International Journal of Food Science and Technology, 2026, 61(1), vvaf265
https://doi.org/10.1093/ijfood/vvaf265
Advance access publication: 18 October 2025
Original Article

Proteomic profiling reveals the role of green tea polyphenols–iron oxide–chitosan nanoparticles in colorectal cancer therapy *via* MAPK/metabolic pathway modulation

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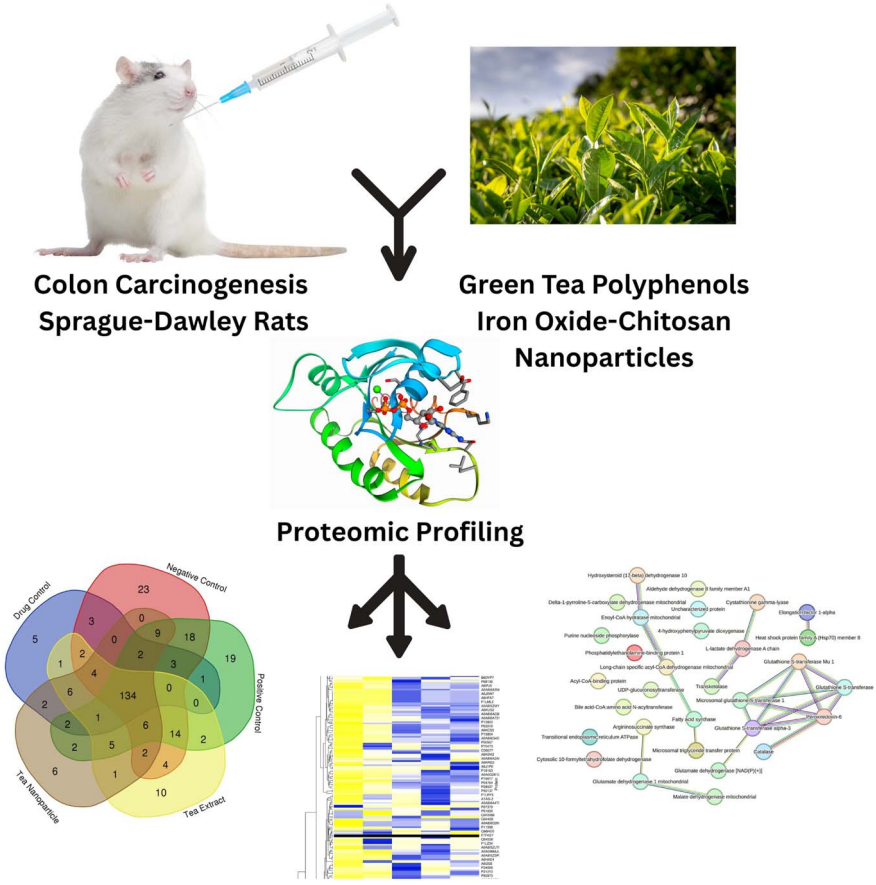
Abstract

This research aimed to study the protein profiles of rat-induced colon carcinogenesis supplemented with green tea extract and iron oxide–chitosan green tea polyphenol nanoparticles. Fifteen male Sprague–Dawley rats were divided into five groups. Colon carcinogenesis was induced with azoxymethane, followed by treatments with 5-fluorouracil, green tea polyphenol extracts, or iron oxide–chitosan–encapsulated tea polyphenol nanoparticles. Liver proteomes were analysed using mass spectrometry, which revealed 2,258 proteins across all groups. Proteomic analysis revealed 20 differentially expressed proteins following the treatment with tea extracts or tea nanoparticles. These proteins were associated with pathways in cancer progression. Notably, six proteins were uniquely expressed in the nanoparticle group, including phosphatidylethanolamine-binding protein 1, which is linked to anticancer potential through modulation of the mitogen-activated protein kinase signalling pathway. These findings suggest that nanoparticles may enhance the therapeutic effects of tea polyphenols against colorectal cancer by more effectively modulating signalling pathways, oxidative stress, and energy metabolism.

Keywords: LC-MS/MS, proteomics, MAPK, colorectal cancer, green tea polyphenols, nanoparticles

Received: 3 September 2025. **Accepted:** 5 December 2025

Graphical abstract



Introduction

Tea is the most widely consumed beverage globally. (Khan & Mukhtar, 2019). It is made from the leaves of the plant *Camellia sinensis*. There are various types of tea, including green tea, black tea, and fermented tea, also known as Pu-erh tea. Green tea is a nonfermented dried tea leaf containing the most oligomeric polyphenols (Wang et al., 2020). Green tea polyphenols are composed of various phytochemicals that possess numerous health-promoting benefits. The main composition of green tea polyphenols is catechins, which make up approximately 59% of total polyphenols (Wang et al., 2020). Four distinctive green tea catechins are (-)-epigallocatechin, (-)-epigallocatechin-3-gallate, (-)-epicatechin, and (-)-epicatechin-3-gallate. Up to 70% of green tea catechins are composed of epigallocatechin gallate (Khan & Mukhtar, 2019; Mujtaba et al., 2023).

These green tea polyphenols have been extensively studied for their chemopreventive effects against various types of cancer (Al-Awawdeh & Shafie, 2025). Thus, green tea polyphenols are promising agents for a natural alternative and supplementary treatment for cancer. The anticancer properties of green tea polyphenols are commonly attributed to their potent ability to scavenge free radicals and significantly reduce oxidative damage (Dai et al., 2022; Yan et al., 2020).

One of the main limitations of green tea polyphenols is due to their low bioavailability. After 6 hr of green tea oral administration, only about 0.16% of catechins were detected in human blood (Warden et al., 2001). Green tea polyphenols' poor bioavailability is attributed to their inability to maintain their chemical structure along the gastrointestinal tract and their failure to cross the intestinal membrane efficiently (Cai et al., 2018).

