₈ H ₅₆ O ₂₉	- 10. 3	933.2426 787.1872 463.0440 301.0770	⁵¹	+	+	+	-	-	-
37.	3.52 1	Nicotinic acid- <i>O</i> -pentoside	Nitrogenous	[M-H] ⁻ 254.0663	C ₁₁ H ₁₂ N O ₆ ⁻	C ₁₁ H ₁₃ NO 6	2.9	124.0392		-	+	-	+	-	-
38.	3.52 6	Quercetin- <i>O</i> -sinapoylhexoside- <i>O</i> -deoxyhexoside	Flavonoid	[M-H] ⁻ 817.2055	C ₃₈ H ₄₁ O ₂ 0 ⁻	C ₃₈ H ₄₂ O ₂₀	- 0.6	609.1470 447.0918		+	+	+	+	-	-
39.	3.54 6	Cyanidin- <i>O</i> -tetrahexoside	Anthocyanin	[M+H] ⁺ 935.2672	C ₃₉ H ₅₁ O ₂ 6 ⁺	C ₃₉ H ₅₀ O ₂₆	1.9	773.1220 611.1612 449.1077 287.0546		+	-	+	-	-	-

40.	3.62	Kaempferol- <i>O</i> -dihexoside -di- <i>O</i> -hexoside.	Flavonoid	[M-H] ⁻ 933.2563	C ₃₉ H ₄₉ O ₂ 6 ⁻	C ₃₉ H ₅₀ O ₂₆	- 1.8	771.1999 609.1450 489.1227 285.0323	52	+	+	+	-	-	-
41.	3.65	Quercetin- <i>O</i> -dihexoside - <i>O</i> -hexosyldeoxyhexoside	Flavonoid	[M-H] ⁻ 933.2478	C ₃₉ H ₄₉ O ₂ 6 ⁻	C ₃₉ H ₅₀ O ₂₆	- 2.1	771.1948 447.0349 301.1754	53	+	+	+	-	-	-
42.	3.67	Kaempferol- <i>O</i> -sinapoylcaffeoyl-tri- <i>O</i> -hexoside	Flavonoid	[M-H] ⁻ 1139.297 4	C ₅₃ H ₅₅ O ₂ 8 ⁻	C ₅₃ H ₅₆ O ₂₈	- 7.8	933.2480 771.1925 609.1419 229.1541		-	-	+	-	-	-
43.	3.68 2	Leucyl-leucyl-glutamic acid	Peptide	[M+H] ⁺ 374.2283	C ₁₇ H ₃₂ N 3O ₆ ⁺	C ₁₇ H ₃₁ N ₃ O ₆	0.7	261.1440 227.175		-	-	-	-	+	-
44.	3.69	Unknown	-	[M-H] ⁻ 229.1584	C ₁₆ H ₂₁ O ⁻	C ₁₆ H ₂₂ O ⁻	5.9	211.1462 185.1673 130.877		+	-	-	+	-	-
45.	3.78 2	kaempferol- <i>O</i> -trihexoside	Phenolic acid	[M-H] ⁻ 591.1721	C ₂₄ H ₃₁ O ₁ 7 ⁻	C ₂₄ H ₃₂ O ₁₇	- 0.3	545.1543 383.1006	54	+	-	-	-	-	-
46.	3.82 8	Unknown	-	[M-H] ⁻ 525.1602	C ₂₄ H ₂₉ O ₁ 3 ⁻	C ₂₄ H ₃₀ O ₁₃	2.2	363.1034		-	-	-	-	+	-
47.	3.84	Kaempferol- <i>O</i> -(methoxycaffeoyl)pentoside- <i>O</i> -trihexoside	Flavonoid	[M-H] ⁻ 1095.285 9	C ₄₈ H ₅₅ O ₂ 9 ⁻	C ₄₈ H ₅₆ O ₂₉	- 2.2	963.2418 771.1985 287.1570	55	-	-	-	+	-	-
48.	3.85 8	Quercetin- <i>O</i> -methoxycaffeoyldeoxyhexoside dihexoside- <i>O</i> -pentoside	Flavonoid	[M-H] ⁻ 1095.281 7	C ₄₈ H ₅₅ O ₂ 9 ⁻	C ₄₈ H ₅₆ O ₂₉	1.3	771.1987 625.1407		-	-	-	+	-	-
49.	3.98	Kaempferol- <i>O</i> -hydroxyferuloyl-tri- <i>O</i> -hexoside	Flavonoid	[M-H] ⁻ 963.2435	C ₄₃ H ₄₇ O ₂ 5 ⁻	C ₄₃ H ₄₈ O ₂₅	- 2.4	639.1725 447.0524 285.1777	56	+	-	-	-	-	-
50.	4.00 1	Glucobrassicin	Glucosinolate	[M-H] ⁻ 447.0535	C ₁₆ H ₁₉ N 2O ₉ S ₂ ⁻	C ₁₆ H ₂₀ N ₂ O ₉ S ₂	- 1.8	179.0574 96.9590	57	+	+	+	+	+	-
51.	4.08 3	Kaempferol- <i>O</i> -sinapoyl-tri-hexosyl- <i>O</i> -pentoside	Flavonoid	[M-H] ⁻ 1109.301 1	C ₄₉ H ₅₇ O ₂ 9 ⁻	C ₄₉ H ₅₈ O ₂₉	- 1.8	977.2579 771.2009 609.1472 285.1264		+	-	+	+	-	+
52.	4.10 2	Isorhamnetin- <i>O</i> -caffeoylpentosyl	Flavonoid	[M-H] ⁻ 1079.286	C ₄₈ H ₅₅ O ₂ 8 ⁻	C ₄₈ H ₅₆ O ₂₈	7.9	771.1951 609.1242		-	+	+	-	-	-

