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Original article

Chemical Profiling and Antioxidant Effects of γ-irradiated *Potentilla Fragarioides* Callus

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ABSTRACT This study investigates the effects of γ-irradiation on the antioxidant properties and metabolite profiles of *Potentilla fragarioides* callus to enhance its bioactive potential. Antioxidant activities, assessed via DPPH and $ABTS^+$ radical scavenging assays, exhibited a dose-dependent improvement, with IC_{50} values significantly decreasing at higher γ-irradiation doses. Metabolomic analysis using UPLC-QTOF/MS identified 40 metabolites, with notable transformations, including the hydrolysis of PC (18 : 2/18 : 2) into lysophospholipids such as lysoPC (18 : 2) and lysoPC (16 : 0), contributing to improved antioxidant activity. Statistical validation through PLS-DA confirmed irradiation-induced biochemical shifts. These findings highlight γ-irradiation as a promising technique for enhancing the functional attributes of plant-based materials, paving the way for applications in pharmaceuticals, nutraceuticals, and functional foods

Key words: *Potentilla fragarioides* callus; γ-Irradiation, Antioxidant, Chemical profile, UPLC-QTOF/MS

1. Introduction

The extracts of *Potentilla fragarioides*, commonly known as cinquefoil, are renowned for their diverse biological activities, including antioxidant, anti-diabetic, and neuroprotective effects, particularly in the context of Alzheimer's disease [1-3]. These effects are primarily attributed to its high content of bioactive compounds, such as tannins, flavonoids, and triterpenoids [4]. Despite these known properties, research utilizing advanced analytical techniques such as UPLC- and GC-mass spectrometry (MS) for the chemical profiling of *P. fragarioides* remains scarce. Moreover, studies focusing on the bioactive potential of its callus tissues are virtually nonexistent. Similarly, the application of γ-irradiation, a technology recognized for its ability to enhance the functional properties of plant materials, has not yet been explored for this plant.

γ-Irradiation is a cutting-edge technology widely applied in food sterilization [5]. Beyond its traditional uses, it is gaining attention for its ability to induce beneficial modifications in the chemical composition of natural products, leading to enhanced functionality [6]. Recent studies have reported structural transformations in natural compounds through γ-irradiation, resulting in novel compounds with improved bioactivities compared to their parent molecules [7-9]. For instance, when γ-irradiation was applied to the callus of *Euphorbia maculata*, a significant enhancement in antioxidant activity was observed, with the key metabolites responsible for this activity identified through LC-QTOF-MS chemical profiling [10]. These findings underscore the potential of γ-irradiation as a powerful tool for augmenting the bioactive properties of plant-derived materials.

In this study, we investigate the effects of γ-irradiation on the antioxidant activity and chemical composition of *P. fragarioides* callus. Using ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF/MS), we performed a detailed chemical profiling to identify the primary compounds affected by γ-irradiation. This research aims to explore the biochemical modifications induced by γ-irradiation and evaluate its potential as an innovative technique for enhancing the functional properties of natural products, thereby contributing to advancements in pharmaceutical

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and nutraceutical applications.

2. Materials and Methods

2.1. Chemicals

The 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-*bis* (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$), methanol (MeOH), ethanol (EtOH), acetonitrile (MeCN), formic acid (HCOOH), and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). All standard compounds were purchased from Sigma-Aldrich, and all other reagents used in this study were of analytical grade

2.2. γ-Irradiation of Potentilla fragarioides callus

Callus of *Potentilla fragarioides* was supplied by the Korean Collection for Type Cultures (KCTC) at the Korea Research Institute of Bioscience and Biotechnology (KRIBB) and utilized in the experiments. γ-Irradiation was performed at room temperature using a cobalt-60 irradiator (model: AECL, IR-79, MDS Nordion International Co. Ltd., Ottawa, ON, Canada), as described in a previously reported method, at the Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongup, Korea [11]. The source activity at the sample position was approximately 320 kCi, delivering a dose rate of 10 kGy/h. Dosimetry was conducted with 5 mm alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), calibrated in accordance with international standards established by the International Atomic Energy Agency (IAEA, Vienna, Austria) to ensure precise dose determination. *P. fragarioides* callus samples (weighing 10 g each) were irradiated with γ-ray doses of 0.05, 0.5, and 10 kGy. Following irradiation, the samples were ground and extracted overnight at ambient temperature with 50 mL of 80% MeOH. The resulting extracts were filtered, and the solvent was removed under reduced pressure to obtain crude extracts of the callus.