Nanoparticle drug delivery employs the encapsulation of a targeted drug to increase its bioavailability (Cai et al., 2018). The nanostructure drug delivery uses various mechanisms to increase bioavailability, such as improving chemical stability, promoting permeability, time-based drug release, and altering the drug's polarity (Cai et al., 2018). Iron oxide nanoparticles with chitosan coating are a promising encapsulation strategy for increasing phytochemical compound applications in biomedicine (Appu et al., 2021). Chitosan, a carbohydrate-based biopolymer, has been extensively studied for its ability to improve epigallocatechin gallate bioavailability due to its unique physicochemical compatibility, cost-effectiveness, and environmentally friendly synthesis (Cai et al., 2018; Niu et al., 2022). Thus, iron oxide–chitosan nanoparticles may be a promising strategy for enhancing the efficacy of green tea polyphenols.

Iron oxide nanoparticles have been previously described as having minimal systemic toxicity, and similarly, chitosan has been studied for its low toxicity and high biodegradability (Al-Awawdeh & Shafie, 2025; Ishak et al., 2025). A prior study using iron oxide–chitosan found no signs of acute toxicity in mice after intravenous injection, with normal histology observed in major organs (Veiseh et al., 2009). Conversely, biodistribution analyses indicate that nanoparticles mainly accumulate in the liver and spleen, with lower levels in various tissues and minimal toxicity (Sonin et al., 2020; Veiseh et al., 2009). Therefore, it is crucial to investigate the potential of chitosan-coated iron oxide nanoparticles in conjunction with green tea polyphenols for managing epidemiologically significant diseases, such as cancer.

Colon cancer is ranked third as the most common type of cancer, and second in the world for causing death related to

cancer (World Health Organization, 2023). In addition, conventional treatment for colorectal cancer, such as the chemotherapeutic drug 5-Fluorouracil, often results in adverse side effects such as drug resistance, nausea, vomiting, diarrhoea, mucositis, and leukaemic blood disorders. Cases of cardiotoxicity and neurotoxicity are also reported in the minority as side effects of 5-Fluorouracil (Jose et al., 2022) Therefore, the supplementary use of green tea polyphenols as a treatment for colorectal cancer is promising since natural products have been known for their potential to supplement conventional drugs with minimal side effects synergistically (Alnuqaydan et al., 2020).

Proteomics is a powerful tool for profiling extensive protein data and identifying proteins associated with significant biological pathways or diseases, such as cancer (Ahmed et al., 2021; Mansor et al., 2023; O'Dwyer et al., 2011). Identification of the signature proteins is essential because it may lead to a deeper understanding of the cancer disease, provide support for other pretranslational omics results, the discovery of therapeutic targets, and the possible improvement of diagnosis or prognosis (Ahmed et al., 2021; Mansor et al., 2023; O'Dwyer et al., 2011).

Therefore, a proteomic approach, among other omics, should be employed to understand and provide evidence for the potential use of green tea polyphenols in treating colorectal cancer. Hence, the present study aims to investigate the protein profiles of rat-induced colon carcinogenesis supplemented with green tea extract and iron oxide–chitosan green tea polyphenol nanoparticles.

Materials and methods

Green tea polyphenols and iron oxide–chitosan green tea polyphenols nanoparticles preparation

Fresh tea leaves (*C. sinensis*) were collected from the BOH Plantation Sdn. Bhd. in Cameron Highlands, Pahang. A total of 5 kg of fresh leaves were collected in March 2019 from Farm 7 of the plantation. The leaves, consisting of one shoot and two or three young leaves from the top branches, were placed in a vacuum plastic bag and subsequently transported to the laboratory. The leaves were dried, ground into powder, and stored at −20 °C. The tea leaves were homogenised, and then the tea polyphenols were extracted using 2.5 g of dried leaves powder in 50 ml of distilled water using a microwave-assisted extraction system (Milestone, Advanced Microwave Laboratory, USA) for four minutes at 80 °C. The extract was filtered and dried. The tea polyphenol extract powder was stored at −20 °C (Serdar et al., 2016). The iron oxide nanoparticles were prepared using the coprecipitation method. (Barahuie et al., 2017). In brief, nanoparticles were made from ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$) and ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) at a ratio of 1:2. 2.982 g $FeCl_2$ and 8.109 g $FeCl_3$ were stirred at 800 rpm and 80 °C in 150 ml deionised water under nitrogen. Twenty millilitres of ammonia was added and stirred for another hour. Then, the pH was adjusted to 10 using NaOH. Next, 100 ml of chitosan solution, 100 ml of 10 mg/ml tea polyphenol extract in deionised water, and 1 ml of 10 mg/ml pentasodium tripolyphosphate hexahydrate in deionised water were added and stirred. After stirring, the mixture was sonicated, centrifuged, and washed using distilled water. Finally, the mixture was dried using an oven (Asey et al., 2019; Khan et al., 2014).

Animal experimental design

The animal experimental design was previously described, with some modifications (Norazalina et al., 2010). Fifteen male Sprague–Dawley rats (4 weeks old, average weight of 50–70 g) were used. The rats were given tap water ad libitum, and free access to standard rat pellets composed of vitamin-free casein (30%), sucrose (52.8%), corn oil (10%), cod liver oil, United States Pharmacopoeia (USP) (0.2%), Alphacel non-nutritive bulk (2%), and salt mixture no. 2 (5%). The rats were randomised into five groups: Negative Control (NC), Positive Control (PC), Drug Control (DC), Tea Extract (TE), and Tea Nanoparticle (TN) (Table 1). All rats, except for NC, were injected intraperitoneally with azoxymethane (AOM) at 15 mg/kg body weight once a week for 2 weeks. After AOM, DC received 5-fluorouracil treatment, whereas TE received 200 mg/kg body weight tea polyphenol extract, and TN received 200 mg/kg body weight iron oxide–chitosan encapsulated tea polyphenols nanoparticles *via* oral administration. NC received a regular diet. The selected dose of 200 mg/kg body weight for tea polyphenols was based on previous studies reporting its effective chemopreventive potential with minimal adverse effects (Srinivasan et al., 2008). After 20 weeks, upon termination, the rats were fasted for 16 hr before being euthanised *via* carbon dioxide (CO_2) asphyxiation. The liver is the primary organ for metabolism and detoxification, serving as an essential site for protein markers of antioxidant and anti-inflammatory activities (Vasilogianni et al., 2022). Therefore, consistent with previous studies, liver tissues were collected for proteomic analysis (Norazalina et al., 2010; Shafie, 2013). Ethical approval was granted by Universiti Putra Malaysia Institutional Animal Care and Use Committee (UPM IACUC) (AUP-R052/2020).