		deoxyhexosyl di- <i>O</i> -hexoside		5				316.1858							
53.	4.13	Lariciresinol- <i>O</i> -dihexoside	Lignan	[M-H] ⁻ 683.2488	C ₃₂ H ₄₃ O ₁₆ ⁻	C ₃₂ H ₄₄ O ₁₆	-1.4	521.2043 359.1515	⁵⁸	+	-	-	-	-	-
54.	4.18 5	Leucyl-leucine	Peptide	[M-H] ⁻ 245.1854	C ₁₂ H ₂₅ N ₂ O ₃ ⁻	C ₁₂ H ₂₆ N ₂ O ₃	2.2	199.1803 132.1008		+	-	-	-	-	-
55.	4.20 7	Kaempferol- <i>O</i> -methoxycaffeoyldeoxyhexoside tri- <i>O</i> -hexoside	Flavonoid	[M-H] ⁻ 947.2477	C ₄₃ H ₄₇ O ₂₄ ⁻	C ₄₃ H ₄₈ O ₂₄	-0.5	917.2372 591.2667 287.1776		+	-	+	-	-	-
56.	4.21 2	Unknown	Nitrogenous	[M-H] ⁻ 452.1662	C ₁₄ H ₃₀ N ₁₅ O ₁₅ ⁻	C ₁₄ H ₃₁ NO ₁₅	-8.9	362.1349 290.1131 184.1002 179.0553	⁵⁹	-	+	-	-	+	-
57.	4.25 0	Kaempferol- <i>O</i> -feruloyldihexoside- <i>O</i> -hexoside	Flavonoid	[M-H] ⁻ 947.2477	C ₄₃ H ₄₇ O ₂₄ ⁻	C ₄₃ H ₄₈ O ₂₄	-1.7	609.1419 286.1752		+	-	-	-	-	-
58.	4.26 0	Methoxytyrosine- <i>O</i> -hexoside	Nitrogenous	[M-H] ⁻ 372.1271	C ₁₆ H ₂₂ N ₉ O ₉ ⁻	C ₁₆ H ₂₃ NO ₉	7.9	328.1058 210.0751		-	+	-	-	+	-
59.	4.26 3	Unknown flavonoid glycoside	Flavonoid	[M-H] ⁻ 917.2363	C ₄₂ H ₄₅ O ₂₃ ⁻	C ₄₂ H ₄₆ O ₂₃	6.9	771.2002 593.2445 315.0755		+	+	+	-	+	-
60.	4.27 0	Unknown	Nitrogenous	[M-H] ⁻ 474.2150	C ₂₅ H ₃₂ N ₈ O ₈ ⁻	C ₂₅ H ₃₃ NO ₈	5.9	384.1751 312.1545		-	+	-	-	+	-
61.	4.27 7	Feruloyl- <i>O</i> -hexoside	Phenolic acid	[M-H] ⁻ 355.1042	C ₁₆ H ₁₉ O ₉ ⁻	C ₁₆ H ₂₀ O ₉	-2.2	193.0520		+	+	+	+	+	+
62.	4.28 8	Delphinidin- <i>O</i> -deoxyhexoside-di- <i>O</i> -hexoside	Anthocyanin	[M+H] ⁺ 773.2213	C ₃₃ H ₄₁ O ₂₁ ⁺	C ₃₃ H ₄₀ O ₂₁	1.4	611.1610 449.1077 303.0494	⁶⁰	-	-	-	-	+	+
63.	4.29 0	Hydroxyglucobrassicin	Glucosinolate	[M-H] ⁻ 463.1705	C ₁₆ H ₁₉ N ₂ O ₁₀ S ₂ ⁻	C ₁₆ H ₂₀ N ₂ O ₁₀ S ₂	5.6	283.1907 224.0989	⁵⁷	+	+	-	-	-	-
64.	4.30 3	(Methylsulfonyl)octylglucosinolate	Glucosinolate	[M-H] ⁻ 508.0958	C ₁₆ H ₃₀ N ₃ O ₁₁ S ₃ ⁻	C ₁₆ H ₃₁ N ₂ O ₁₁ S ₃	5.6	464.10 60 266.08 62	⁶¹	-	-	-	-	+	-
65.	4.38 0	Methylsulfinyloctylglucosinolate (Glucohirsutin)	Glucosinolate	[M-H] ⁻ 492.0462	C ₁₆ H ₃₀ N ₃ O ₁₀ S ₃ ⁻	C ₁₆ H ₃₁ NO ₁₀ S ₃ ⁻	-0.7	411.0462 259.0513		+	-	+	-	-	-