2.3. Evaluation of antioxidant activity

The DPPH radical scavenging activity was evaluated following the method outlined by Blois [12], with slight modifications. A 0.2 mM DPPH solution was prepared in EtOH, and test extracts were dissolved in 70% EtOH to achieve a final concentration of 200 μg/mL. A 60 μL portion of the DPPH solution was combined with 120 μL of each test extract, and the mixtures were incubated in the dark for 15 min. After incubation, the absorbance reduction was recorded at 517 nm using an ELISA reader.

The $ABTS^+$ radical was produced by reacting 5 mL of $ABTS^+$ solution (7 mM in EtOH) with 5 mL of 2.4 mM potassium persulfate ($K_2S_2O_8$). This reaction mixture was kept in the dark at 25°C for 24 h prior to use. For the $ABTS^+$ radical scavenging assay, 100 μL of the prepared $ABTS^+$ solution was dispensed into a 96-well plate, where it was mixed with varying concentrations of callus extracts or a positive control (L-ascorbic acid). The mixtures were stirred for 30 s and then incubated at 25°C for 30 min [13].

$$\text{Radical scavenging activity (\%)} = [1 - (A_2/A_1)] \times 100$$

where A_1 is the absorbance of the control (without the sample) and A_2 is the absorbance of the test sample. The half-maximal inhibitory concentration (IC_{50}) value was calculated by linear regression analysis of activity under the assay conditions. L-Ascorbic acid was used as a positive control.

2.4. Ultra-performance liquid chromatography-quadrupole time-of-flight/mass spectrometry (UPLC-QTOF/MS) analysis

UPLC-QTOF/MS analysis was conducted using a Xevo system (Waters, Milford, MA, USA). Samples (5 μL) were injected into an Acquity UPLC BEH C_{18} column (2.1 × 100 mm, 1.7 μm particle size; Waters). The column was equilibrated with 0.1% HCOOH in water (solvent A) and eluted with MeCN (solvent B) under a gradient program at a flow rate of 0.35 mL/min. The gradient conditions were as follows: 0-2.5 min, 85% A; 2.5-7.5 min, 80% A; 7.5-12.5 min, 70% A; 12.5-13.5 min, 100% A; 13.5-20.0 min, 0% A. Eluted metabolites were detected using QTOF/MS operated in positive electrospray ionization (ESI) mode. The capillary voltage was set at 3 kV, with a sampling cone voltage of 30 V. The source temperature was maintained at 100°C, while the desolvation gas flow rate and temperature were 800 L/h and 400°C, respectively. TOF/MS data were collected across an *m/z* range of 50-1500 with a scan time of 0.2 s. MS/MS spectra were acquired for the *m/z* range of 50-1000 using a collision energy ramp of 10-30 eV. This provided data on *m/z*, retention time (RT), and ion intensity. Data extraction, normalization, and alignment were processed using UNIFI software (Waters, Milford, MA, USA). Metabolite identification was performed by referencing multiple resources, including the Human Metabolome Database (HMDB; accessed March 10, 2022), METLIN (accessed March 10, 2022), ChemSpider (accessed March 10, 2022), relevant literature, and authentic standards.