Protein extraction and digestion

Extraction and digestion were carried out according to a previous study (Lee, 2017). Liver tissue samples were homogenised, lysed, and centrifuged to remove debris. Proteins were quantified at 280 nm and digested using trypsin. Disulfide bonds were reduced using dithiothreitol and alkylated with iodoacetamide. Digested proteins were purified using spin columns and stored at −80 °C for subsequent analysis.

Liquid chromatography–mass spectrometry analysis

Analysis was done using the previously described method (Liew et al., 2022). Nanoflow-ESI liquid chromatography–tandem mass spectrometry (LC/MS/MS) was used (Agilent, USA). Agilent Large Capacity Chip was used, along with an enrichment and analytical column (Zorbax 300SB-C18). Solvent A was 0.1% formic acid in water, and Solvent B was 0.1% formic acid in 90% acetonitrile. Gradient: 5% B (30 min), 5%–75% B (30 min), then 5% B (8 min). Spectra were acquired in positive ion mode over a mass-to-charge range of 110–3,000 m/z. Data were processed with Agilent MassHunter Workstation.

Protein identification and quantification

The procedure was done following a previous study (Liew et al., 2022). Data processing and protein identification were performed using PEAKS Studio X (Bioinformatics Solution Inc.) against UniProtKB (https://www.uniprot.org/), a comprehensive protein database. Agilent MassHunter Qualitative analysis software (Agilent Technologies, USA) was used to obtain the sequence tag for amino acids from the mass spectrometry spectra. The sequence tag was used for protein identification and homology search using PEAKS Studio X, with label-free quantification employed. Carbamidomethylation was predetermined as the fixed modification parameter with a maximum number of missed cleavages equal to 2. The digestive enzyme was set as trypsin. The identified protein profiles from each group were sorted based on

Table 1. Experimental design of animal study.

Group	Treatment description
NC	Regular diet for 20 weeks + no AOM injection
PC	Regular diet for 20 weeks + AOM injection (15 mg/kg, once weekly for 2 weeks)
DC	Regular diet for 20 weeks + AOM injection (15 mg/kg, once weekly for 2 weeks) + 5-fluorouracil after AOM
TE	Regular diet for 20 weeks + AOM injection (15 mg/kg, once weekly for 2 weeks) + tea polyphenol extract (200 mg/kg, oral)
TN	Regular diet for 20 weeks + AOM injection (15 mg/kg, once weekly for 2 weeks) + iron oxide–chitosan encapsulated tea polyphenols nanoparticles (200 mg/kg, oral)

Note. AOM = azoxymethane; DC = drug control; NC = negative control; PC = positive control; TE = tea extract; TN = tea nanoparticle.

protein name, peptide sequences, accession numbers, lengths, mass-to-charge (m/z) ratios, and protein abundances.

Bioinformatics analysis

First, all proteins were filtered for high-confidence identification using previously described criteria (Ibrahim et al., 2024; Weiner et al., 2022). The filters used were a false discovery rate (FDR) of <1% to reduce false positives, a threshold of $-10\log(P) > 20$ to ensure statistical confidence, and proteins must be detected in at least two out of three replicates to avoid random error. Additionally, protein coverage was required to be ≥1%, and unique peptide coverage was needed to be ≥2 to prevent false identification. These cut-offs were used in proteomics studies to ensure high-confidence identification and reproducibility. Second, the identified proteins were subjected to differential expression analysis using previously described methods (Kim et al., 2012; Li et al., 2022; Ibrahim et al., 2024). Each protein was compared between groups using one-way ANOVA with Tukey's post hoc test, followed by fold change analysis with the NC group as the baseline. Proteins with significant post hoc differences ($p < .05$) and fold-change values ≤0.83 or ≥1.20 relative to NC were defined as differentially dysregulated. Third, uniquely expressed proteins were identified. The proteins were pooled across groups, and intersections were visualised using Create-A-Venn (http://bioinformatics.psb.ugent.be/webtools/Venn/). Fourth, functional enrichment analysis was performed using UniProt, Gene Ontology (QuickGO), and Protein Analysis Through Evolutionary Relationships (PANTHER) to classify proteins by cellular components, families, molecular functions, and biological processes, reported as percentages. Protein–protein interaction networks were generated with Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (score ≥ 0.700), and curated pathway analysis was conducted using Kyoto Encyclopedia of Genes and Genomes (KEGG), with pathways, matching proteins, and FDR values reported as significant at FDR < 0.05 (Liew et al., 2022).

Statistical analysis

One-way ANOVA and Tukey's post hoc were performed using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA), and fold-change analysis was done using Microsoft Excel to determine the differentially expressed proteins among the groups. Data were presented as mean ± standard deviation, and a p-value of <.05 was considered statistically significant.