66.	4.399	Kaempferol- <i>O</i> -pentosyldeoxyhexoside-di- <i>O</i> -hexoside	Flavonoid	[M-H] ⁻ 887.2451	C ₃₈ H ₄₇ O ₂ 4 ⁻	C ₃₈ H ₄₈ O ₂₄	1.2	755.2030 593.1496 433.2074 431.1582		-	-	-	-	+	-
67.	4.427	Quercetin- <i>O</i> -feruloyl- <i>O</i> -di-pentoside	Flavonoid	[M-H] ⁻ 741.1876	C ₃₅ H ₃₃ O ₁ 8 ⁻	C ₃₅ H ₃₄ O ₁₈	10.0	609.1361 433.0756		-	-	-	-	+	-
68.	4.420	Gluconasturtiin	Glucosinolate	[M-H] ⁻ 422.0596	C ₁₅ H ₂₀ N O ₉ S ₂ ⁻	C ₁₅ H ₂₁ NO ₉ S ₂	- 2.7	342.1027 404.0490 216.0169		-	+	-	+	-	-
69.	4.440	Sinapoyl- <i>O</i> -hexoside (Raphanusol B)	Sinapic acid derivative	[M-H] ⁻ 385.1133	C ₁₇ H ₂₁ O ₁ 0 ⁻	C ₁₇ H ₂₂ O ₁₀	- 1.9	223.0560		+	+	+	+	+	+
70.	4.443	Unknown	Unknown	[M-H] ⁻ 439.2120 2	C ₂₆ H ₃₁ O ₆ -	C ₂₆ H ₃₂ O ₆	5.6	349.1752 277.1576		-	-	-	-	+	+
71.	4.488	Kaempferol- <i>O</i> -hexosyl- <i>O</i> -deoxyhexosylhexoside	Flavonoid	[M-H] ⁻ 755.2037	C ₃₃ H ₃₉ O ₂ 0 ⁻	C ₃₃ H ₄₀ O ₂₀	2.1	593.1507 447.0935 285.0431		-	-	-	-	+	+
72.	4.491	Delphinidin di- <i>O</i> -hexosidedi- <i>O</i> -deoxyhexoside	Anthocyanin	[M+H] ⁺ 919.2750	C ₃₉ H ₅₁ O ₂ 5 ⁺	C ₃₉ H ₅₀ O ₂₅	- 3.9	757.2183 611.1595 304.1743		-	-	-	+	+	-
73.	4.493	Methoxytyrosin	Nitrogenous	[M-H] ⁻ 210.0787	C ₁₀ H ₁₂ N O ₄ ⁻	C ₁₀ H ₁₃ NO 4	- 3.8	124.0412		-	+	-	-	+	-
74.	4.520	Quercetin di- <i>O</i> -hexoside	Flavonoid	[M-H] ⁻ 625.1367	C ₂₇ H ₂₉ O ₁ 7 ⁻	C ₂₇ H ₃₀ O ₁₇	6.9	463.0878 301.0336		+	-	-	-	+	+
75.	4.555	Kaempferol- <i>O</i> -pentosylhexoside- <i>O</i> -deoxyhexoside	Flavonoid	[M-H] ⁻ 725.1937	C ₃₂ H ₃₇ O ₁ 9 ⁻	C ₃₂ H ₃₈ O ₁₉	- 0.3	579.1279 417.0823		-	-	-	-	+	-
76.	4.560	Glucoarabin (methylsulfinyl)nonylglucosinolate	Glucosinolate	[M-H] ⁻ 506.1190	C ₁₇ H ₃₂ N O ₁₀ S ₃ ⁻	C ₁₇ H ₃₃ NO 10S ₃	0.7	488.10 84 426.16 21 96.959 5	61	-	-	-	-	+	-
77.	4.589	Cyanidin- <i>O</i> -deoxyhexosyl- <i>O</i> -hexosylpentoside	Anthocyanin	[M+H] ⁺ 727.2076	C ₃₂ H ₃₉ O ₁ 9 ⁺	C ₃₂ H ₃₈ O ₁₉	1.1	565.1534 433.1121 287.0540			-	-	-	+	-
78.	4.60	Prinsepiol- <i>O</i> -hexoside	Lignan	[M-H] ⁻	C ₂₆ H ₃₁ O ₁	C ₂₆ H ₃₂ O ₁₃	1.8	389.1223		-	-	-	+	+	-