Table 1. Comparison of radical scavenging activities of γ-irradiated *Potentilla fragarioides* callus for different dose

γ-Irradiation dose (kGy)	IC_{50} values (μg/mL)[a]	
	DPPH radical	$ABTS^+$ radical
0 (control)	179.6±6.8	68.2±2.1
0.05	117.1±2.6	67.0±2.1
0.5	109.0±2.2	47.9±1.3
10	95.6±2.5	37.5±1.8
L-Ascorbic acid [b]	67.5±1.3	26.7±0.5

[a]The values (μg/mL) are defined as the concentration that results in a 50% decrease of DPPH and $ABTS^+$ radicals. Activity data are mean±Standard deviation (SD) from triplicate experiments ($p<0.05$).
[b]Used as a positive control.

2.5. Statistical analysis

The MS datasets processed through UNIFI software were analyzed using multivariate statistical techniques with SIMCA-P^+ version 12.0.1 (Umetrics, Umeå, Sweden). Partial least squares discriminant analysis (PLS-DA) was employed to visualize differences among *P. fragarioides* callus groups. The quality of the PLS-DA models was assessed based on the goodness of fit metrics (R^2X and R^2Y) and predictive capability (Q^2), with further validation performed through cross-validation and a permutation test ($n=200$). Metabolites responsible for group differentiation were identified using a combination of the variable importance in projection (VIP) score (>0.7) obtained from PLS-DA and statistical significance determined by one-way analysis of variance (ANOVA) with Duncan's test ($p<0.05$), performed in SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Antioxidant effect of Potentilla fragarioides callus after different doses of γ-irradiation

Potentilla fragarioides extracts are known for their diverse functional properties, such as antioxidant and anti-diabetic activities [1,2]. However, studies focusing on the callus of *P. fragarioides* remain limited. In this research, we investigated the antioxidant properties of *P. fragarioides* callus exposed to various doses of γ-irradiation. Table 1 presents the antioxidant activities of the callus evaluated at irradiation doses of 0, 0.05, 0.5, and 10 kGy. Notably, the antioxidant activity demonstrated the greatest improvement at higher irradiation doses. The DPPH radical scavenging activity exhibited a dose-dependent enhancement, with IC_{50} values decreasing from 179.6±6.8 μg/mL at 0 kGy to 95.6±2.5 μg/mL at 10 kGy (Table 1). Similarly, $ABTS^+$ radical scavenging activity improved significantly, as IC_{50} values decreased from 68.2±2.1 μg/mL at 0 kGy to 37.5±1.8 μg/mL at 10 kGy, suggesting a clear relationship between γ-irradiation dose and antioxidant capacity (Table 1).

These results indicate that γ-irradiation may promote the synthesis or activation of antioxidant compounds within the callus, with higher doses producing more pronounced effects. This highlights the potential of γ-irradiation as a promising technique for enhancing the bioactive properties of plant-based materials, potentially benefiting pharmaceutical and nutraceutical applications [14]. To explore the underlying mechanisms, we propose that γ-irradiation alters key phytochemicals in the callus. As a next step, we plan to perform UPLC-QTOF/MS analysis to identify and characterize the specific compounds affected by irradiation.