Results

The LC-MS/MS analysis revealed a total of 2,258 proteins across all experimental groups. After preprocessing for high-quality identifications and consistency, 287 proteins were retained for downstream analysis. Differential protein analysis identified 20 proteins that were significantly dysregulated. Then, unique protein analysis further revealed 16 proteins that were uniquely expressed following TE or TN treatment, with only two proteins uniquely expressed in the TN group. Finally, one protein was identified as a potential biomarker due to its previously recorded significance in anticancer pathways. Figure 1 illustrates the flowchart of the number of proteins at each stage of the analysis.

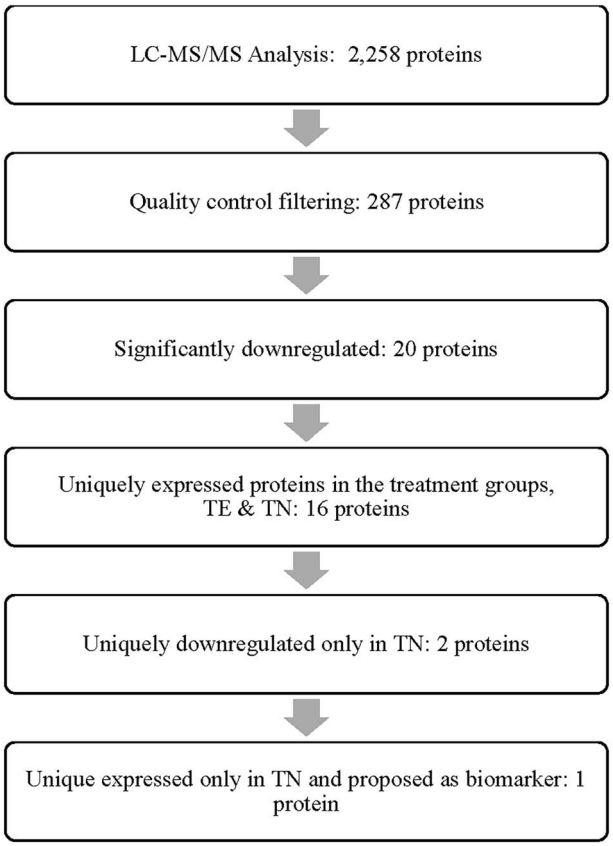


Figure 1. Flowchart of the number of proteins after subsequent analysis.

Differential protein expression

The expression of the proteins across the control and treatment groups was visualised using a heatmap (Figure 2). As depicted in the heatmap, hierarchical clustering of protein expression revealed decreased expression across treatment groups compared to the control groups.

Differential protein expression analysis using one-way ANOVA, post hoc analysis, and fold-change analysis identified 20 proteins across the treatment groups that met the criteria for statistical significance in post hoc analysis ($p < .05$) and the fold-change thresholds (≤0.83 or ≥1.20) relative to NC as the baseline (Table 2).