	9			551.1760	3 ⁻										
79.	4.62 5	Isorhamnetin- <i>O</i> - deoxyhexoside- <i>O</i> -di- hexoside	Flavonoid	[M+H] ⁺ 787.2273	C ₃₄ H ₄₃ O ₂ 1 ⁺	C ₃₄ H ₄₂ O ₂₁	2.4	625.1807 463.1213 317.0653		-	-	-	-	+	-
80.	4.64 3	Kaempferol- <i>O</i> -acyl- <i>O</i> - di-pentose	Flavonoid	[M-H] ⁻ 725.1912	C ₃₂ H ₃₇ O ₁ 9 ⁻	C ₃₂ H ₃₈ O ₁₉	2.3	725.1911 2 593.1489 417.0817 285.0396		-	-	-	-	+	-
81.	4.64 9	Cyanidin- <i>O</i> - deoxyhexosyl- <i>O</i> - pentose	Anthocyanin	[M+H] ⁺ 565.1555	C ₂₆ H ₂₉ O ₁ 4 ⁺	C ₂₆ H ₂₈ O ₁₄	- 0.6	433.1118 287.0542		-	-	-	-	+	-
82.	4.65 8	Delphinidin- <i>O</i> - deoxyhexosyl- <i>O</i> - pentose	Anthocyanin	[M+H] ⁺ 581.1501	C ₂₆ H ₂₉ O ₁ 5 ⁺	C ₂₆ H ₂₈ O ₁₅	0.1	449.1017 303.0493		-	-	-	-	+	-
83.	4.78 6	Quercetin- <i>O</i> - deoxyhexosylhexoside	Flavonoid	[M-H] ⁻ 771.1953	C ₃₃ H ₃₉ O ₂ 1 ⁻	C ₃₃ H ₄₀ O ₂₁	4.8	301.1190		+	-	-	-	+	-
84.	4.83	Quercetin- <i>O</i> - deoxyhexosyl- <i>O</i> - hexoside	Flavonoid	[M-H] ⁻ 609.08	C ₂₇ H ₂₉ O ₁ 6 ⁻	C ₂₇ H ₃₀ O ₁₆	- 1.8	463.1785 301.1631		+	+	-	-	+	-
85.	4.94	Kaempferol- <i>O</i> - (methoxycaffeoyl)- <i>O</i> - dihexoside	Flavonoid	[M-H] ⁻ 801.1896	C ₃₇ H ₃₇ O ₂ 0 ⁻	C ₃₇ H ₃₈ O ₂₀	- 2.0	609.1478 287.1517		-	-	-	-	+	-
86.	4.96 9	Kaempferol- <i>O</i> - deoxyhexosyl- <i>O</i> - pentose	Flavonoid	[M-H] ⁻ 563.1418 [M+H] ⁺ 565.1553	C ₂₆ H ₂₇ O ₁ 4 ⁻ C ₂₆ H ₂₉ O ₁ 4 ⁺	C ₂₆ H ₂₈ O ₁₄	- 2.3 0.7	431.019		-	-	-	-	+	-
87.	5.00 3	Delphinidin- <i>O</i> - deoxyhexoside	Anthocyanin	[M+H] ⁺ 449.1070	C ₂₁ H ₂₉ O ₁ 4 ⁺	C ₂₁ H ₂₈ O ₁₄	1.2	303.0495	29	-	-	-	+	+	-
88.	5.03 7	Cyanidin- <i>O</i> -pentose	Anthocyanin	[M+H] ⁺ 419.097	C ₂₀ H ₁₉ O ₁ 0 ⁺	C ₂₀ H ₁₈ O ₁₀	2.0	287.0541		-	-	-	+	+	-
89.	5.09	Isorhamnetin- <i>O</i> - rutinoside	Flavonoid	[M-H] ⁻ 623.095 [M+H] ⁺ 625.1752	C ₂₈ H ₃₁ O ₁ 6 ⁻ C ₂₈ H ₃₃ O ₁ 6 ⁺	C ₂₈ H ₃₂ O ₁₆	1.6 1.5	447.0127		+	-	-	-	+	-
90.	5.13 8	Unknown acylated flavonoid glycoside	Flavonoid	[M-H] ⁻ 739.2076	C ₃₃ H ₃₉ O ₁ 9 ⁻ C ₃₃ H ₃₉ O ₁	C ₃₃ H ₄₀ O ₁₉	- 1.5	623.1606 563.1396 392.1026		-	-	-	-	+	-