3.2. Identification of metabolites produced in P. fragarioides callus by γ-irradiation

In the present study, UPLC-QTOF/MS was used to identify the predominant compounds present in plant-derived callus of *Potentilla fragarioides*. The identification of major metabolites was achieved by comparing their retention times and MS data with authentic references and previously reported data in the literature (Table 2). Moreover, the MS database for HMDB database, METLIN database, and ChemSpider database were used to confirm the results. A total of 40 metabolites were found in *P. fragarioides* callus, including 1 sesquiterpenoid (pteroside B), 8 amino acid derivatives (*N*-phenylacetylglutamic acid, *N*-(1-deoxy-1-fructosyl)valine, *N*-(1-deoxy-1-fructosyl)isoleucine, *N*-(1-deoxy-1-fructosyl)phenylalanine, $_L$-phenylalanine, *N*-(1-deoxy-1-fructosyl)tryptophan, $_L$-tryptophan, and γ-glutamylleucine), 11 flavonoids (isovitexin 6''-rhamnoside, (±)-catechin, kaempferol 3-sophoroside-7-glucuronide, (±)-epicatechin 3-glucuronide, (±)-epicatechin, quercetin 3-(2'',6''-digalloyl) galactoside, quercetin, quercetin 3-(6'''-*p*-coumarylglucosyl) (1→2)-rhamnoside 7-glucoside, pelargonidin 3-(6''-malonyl) glucoside, isovitexin 7-(6'''-feruloyl)glucoside, and isovitexin 2''-(6'''-feruloyl)glucoside), 1 phenolic acid (methyl gallate), 4 triterpenoids (12-hydroxy-11-methoxy-8,11,13-abietatrien-20-oic acid, euscaphic acid, 2-hydroxy linolenic acid, and (17α,23S)-epoxy-29-hydroxy-27-norlanosta-1,8-diene-3,15,24-trione), 11 lipids (eicosanedioic acid, heptadeca-sphing-4-enine, dehydrophytosphingosine, docosanedioic acid, lysoPC (18:3), PA (20:5/14:1), lysoPC (18:2), lysoPC (16:0), phytosphin-

Table 2. Identification of differential metabolites in the non-irradiated and irradiated groups of *Potentilla fragarioides* callus by UPLC-Q-TOF/MS/MS

Metabolite No.	Retention time (min)	Experimental mass (*m/z*)	Ion type	Fragment ions (*m/z*)	Identification	Non-irradiated	Irradiated
1	0.65	381.08	$[M+H]^+$	365, 309, 219, 175	Pteroside B	+	–
2	0.66	266.12	$[M+H]^+$	248, 230	*N*-Phenylacetylglutamic acid	–	+
3	0.69	280.14	$[M+H]^+$	262, 244, 234, 216	*N*-(1-Deoxy-1-fructosyl)valine	–	+
4	0.88	294.15	$[M+H]^+$	276, 258, 230, 86	*N*-(1-Deoxy-1-fructosyl)isoleucine	+	+
5	1.14	328.14	$[M+H]^+$	310, 292, 264, 120	*N*-(1-Deoxy-1-fructosyl)phenylalanine	+	+
6	1.19	166.09	$[M+H]^+$	120, 103, 93	L-Phenylalanine	+	+
7	1.55	367.15	$[M+H]^+$	349, 321, 303	*N*-(1-Deoxy-1-fructosyl)tryptophan	+	+
8	1.72	205.10	$[M+H]^+$	188, 170, 146, 118	L-Tryptophan	+	+
9	1.83	579.14	$[M+H]^+$	291, 163	Isovitexin 6"-rhamnoside	+	–
10	1.97	261.15	$[M+H]^+$	244, 198, 171, 132	γ-Glutamylleucine	+	+
11	2.04	291.09	$[M+H]^+$	261, 249, 165, 139	(±)-Catechin	+	–
12	2.27	185.04	$[M+H]^+$	153, 109	Methyl gallate	–	+
13	2.43	787.10	$[M+H]^+$	769, 595, 177	Kaempferol 3-sophoroside-7-glucuronide	+	–
14	2.67	467.08	$[M+H]^+$	291, 273, 177, 139	(±)-Epicatechin 3-glucuronide	+	+
15	2.73	291.09	$[M+H]^+$	207	(±)-Epicatechin	+	+
16	3.66	769.09	$[M+H]^+$	617, 303	Quercetin 3-(2",6"-digalloyl)galactoside	+	+
17	3.88	303.02	$[M+H]^+$	207, 153	Quercetin	+	+
18	4.42	919.08	$[M+H]^+$	465, 303	Quercetin 3-(6"'-*p*-coumarylglucosyl)(1→2)-rhamnoside 7-glucoside	+	+
19	4.89	520.20	$[M+H]^+$	91	Pelargonidin 3-(6"-malonyl)glucoside	+	+
20	5.14	771.11	$[M+H]^+$	433, 343	Isovitexin 7-(6"'-feruloyl)glucoside	+	+
21	5.83	771.11	$[M+H]^+$	339	Isovitexin 2"-(6"'-feruloyl)glucoside	+	–
22	10.45	347.22	$[M+H]^+$	329, 311, 301, 165	12-Hydroxy-11-methoxy-8,11,13-abietatrien-20-oic acid	+	+
23	13.42	489.36	$[M+H]^+$	471, 453, 425, 389	Euscaphic acid	+	+
24	13.80	295.23	$[M+H]^+$	277, 259, 177, 149	2-Hydroxy linolenic acid	+	–
25	13.89	469.33	$[M+H]^+$	451, 433, 393	(17α,23*S*)-Epoxy-29-hydroxy-27-norlanosta-1,8-diene-3,15,24-trione	+	+
26	14.12	343.30	$[M+H]^+$	307, 227	Eicosanedioic acid	+	+
27	14.21	288.25	$[M+H]^+$	240	Heptadeca-sphing-4-enine	+	+
28	14.23	316.28	$[M+H]^+$	298, 280, 237, 195	Dehydrophytosphingosine	–	+
29	14.44	371.33	$[M+H]^+$	279, 227	Docosanedioic acid	–	+
30	14.54	518.32	$[M+H]^+$	184	LysoPC (18:3)	–	+
31	14.65	665.41	$[M+H]^+$	539	PA (20:5/14:1)	+	+
32	14.72	520.34	$[M+H]^+$	184	LysoPC (18:2)	–	+
33	14.88	496.34	$[M+H]^+$	184	LysoPC (16:0)	–	+
34	15.08	398.23	$[M+H]^+$	280, 85	Phytosphingosine-1-phosphate	+	+
35	15.21	621.27	$[M+H]^+$	593, 561, 473	Methyl pheophorbide B	+	–
36	15.30	593.28	$[M+H]^+$	531, 487, 443	Pheophorbide A	+	+
37	15.44	623.29	$[M+H]^+$	573, 545	Pheophorbide	+	+
38	15.56	607.29	$[M+H]^+$	547, 505, 457, 445	Pheophorbide B	+	+
39	16.03	338.34	$[M+H]^+$	321, 303	13-Docosenamide	+	–
40	16.06	782.57	$[M+H]^+$	184	PC (18:2/18:2)	+	–