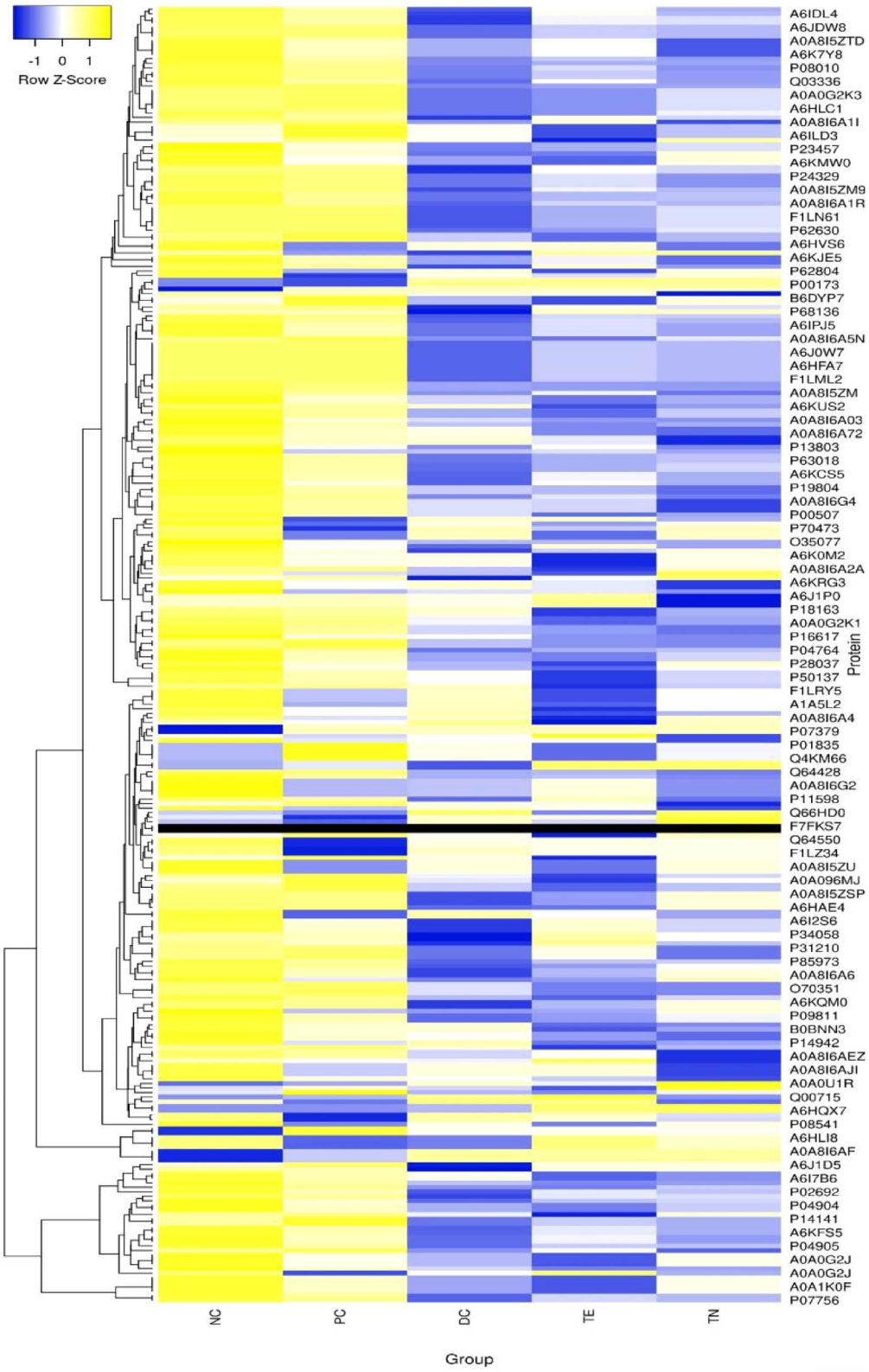


Figure 2. Heatmap showing the relative protein expression levels across the sample groups. Higher and lower expression levels are represented by relative intensity values. The Y-axis represents the proteins. Each row corresponds to a specific protein identified in the analysis. The X-axis represents the sample groups. Each column corresponds to one of the experimental groups: negative control (NC), positive control (PC), drug control (DC), tea extract (TE), and tea nanoparticle (TN).

Table 2. Significantly dysregulated proteins across experimental groups.

No.	Protein name	Protein ID	Average protein abundance (Log2)					*p*-value	Fold change analysis				
			NC (baseline)	PC	DC	TE	TN		NC (baseline)	: PC	: DC	: TE	: TN
1.	3-Hydroxyacyl-Coa Dehydrogenase Type-2	O70351	14.24^{a}	14.32^{a}	13.19^{ab}	$*12.55^{b}$	$*12.65^{b}$	0.026	1.00	: 1.07	: 0.44 (↓)	: *0.29 (↓)	: *0.30 (↓)
2.	Aldehyde Dehydrogenase 8 Family Member A1	D3ZXY4	15.69^{a}	14.83^{ab}	13.94^{ab}	$*13.58^{b}$	$*13.83^{b}$	0.020	1.00	: 0.53 (↓)	: 0.29 (↓)	: *0.27 (↓)	: *0.28 (↓)
3.	Argininosuccinate Synthase	A6JU24	18.83^{a}	18.35^{ab}	$*16.96^{bc}$	$*16.83^{c}$	$*17.34^{bc}$	0.003	1.00	: 0.72 (↓)	: *0.29 (↓)	: *0.28 (↓)	: *0.35 (↓)
4.	Bile Acid-Coa: Amino Acid N-Acyltransferase	Q63276	16.82^{a}	15.76^{ab}	14.10^{ab}	14.85^{ab}	$*13.52^{b}$	0.044	1.00	: 0.72 (↓)	: 0.18 (↓)	: 0.31 (↓)	: *0.16 (↓)
5.	Bile Acid-Coenzyme A: Amino Acid N-Acyltransferase	A6KJE5	16.82^{a}	15.76^{ab}	14.10^{ab}	14.85^{ab}	$*13.52^{b}$	0.044	1.00	: 0.72 (↓)	: 0.18 (↓)	: 0.31 (↓)	: *0.16 (↓)
6.	Cystathionine Gamma-Lyase	P18757	14.93^{a}	14.30^{a}	12.86^{ab}	$*12.12^{b}$	13.08^{ab}	0.010	1.00	: 0.59 (↓)	: 0.25 (↓)	: *0.17 (↓)	: 0.26 (↓)
7.	Enoyl Coenzyme A Hydratase Short Chain 1 Mitochondrial Isoform CRA_A	A6HXG6	15.12^{a}	14.53^{a}	14.46^{a}	14.05^{ab}	$*12.94^{b}$	0.005	1.00	: 0.62 (↓)	: 0.57 (↓)	: 0.47 (↓)	: *0.20 (↓)
8.	Enoyl-CoA Hydratase Mitochondrial	P14604	15.12^{a}	14.53^{a}	14.46^{a}	14.05^{ab}	$*12.94^{b}$	0.005	1.00	: 0.62 (↓)	: 0.57 (↓)	: 0.47 (↓)	: *0.20 (↓)
9.	Glutathione S-Transferase	B6DYP8	17.89^{a}	17.29^{ab}	16.73^{ab}	$*16.57^{b}$	16.86^{ab}	0.048	1.00	: 0.70 (↓)	: 0.44 (↓)	: *0.42 (↓)	: 0.48 (↓)
10.	Glutathione S-Transferase Alpha-3	P04904	17.89^{a}	17.29^{ab}	16.73^{ab}	$*16.57^{b}$	16.86^{ab}	0.048	1.00	: 0.70 (↓)	: 0.44 (↓)	: *0.42 (↓)	: 0.48 (↓)
11.	Heat Shock Protein Family A Hsp70 Member 8	A0A8I6A5N6	14.86^{ab}	15.09^{a}	13.72^{ab}	$*13.60^{b}$	14.19^{ab}	0.019	1.00	: 1.23 (↑)	: 0.45 (↓)	: *0.46 (↓)	: 0.65 (↓)
12.	Hydroxyacyl-Coenzyme A Dehydrogenase Type II	B0BMW2	14.24^{a}	14.32^{a}	13.19^{ab}	$*12.55^{b}$	$*12.65^{b}$	0.026	1.00	: 1.07	: 0.44 (↓)	: *0.29 (↓)	: *0.30 (↓)
13.	Hydroxysteroid (17-Beta) Dehydrogenase 10	A0A8I6AFS1	14.24^{a}	14.32^{a}	13.19^{ab}	$*12.55^{b}$	$*12.65^{b}$	0.026	1.00	: 1.07	: 0.44 (↓)	: *0.29 (↓)	: *0.30 (↓)
14.	Microsomal Glutathione S-Transferase 1	A0A8I6ALY9	14.11^{a}	14.34^{a}	13.51^{ab}	$*13.14^{b}$	13.49^{ab}	0.043	1.00	: 1.15	: 0.63 (↓)	: *0.48 (↓)	: 0.62 (↓)
15.	Microsomal Glutathione S-Transferase 1 Isoform CRA_A	A6IML5	14.11^{a}	14.34^{a}	13.51^{ab}	$*13.14^{b}$	13.49^{ab}	0.043	1.00	: 1.15	: 0.63 (↓)	: *0.48 (↓)	: 0.62 (↓)
16.	Peroxiredoxin-6	A0A8I6A038	15.64^{a}	14.54^{ab}	14.15^{ab}	$*13.74^{b}$	$*13.71^{b}$	0.032	1.00	: 0.47 (↓)	: 0.34 (↓)	: *0.30 (↓)	: *0.25 (↓)
17.	Transketolase	P50137	15.53^{a}	14.74^{a}	14.21^{ab}	$*12.58^{b}$	13.79^{ab}	0.007	1.00	: 0.62 (↓)	: 0.38 (↓)	: *0.15 (↓)	: 0.28 (↓)
18.	Transketolase Isoform CRA_A	A6KG34	15.53^{a}	14.74^{a}	14.21^{ab}	$*12.58^{b}$	13.79^{ab}	0.007	1.00	: 0.62 (↓)	: 0.38 (↓)	: *0.15 (↓)	: 0.28 (↓)
19.	Transketolase Isoform CRA_B	A6KG36	15.53^{a}	14.74^{a}	14.21^{ab}	$*12.58^{b}$	13.79^{ab}	0.007	1.00	: 0.62 (↓)	: 0.38 (↓)	: *0.15 (↓)	: 0.28 (↓)
20.	UDP-Glucuronosyltransferase 2B2	P08541	13.66^{a}	$*11.69^{b}$	12.55^{ab}	$*11.60^{b}$	12.60^{ab}	0.014	1.00	: *0.27 (↓)	: 0.45 (↓)	: *0.28 (↓)	: 0.47 (↓)