				[M+H] ⁺ 741.2272	9 ⁺			193.0504							
91.	5.13 9	Cyanidin-di- <i>O</i> - deoxyhexoside	Anthocyanin	[M+H] ⁺ 579.1708	C ₂₇ H ₃₁ O ₁₄ 4 ⁺	C ₂₇ H ₃₀ O ₁₄	5.3	433.1126 287.0550	31	-	-	-	+	+	-
92.	5.14 2	Kaempferol- <i>O</i> - hexosyl- <i>O</i> -di- deoxyhexoside	Flavonoid	[M-H] ⁻ 739.2100 [M+H] ⁺ 741.2012	C ₃₃ H ₃₉ O ₁ 9 ⁻	C ₃₃ H ₄₀ O ₁₉	3.1	593.1515 431.0960 285.0349		-	-	-	-	+	-
93.	5.17 6	Quercetin- <i>O</i> -tri- deoxyhexoside	Flavonoid	[M-H] ⁻ 739.2052	C ₃₃ H ₃₉ O ₁ 9 ⁻	C ₃₃ H ₄₀ O ₁₉	3.1	447.0892 301.0348		-	-	-	-	+	-
94.	5.26	Syringic acid- <i>O</i> - hexoside	Phenolic acid	[M-H] ⁻ 359.0984	C ₁₅ H ₁₉ O ₁ 0 ⁻	C ₁₅ H ₂₀ O ₁₀	- 0.2	197.04 55179. 0349		-	-	+	+	+	-
95.	5.34	Kaempferol- <i>O</i> - (pentosyl-(coumaroyl)- pentoside)	Flavonoid	[M-H] ⁻ 771.1798	C ₃₆ H ₃₅ O ₁ 9 ⁻	C ₃₆ H ₃₆ O ₁₉	- 2.1	639.1587 385.1152 315.1820 163.0409 119.0510		+	-	-+	+	-	-
96.	5.37 9	Isorhamnetin- <i>O</i> - deoxyhexosyl- <i>O</i> - pentoside	Flavonoid	[M+H] ⁺ 595.1650	C ₂₇ H ₃₁ O ₁₄ 5 ⁺	C ₂₇ H ₃₀ O ₁₅	1.2	463.1226 317.0649		-	-	-	+	-	-
97.	5.39	Neoglucobrassicin	Glucosinolate	[M-H] ⁻ 477.0646	C ₁₇ H ₂₁ N 2O ₁₀ S ₂ ⁻	C ₁₇ H ₂₂ N ₂ O ₁₀ S ₂	2.3	446.0445 283.9877 96.9609	57	+	+	-	-	-	-
98.	5.42	Quercetin- <i>O</i> - glucuronide	Flavonoid	[M-H] ⁻ 447.0392	C ₂₁ H ₁₇ O 13 ⁻	C ₂₁ H ₁₈ O ₁ 3	8.3	175.0392		+	-	-	+	-	-
99.	5.43	Isorhamnetin- <i>O</i> - pentosyl-tri- <i>O</i> -hexoside	Flavonoid	[M-H] ⁻ 933.1791	C ₃₉ H ₄₉ O ₂ 6 ⁻	C ₃₉ H ₅₀ O ₂₆	5.4	771.1789 639.1603 477.0645 315.1823		-	-	-	-	+	-
100	5.62 0	Quercetin- <i>O</i> -hexoside	Flavonoid	[M-H] ⁻ 463.0892	C ₂₁ H ₁₉ O ₁ 2 ⁻	C ₂₁ H ₂₀ O ₁₂	- 2.3	301.0351		+	+	-	-	-	-
101	5.82 0	p-Coumaroyl-malic acid	Phenolic acid	[M-H] ⁻ 279.0526	C ₁₃ H ₁₁ O ₇ -	C ₁₃ H ₁₂ O ₇	5.5	133.0154		-	-	-	+	+	-
102	5.93 3	Ferulic acid	Phenolic acid	[M-H] ⁻ 193.0506	C ₁₀ H ₉ O ₄ ⁻	C ₁₀ H ₁₀ O ₄	0.1	178.0278 134.0377		-	-	-	+	+	-
103	5.93 5	Hydroxycoumarin- <i>O</i> - hexoside	Coumarin	[M-H] ⁻	C ₁₅ H ₁₅ O ₉ -	C ₁₅ H ₁₆ O ₉	- 1.3	177.0184		-	-	+	-	-	-