+: detected
–: not detected.

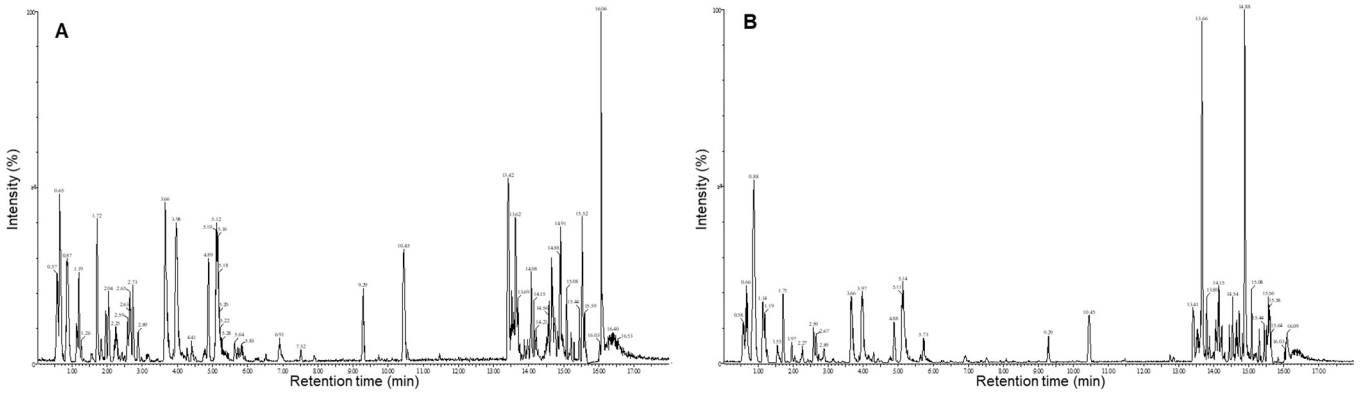

Fig. 1. TIC chromatograms of non-irradiated (A) and irradiated (B) *Potentilla fragarioides* callus.