Note. DC = drug control; NC = negative control; PC = positive control; TE = tea extract; TN, tea nanoparticle. Tukey post hoc analysis: different superscript letters indicate significant difference of 'Average Protein Abundance (Log2)' between groups within the same row ($p < .05$). Fold change analysis: (↑) indicates upregulation, and (↓) indicates downregulation (threshold values of ≥1.20) and (↓) (threshold values of ≤0.83). *Proteins with significant post hoc differences ($p < .05$) and fold-change values ≤0.83 or ≥1.20 relative to NC were defined as differentially dysregulated.

Overall, TE and TN treatments showed a consistent downregulatory trend. The magnitude of downregulation effect differed slightly between the two treatment groups; however, no statistically significant differences were observed between TE and TN. A total of 12 proteins exhibited significant downregulation in at least one treatment group compared to NC. Among these, five proteins were uniquely downregulated in the TE group, namely, Cystathionine gamma-lyase, Glutathione S-transferase, Microsomal glutathione S-transferase 1, Heat shock protein family A Hsp70 member 8, and Transketolase. Interestingly, only one protein, Enoyl-CoA hydratase mitochondrial, was significantly downregulated in the TN group. Six proteins were downregulated in both TE and TN groups, including Peroxiredoxin-6, 3-hydroxyacyl-CoA dehydrogenase type-2, Aldehyde dehydrogenase 8 family member A1, Argininosuccinate synthase, Hydroxyacyl-coenzyme A dehydrogenase type II, and Hydroxysteroid 17β dehydrogenase 10. From the DC group, only Argininosuccinate synthase showed significant downregulation relative to NC. Interestingly, similar downregulatory trends were also observed in the TE and TN groups, and their downregulatory effect did not differ significantly from one another. Notably, the TN group demonstrated a greater downregulatory trend for Peroxiredoxin-6 compared to TE, though this difference was not statistically significant. Interestingly, Enoyl-CoA hydratase mitochondrial exhibited significant downregulation ($p < .05$) only in the TN group relative to NC, while comparable but nonsignificant trends were observed in the DC and TE groups. Overall, these findings indicate that TE and TN exert similar overall downregulation effects, with TN showing a slightly stronger Peroxiredoxin-6 and Enoyl-CoA hydratase mitochondrial but not a significant downregulatory magnitude.

Uniquely expressed proteins

The Venn diagram (Figure 3) revealed 10 proteins uniquely expressed in TE, which are Transitional endoplasmic reticulum ATPase (P46462), Cytosolic 10-formyltetrahydrofolate dehydrogenase (P28037), Diazepam binding inhibitor acyl-CoA binding protein (A0A8I5ZTD4), Microsomal triglyceride transfer protein large subunit (D4A1W8), Uncharacterised protein (A0A0U1RVK8), L-lactate dehydrogenase A chain (P04642), RCG37628 (A6K7Y8), Elongation factor 1-alpha (A0A8I6AQI9), LRRGT00046 (Q6TXF3), and Acyl-CoA-binding protein (P11030).

On the other hand, six proteins are uniquely expressed in TN, which are Phosphatidylethanolamine binding protein 1 (A0A8I5ZLC1), Delta-1-pyrroline-5-carboxylate dehydrogenase mitochondrial (P0C2X9), Multifunctional fusion protein (A0A0G2JUB9), Phosphatidylethanolamine-binding protein 1 (P31044), Phosphatidylethanolamine binding protein one isoform CRA_c (A6J1P0), and Fatty acid synthase (A0A8I6A2Z2).

Functional and pathway enrichment

Functional enrichment analysis revealed that the differentially and uniquely expressed proteins were associated with various molecular and biological functions, as depicted in Figure 4.