				339.0662											
104	5.95 0	Sinapoyl- <i>O</i> -malate	Phenolic acid	[M-H] ⁻ 339.0741	C ₁₅ H ₁₅ O ₉ -	C ₁₅ H ₁₆ O ₉ ⁻	- 5.8	223.0618		-	-	-	+	-	-
105	6.01 0	Feruloyl- <i>O</i> -malate	Phenolic acid	[M-H] ⁻ 309.029	C ₁₄ H ₁₃ O ₈ -	C ₁₄ H ₁₄ O ₈	6.4	193.05 01115. 0031	³⁴	+	+	+	+	-	-
106	6.05 0	Ferulic acid derivative	Phenolic acid	[M-H] ⁻ 339.0737	C ₁₅ H ₁₅ O ₉ -	C ₁₅ H ₁₆ O ₉	- 5.8	193.0517	⁶²	-	-	-	+	+	-
107	6.07 0	Unknown	-	[M-H] ⁻ 619.064	C ₂₈ H ₂₇ O ₁ 6 ⁻	C ₂₈ H ₂₈ O ₁₆	0.1	457.1032		+	-	-	-	-	-
108	6.18 3	Isorhamnetin- <i>O</i> -(p-coumaroyl)- <i>O</i> -hexoside	Flavonoid	[M-H] ⁻ 755.1768	C ₃₆ H ₃₅ O ₁ 8 ⁻	C ₃₆ H ₃₆ O ₁₈	8.0	315.1793		-	+	-	-	-	-
109	6.30 0	Caffeoyl- <i>O</i> -hexoside	Phenolic acid	[M-H] ⁻ 341.0915	C ₁₅ H ₁₇ O ₉ -	C ₁₅ H ₁₈ O ₉	- 10. 3	180.9824		+	-	+	-	+	+
110	6.45 0	Diferuloyl- <i>O</i> -malate	Phenolic acid	[M-H] ⁻ 505.1362	C ₂₄ H ₂₅ O ₁ 2 ⁻	C ₂₄ H ₂₆ O ₁₂	- 2.6	389.1277 193.0542		+	-	-	+	+	-
111	6.62 0	Quercetin- <i>O</i> -deoxyhexoside	Flavonoid	[M-H] ⁻ 447.0931	C ₂₁ H ₁₉ O ₁ 1 ⁻	C ₂₁ H ₂₀ O ₁₁	0.3	301.0588		+	+	-	-	-	-
112	6.64 0	Kaempferol- <i>O</i> -hexoside	Flavonoid	[M-H] ⁻ 447.0934	C ₂₁ H ₁₉ O ₁₁ ⁻	C ₂₁ H ₂₀ O ₁₁	- 0.2	285.0410		+	+	+	+	+	+
113	6.92 0	Rhamnetin - <i>O</i> -hexoside	Flavonoid	[M-H] ⁻ 477.053	C ₂₂ H ₂₁ O ₁ 2 ⁻	C ₂₂ H ₂₂ O ₁₂	1.7			+	+	-	-	+	-
114	7.20 2	Kaempferol- <i>O</i> -pentoside	Flavonoid	[M-H] ⁻ 417.0823	C ₂₀ H ₁₇ O ₁ 0 ⁻	C ₂₀ H ₁₈ O ₁₀	0.4	284.0318		-	-	-	-	+	-
115	7.53 0	Azelaic acid	Dicarboxylic acid	[M-H] ⁻ 187.0969	C ₉ H ₁₅ O ₄ ⁻	C ₉ H ₁₆ O ₄	3.8	169.0888 125.0965		+	+	+	-	+	-
116	7.79 0	Oxododecanedioic acid	Fatty acid	[M-H] ⁻ 243.1232	C ₁₂ H ₁₉ O ₅ -	C ₁₂ H ₂₀ O ₅	2.7	225.1126 207.1027 181.1233		-	+	+	-	-	-
117	8.10 0	Dihydroxy-dioxooctadecanoic acid	Fatty acid	[M-H] ⁻ 343.2110	C ₁₈ H ₃₁ O ₆ -	C ₁₈ H ₃₂ O ₆	4.8	255.1629 229.1431		+	+	-	-	+	-
118	8.35 4	Kaempferol- <i>O</i> -deoxyhexoside	Flavonoid	[M-H] ⁻ 431.0994 [M+H] ⁺ 433.1123	C ₂₁ H ₁₉ O ₁ 0 ⁻ C ₂₁ H ₂₁ O ₁ 0 ⁺	C ₂₁ H ₂₀ O ₁₀	- 2.4 1.4	285.0403		+	+	+	+	+	+

119	10.3 93	Kaempferol	Flavonoid	$[M-H]^-$ 285.0402	$C_{15}H_9O_6^-$	$C_{15}H_{10}O_6$	0.8	257.04 49 163.00 31 125.02 38		-	-	+	-	+	-
120	11.1 83	Apigenin- <i>O</i> - deoxyhexosyl-hexoside	Flavonoid	$[M-H]^-$ 577.1608	$C_{27}H_{29}O_{14}^-$	$C_{27}H_{30}O_{14}$	- 7.8	269.0452		-	-	-	+	-	-
121	11.2 77	Epoxyoctadecatrienoic acid	Fatty acid	$[M+H]^+$ 293.2097	$C_{18}H_{29}O_3^+$	$C_{18}H_{28}O_3$	4.9	275.1997 257.1890 163.1113 97.1011	⁶³	+	-	+	+	+	+
122	11.2 92	Indol acetic acid	Nitrogenou s	$[M+H]^+$ 176.0704	$C_{10}H_9NO_2^+$	$C_{10}H_8NO_2$	3.3	161.0467 133.0519		+	-	-	-	+	-
123	11.3 13	Oxo-phytodienoic acid	fatty acid	$[M+H]^+$ 275.003	$C_{18}H_{27}O_2^+$	$C_{18}H_{26}O_2$	3.0	257.1897 239.1783		+	-	+	+	+	+
124	11.3 45	Trihydroxyoctadecadie noic acid	Fatty acid	$[M-H]^-$ 327.2172	$C_{18}H_{31}O_5^-$	$C_{18}H_{32}O_5$	1.6	309.2078 291.1971		+	+	+	+	+	+
125	11.4 65	Methoxyspirobrassinin	Glucosinola te	$[M-H]^-$ 279.1610	$C_{12}H_{27}N_2OS_2^-$	$C_{12}H_{28}N_2OS_2$	- 7.3	261.1495 207.1051 97.0652		+	+	+	+	+	+
126	11.6 89	Trihydroxyoctadeceno ic acid	Fatty acid	$[M-H]^-$ 329.2327	$C_{18}H_{33}O_5^-$	$C_{18}H_{34}O_5^-$	2	229.1056 211.1254		+	+	+	+	+	+
127	12.5 4	Hydroxy- oxooctadecatrienoic acid	Fatty acid	$[M-H]^-$ 307.1914 $[M+H]^+$ 309.2051	$C_{18}H_{27}O_4^-$ $C_{18}H_{29}O_4^+$	$C_{18}H_{28}O_4$	0.3 3.1	289.1532 235.1342		+	+	+	+	+	+
128	13.0 39	Gallocatechin- (epi)catechin-(epi)catechin	Flavonoid	$[M-H]^-$ 881.4929	$C_{45}H_{37}O_{19}^-$	$C_{45}H_{38}O_{19}$	7.8	593.2638 305.1762	⁶⁴ ⁶⁵	+	-	-	+	+	+
129	13.2 9	Octadecenedioic acid	Fatty acid	$[M+H]^+$ 311.189	$C_{27}H_{31}O_{15}^+$	$C_{27}H_{30}O_{15}$	3.8	223.2354		+	+	+	+	+	+
130	13.9	Unknown	Triterpenoi d	$[M-H]^-$ 721.3655	$C_{34}H_{57}O_{16}^-$	$C_{34}H_{58}O_{16}$	3.7	675.3602 415.1436 397.1358 277.2171 235.0803	⁶⁶	+	+	-	-	-	-