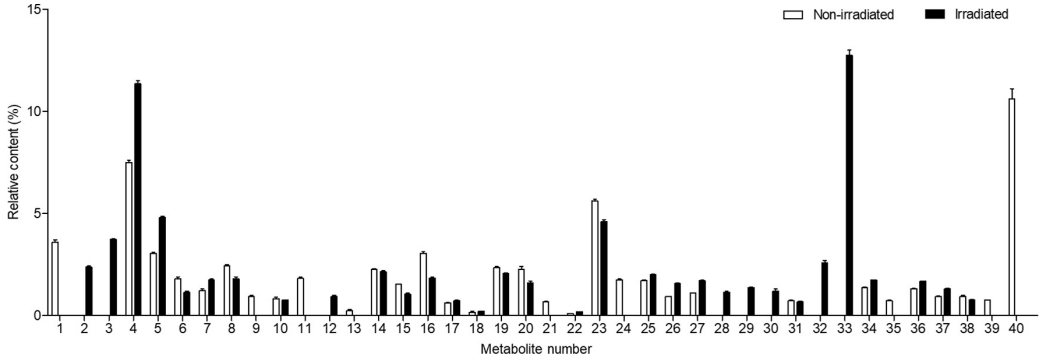

Fig. 2. Changes in metabolites (%) of non-irradiated and irradiated *Potentilla fragarioides* callus. Refer to Metabolite number in Table 2.

gosine-1-phosphate, 13-docosenamide, and PC (18:2/18:2)), and 4 chlorophylls (methyl pheophorbide B, pheophorbide A, pheophorbide, and pheophorbide B).

9 Metabolites (pteroside B, isovitexin 6''-rhamnoside, (±)-catechin, kaempferol 3-sophoroside-7-glucuronide, isovitexin 2''-(6'''-feruloyl)glucoside, 2-hydroxy linolenic acid, methyl pheophorbide B, 13-docosenamide, and PC (18:2/18:2)) were detected in the callus of *P. fragarioides* before γ-irradiation, but it was not detected after γ-irradiation at a dose of 10 kGy. On the other hand, 8 metabolites (*N*-phenylacetylglutamic acid, *N*-(1-deoxy-1-fructosyl)valine, methyl gallate, dehydrophytosphingosinem, docosanedioic acid, lysoPC (18:3), lysoPC (18:2), and lysoPC (16:0)), were detected after γ-irradiation (Table 2).

In particular, PC (18:2/18:2) was detected at a content of 10.6% in the non-irradiated group but was not detected after γ-irradiation. Conversely, lysoPC (16:0), which was not detected in the non-irradiated group, showed a content of 12.7% after γ-irradiation. This suggests that the chemical structure of PC (18:2/18:2), in which two lipids are ester-bonded to glycerol, underwent hydrolysis due to γ-irradiation, resulting in structural transformation to lysophospholipids such as lysoPC (18:3), lysoPC (18:2), and lysoPC (16:0), with only one lipid bound (Fig. 2).

The observed changes in metabolite content before and after γ-irradiation, as shown in Fig. 2, indicate that gamma irradiation triggers notable biochemical transformations in *P. fragarioides* callus. These biochemical shifts may underlie the enhanced antioxidant activities documented in Table 1. To delve deeper into the specific alterations in metabolites, we conducted LC-QTOF/MS analysis, which provided detailed insights into the metabolite profile changes following irradiation (Table 2 and Fig. 2). The analysis confirmed that the modifications in metabolite composition are both substantial and potentially applicable for practical uses. The rise in lysophospholipids, alongside the observed boost in antioxidant activity, highlights the potential of γ-irradiation as a method for augmenting the bioactive properties of plant-derived callus. Prior research has identified lysophospholipids across diverse tissues and cell types, where they are involved in critical physiological processes such as vascular development, reproduction, myelination, and even pathological conditions like neurological disorders and cancer [15]. Given