For molecular functions, most proteins were involved in catalytic activity (GO:0003824), accounting for 71.80% of the identified functions. Other significant molecular functions included binding (GO:0005488) (23.80%), molecular transducer activity (GO:0060089) (1.80%), transporter activity (GO:0005215) (1.50%), antioxidant activity (GO:0016209) (0.70%), and adenosine triphosphate (ATP)-dependent activity (GO:0140657) (0.40%).

For biological functions, the proteins are primarily involved in cellular processes (GO:0009987) (42.30%) and metabolic processes (GO:0008152) (40.90%). Additional biological functions included

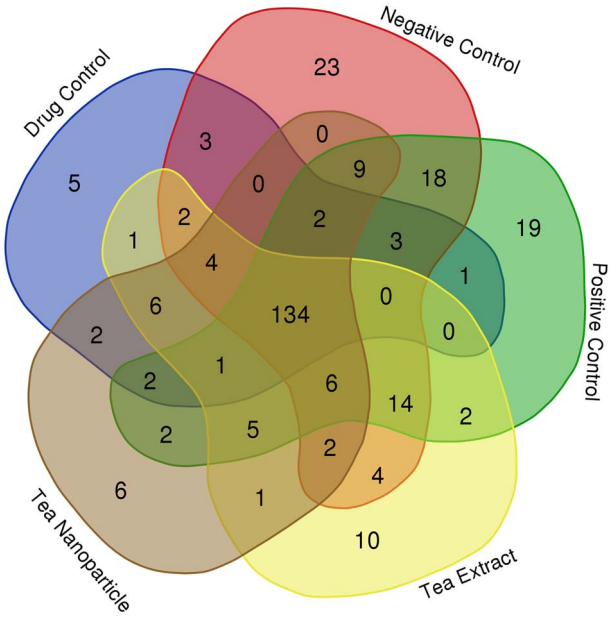


Figure 3. Venn diagram of protein distribution across experimental groups.

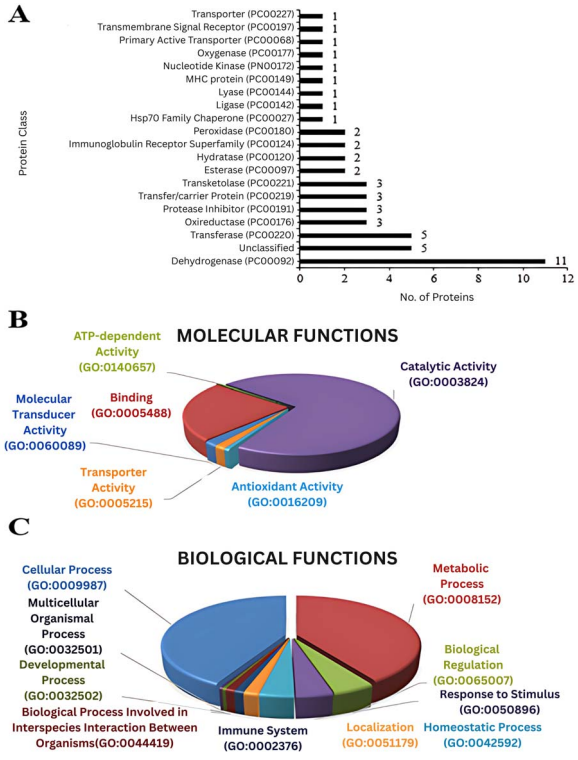


Figure 4. (A) Protein classes from the differentially expressed proteins and uniquely expressed proteins in the treatment groups. (B) Classifications of the differentially and uniquely expressed proteins according to molecular functions. (C) Classifications of the differentially and uniquely expressed proteins according to biological functions.

biological regulation (GO:0065007) (4.70%), response to stimulus (GO:0050896) (4.00%), homeostatic processes (GO:0042592) (3.80%), localisation (GO:0051179) (1.60%), immune system processes (GO:0002376) (1.00%), biological processes involved in interspecies interactions between organisms (GO:0044419)

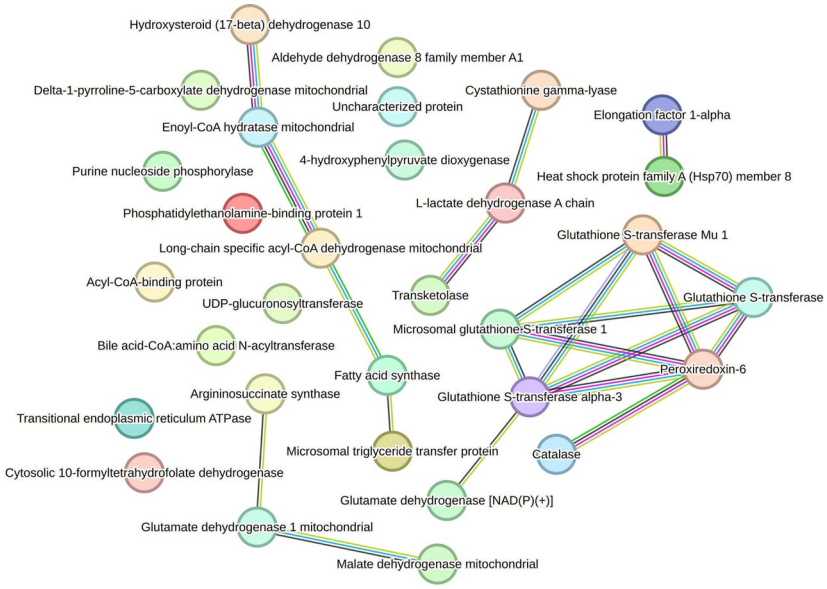


Figure 5. Protein–protein interaction network of unique and differentially expressed proteins.

(1.00%), developmental processes (GO:0032502) (0.40%), and multicellular organismal processes (GO:0032501) (0.40%).