131	13.9 52	Octadecanedioic acid	Fatty acid	[M-H] ⁻ 313.2375	C ₁₈ H ₃₃ O ₄ -	C ₁₈ H ₃₄ O ₄	2.9	225.2582		+	+	+	+	+	+
132	14.4 4	Hydroxylinolenic acid	Fatty acid	[M-H] ⁻ 293.2117	C ₁₈ H ₂₉ O ₃ -	C ₁₈ H ₃₀ O ₃	1.6	275.1346 235.2431		+	+	+	+	+	+
133	14.6 66	Unknown	Steroid	[M+H] ⁺ 391.2453	C ₂₃ H ₃₅ O ₅ +	C ₂₃ H ₃₄ O ₅	6.7	373.2593	⁶⁷	+	+	+	+	+	+
134	14.7 35	Unidentified Brassinosteroid	Steroid	[M+H] ⁺ 417.2390	C ₂₈ H ₃₃ O ₃ +	C ₂₈ H ₃₂ O ₃	8.3	399.3590		+	+	-	+	-	-
135	14.8 22	Octadecenedioic acid	Fatty acid	[M-H] ⁻ 311.2229	C ₁₈ H ₃₁ O ₄ -	C ₁₈ H ₃₂ O ₄	- 0.2	293.2102		+	+	+	+	+	+
136	14.8 32	Monolinolenin	Fatty acid	[M+H] ⁺ 353.2682	C ₂₁ H ₃₇ O ₄ +	C ₂₁ H ₃₆ O ₄	1.3	335.2571 261.2208	⁶⁸	+	+	+	+	+	+
137	15.0 4	Hydroxylinoleic acid	Fatty acid	[M-H] ⁻ 295.2276	C ₁₈ H ₃₁ O ₃ -	C ₁₈ H ₃₂ O ₃	0.9	277.1036 195.1236		+	+	+	+	+	+
138	15.5 09	Lupeol	Triterpene	[M+H] ⁺ 425.3753	C ₃₀ H ₄₉ O +	C ₃₀ H ₄₈ O	5.9	407.2772	⁶⁹	+	-	-	+	-	+
139	15.6 27	Hydroxystigmastenone	Steroid	[M+H] ⁺ 429.3706	C ₂₉ H ₄₉ O ₂ +	C ₂₉ H ₄₈ O ₂	4.9	411.3592 393.3108		+	-	+	-	+	-
140	15.8 12	Stearidonic acid	Fatty acid	[M+H] ⁺ 277.2155	C ₁₈ H ₂₉ O ₂ +	C ₁₈ H ₂₈ O ₂	1.5			+	-	+	+	-	+
141	15.9 3	Linolenoylglycerol	Fatty acid	[M-H] ⁻ 351.2546	C ₂₁ H ₃₅ O ₄ -	C ₂₁ H ₃₆ O ₄	- 0.7	333.249		+	+	+	+	+	+
142	15.9 63	SQDG (16:1/16:0)	Sulfolipid	[M-H] ⁻ 791.4956	C ₄₁ H ₇₅ O ₁ 2S ⁻	C ₄₁ H ₇₆ O ₁₂ S	3.6	537.3298 381.3379 249.1867	⁷⁰ 36	+	+	-	-	-	-
143	16.0 1	SQDG (18:3/16:0)	Sulfolipid	[M-H] ⁻ 817.424	C ₄₃ H ₇₇ O ₁ 2S ⁻	C ₄₃ H ₇₈ O ₁₂ S	1.4	561.3261 539.3286 297.2425		+	+	-	+	-	-
144	16.1 1	Unknown SQDG(18:1/16:1)	Sulfolipid	[M-H] ⁻ 819.436	C ₄₃ H ₇₉ O ₁ 2S ⁻	C ₄₃ H ₇₈ O ₁₂ S	7.8	566.3446 540.3283 279.2322		+	+	-	+	+	+
145	16.3 37	Palmitate- <i>O</i> -hexoside	Fattyacid	[M-H] ⁻ 417.2856	C ₂₂ H ₄₁ O ₇ -	C ₂₂ H ₄₂ O ₇	0.5	255.2320		+	+	-	+	+	+
146	16.8 2	Linolenic acid	Fatty acid	[M-H] ⁻ 277.187	C ₁₈ H ₂₉ O ₂ -	C ₁₈ H ₃₀ O ₂	0.4	215.2163		+	+	+	+	+	+