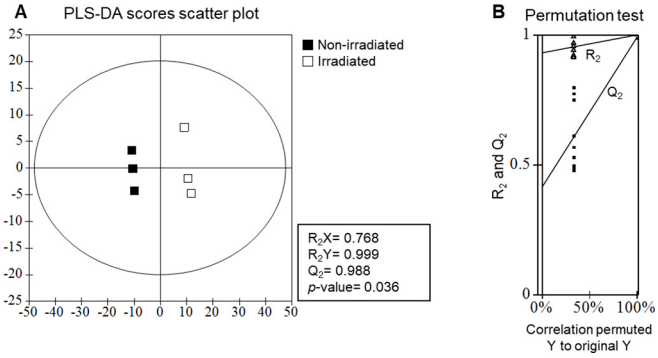

Fig. 3. PLS-DA scores scatter plot (A) and permutation test (B) analysis of metabolite in non-irradiated and irradiated *Potentilla fragarioides* callus.

these findings, gamma irradiation holds promise for applications in industries focused on natural products and food, where enhancing antioxidant properties is highly advantageous [16]. By fine-tuning the conditions of gamma irradiation, it becomes feasible to produce plant extracts with enhanced health-promoting properties, making this a powerful tool for creating functional foods and nutraceuticals. This study adds to the growing body of research supporting the utility of gamma irradiation for improving the functional attributes of natural products, thereby opening up new possibilities for their application across various domains.

3.3. Statistical analysis of changes in P. fragarioides callus by γ−irradiation

Fig. 3 illustrates the pronounced influence of γ-irradiation on the metabolite profiles of *Potentilla fragarioides* callus, as visualized through a PLS-DA scores scatter plot and permutation test analysis. The scatter plot distinctly separates the non-irradiated samples from those treated with 10 kGy γ-irradiation, emphasizing significant differences in their metabolite compositions. The permutation test further validates the PLS-DA model's reliability and statistical relevance, showing high R^2X (0.768) and Q^2 (0.998) values, along with a *p*-value of 0.036. These results confirm that the metabolic alterations observed are induced by gamma irradiation rather than random variation.

In the cross-validation conducted using the permutation test, R^2 is considered suitable when it is 0.5 or below, and Q^2 is deemed acceptable when it is –0.05 or lower. In this study, although the R^2 value is relatively elevated, the statistical analysis is interpreted as robust and free from significant issues. The distinct separation in metabolite profiles between non-irradiated and irradiated E. maculate callus, as demonstrated in Fig. 3, underscores that γ-irradiation induces targeted biochemical modifications. These changes, confirmed by LC-QTOF/MS analysis, highlight the potential of γ-irradiation as a method for enhancing the bioactive properties of plant-derived materials. Such advancements offer promising applications in the natural products and food industries, enabling the development of products with superior health-promoting benefits.

4. Conclusions

This study explored the effects of γ-irradiation on the antioxidant activity and metabolite profiles of *Potentilla fragarioides* callus, revealing its potential as a tool for enhancing bioactive properties. Antioxidant activity, measured through DPPH and $ABTS^+$ radical scavenging assays, significantly increased with higher γ-irradiation doses, as IC_{50} values improved from 179.6 to 95.6 μg/mL and 68.2 to 37.5 μg/mL, respectively. Metabolomic analysis using UPLC-QTOF/MS identified 40 metabolites, with notable shifts in metabolite composition post-irradiation, such as the conversion of PC (18 : 2/18 : 2) into lysophospholipids such as lysoPC (16 : 0), enhancing antioxidant properties. Statistical validation via PLS-DA confirmed the biochemical modifications were irradiation-induced. These findings underscore the potential of γ-irradiation to augment the health-promoting properties of plant-derived materials, presenting opportunities for pharmaceutical, nutraceutical, and functional food applications.

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