147	17.000	Hydroxyl palmitic acid	Fatty acid	[M-H] ⁻ 271.2279	C ₁₆ H ₃₁ O ₃ -	C ₁₆ H ₃₂ O ₃	- 0.0	225.2220		+	+	+	+	+	+
148	17.43	Linoleic acid	Fatty acid	[M-H] ⁻ 279.2326	C ₁₈ H ₃₁ O ₂ -	C ₁₈ H ₃₂ O ₂	1.4	261.2218 235.2425 171.1385		+	+	+	+	+	+
149	17.982	Stigmasterone	Steroid	[M+H] ⁺ 411.3620	C ₂₉ H ₄₇ O +	C ₂₉ H ₄₆ O	0.4	393.3621 313.3610	⁷¹	+	-	-	-	-	+

Table 2. Statistical significance of marker metabolites identified in cruciferous vegetables detected in UPLC-MS/MS analysis in negative mode

Metabolite	F-statistic	p-value	FDR adj. p-value	Effect Size (η ²)	Significance
Quercetin-O-deoxyhexosyl-O-hexoside	3803.69	< 0.001	< 0.001	0.999	***
Ferulic acid	2263.14	< 0.001	< 0.001	0.999	***
Trihydroxyoctadecadienoic acid	1715.99	< 0.001	< 0.001	0.999	***
Rhamnetin -O-hexoside	1421.77	< 0.001	< 0.001	0.998	***
Feruloyl malate	1389.33	< 0.001	< 0.001	0.998	***
Quercetin hexoside deoxyhexoside-O-hexoside	1205.96	< 0.001	< 0.001	0.998	***

Table 2. Statistical significance of marker metabolites identified in cruciferous vegetables detected in UPLC-MS/MS analysis in negative mode

Metabolite	F-statistic	p-value	FDR adj. p-value	Effect Size (η^2)	Significance
Isorhamnetin-O-hexosyl pentoside-O-hexoside	802.46	< 0.001	< 0.001	0.997	***
Kaempferol-O-hexosylhexoside	569.38	< 0.001	< 0.001	0.996	***
Isorhamnetin-O-(coumaroyl)-hexoside	536.39	< 0.001	< 0.001	0.996	***
Gluconic acid	522.56	< 0.001	< 0.001	0.995	***
Gluconapin	381.36	< 0.001	< 0.001	0.994	***
p-Coumaric acid	207.03	< 0.001	< 0.001	0.989	***
Malic acid	186.54	< 0.001	< 0.001	0.987	***
Palmitoleic-linolenic-O- hexoside	65.61	< 0.001	< 0.001	0.965	***
Hydroxylinolenic acid	13.02	< 0.001	< 0.001	0.844	***

ANOVA results for metabolites with FDR-adjusted $p < 0.05$

**** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$*

η^2 = eta-squared (effect size)

$n = 3$ biological replicates per species

Table 3. Statistical significance of marker metabolites identified in cruciferous vegetables detected in UPLC-MS/MS analysis in positive mode

Metabolite	F-statistic	p-value	FDR adj. p-value	Effect Size (η^2)	Significance
Delphinidin -O-deoxyhexoside	585.30	< 0.001	< 0.001	0.996	***
Cyanidin-O-tetra-hexoside	500.48	< 0.001	< 0.001	0.995	***
Methylmalonylcarnitine	413.59	< 0.001	< 0.001	0.994	***
Oxo-phytodienoic acid	338.71	< 0.001	< 0.001	0.993	***
Fructosyl-valine	243.01	< 0.001	< 0.001	0.990	***
Delphinidin-O-deoxyhexoside-di-O-hexoside	170.20	< 0.001	< 0.001	0.986	***
Hexose phenylalanine	159.39	< 0.001	< 0.001	0.985	***
Indole-acetic acid	157.89	< 0.001	< 0.001	0.985	***
Monolinolenin	121.73	< 0.001	< 0.001	0.981	***
Stearidonic acid	91.55	< 0.001	< 0.001	0.974	***
Glutaroyl-carnitine	29.89	< 0.001	< 0.001	0.926	***

ANOVA results for metabolites with FDR-adjusted $p < 0.05$

**** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$*

η^2 = eta-squared (effect size)

$n = 3$ biological replicates per species