The differentially expressed proteins and uniquely expressed proteins in the treatment groups were subjected to pathway enrichment analysis. The protein–protein interactions were compiled using the STRING web tools and presented in Figure 5. The network comprises 31 nodes connected by 21 interactions. The network has an observed enrichment p-value of 6.66×10^{-15}. This significant enrichment suggests that the identified proteins are not randomly associated but are biologically interconnected.

A total of 23 pathways were found to be significantly enriched (FDR < 0.05) based on KEGG pathway analysis. The significant pathways identified through this analysis are summarised in Table 3.

Notably, most of the proteins were matched to the metabolic pathway (rno01100), showing the highest significance with an FDR of 7.39×10^{-19}, involving 20 observed proteins from a background of 1,431 proteins. Other than that, nine pathways showed high levels of significance (FDR < 0.001) with a high number of proteins matched ($n > 3$).

It is essential to comprehensively interpret the protein expression trends, enriched pathways, and their known associations with cancer, enabling a more straightforward understanding of how the treatment affects key biological processes involved in colon carcinogenesis. Table 4 summarises the differentially and uniquely expressed proteins in the treatment groups, along with the enriched pathways and previous association with cancer progression.

Discussion

Pathway enrichment reveals that the mechanisms associated with the downregulated proteins in anticancer are categorised into three types: antioxidant defence and detoxification, stress response, and metabolic activity. Among these, antioxidant defence and detoxification demonstrated the strongest association, as evidenced by the high number of downregulated proteins involved in these pathways.

Previous studies have demonstrated tea polyphenols' potential to enhance antioxidant defence and regulate detoxification as an anticancer mechanism (Xu et al., 2022). Following TE treatment, several glutathione S-transferases were downregulated. Previously, overexpression of this protein was associated with chemotherapy resistance and poor prognosis in colorectal cancer (Johansson et al., 2007). Other antioxidant-related enzymes like aldehyde dehydrogenase and cystathionine gamma-lyase were also downregulated, suggesting reduced oxidative stress (Tunçer et al., 2019; Zuhra et al., 2020). Antioxidant enzymes such as peroxiredoxin-6 and transketolase were downregulated in both the TE and TN groups. Previous studies have observed overexpression of these enzymes as a marker of oxidative damage (Falidas et al., 2021; Li et al., 2022). Therefore, downregulation of these proteins suggests modulation of antioxidant defences. Notably, the TN group showed a greater downregulatory trend of peroxiredoxin-6; however, the difference was not statistically significant. Nevertheless, this trend may suggest a potentially stronger modulatory effect on oxidative stress pathways from the TN group.

Tea polyphenols such as epigallocatechin gallate have been shown to modulate cellular stress response mechanisms by targeting molecular chaperones and signalling pathways (Ishak et al., 2025). Tea polyphenols have been found to reduce the expression of heat shock proteins, which are often upregulated in cancer cells to maintain protein stability and survival under stress (Ishak et al., 2025). In this study, the heat shock protein family A, Hsp70 member 8, was found to be downregulated following TE treatment and upregulated in the untreated control group. Elevated expression of the Heat Shock Protein Family was associated with tumour progression and a poor prognosis in colorectal cancer (Jiang et al., 2021). Thus, downregulation of this protein may elucidate the potential anti-cancer mechanism of TE by attenuating cancer cell survivability.

Tea polyphenols have demonstrated anticancer properties by inhibiting dysregulated metabolic pathways involved in colorectal cancer (Wang et al., 2021). Previously, epigallocatechin gallate was reported to inhibit colorectal cancer cells' viability by inhibiting key pathways involving fatty acid synthesis, fatty acid

Table 3. Enriched pathways identified using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis from differentially and uniquely expressed proteins.

No.	Pathways	KEGG ID	Observed	Back-ground	False discovery rate	Matching proteins	
						Differentially expressed proteins	Uniquely expressed proteins
1	Metabolic pathways	rno01100	20	1,431	7.39E-19	B6DYP8, A0A8L2Q085, P08541, Q63276, A0A8I6ALY9, P04762, P85973, A6KFS5, P15650, P04904, P50137, D3ZXY4, P14604, A0A8I6A038, P04905, A0A8I6AFS1, P18757, P10860, A6JU24	A0A0U1RVK8, P04642, FA0A8I6A2Z2
2	Metabolism of xenobiotics by cytochrome P450	rno00980	5	52	2.46E-06	B6DYP8, P08541, A0A8I6ALY9, P04904, P04905	–
3	Drug metabolism—cytochrome P450	rno00982	5	52	2.46E-06	B6DYP8, P08541, A0A8I6ALY9, P04904, P04905	–
4	Glutathione metabolism	rno00480	5	62	2.81E-06	B6DYP8, A0A8I6ALY9, P04904, A0A8I6A038, P04905	–
5	Fluid shear stress and atherosclerosis	rno05418	6	134	2.81E-06	B6DYP8, A0A8I6ALY9, A6KFS5, P04904, P04905, A6JU24	–
6	Drug metabolism—other enzymes	rno00983	5	70	3.32E-06	B6DYP8, P08541, A0A8I6ALY9, P04904, P04905	–
7	Chemical carcinogenesis	rno05204	5	70	3.32E-06	B6DYP8, P08541, A0A8I6ALY9, P04904, P04905	–
8	Hepatocellular carcinoma	rno05225	5	161	1.20E-04	B6DYP8, A0A8I6ALY9, A6KFS5, P04904, P04905	–
9	Carbon metabolism	rno01200	4	112	2.32E-05	P04762, P50137, P14604, P10860	–
10	Platinum drug resistance	rno01524	4	73	1.30E-04	B6DYP8, A0A8I6ALY9, P04904, P04905	–
11	Cysteine and methionine metabolism	rno00270	2	46	1.30E-03	P18757	P04642
12	Tryptophan metabolism	rno00380	3	47	1.30E-03	P04762, D3ZXY4, P14604	–
13	Fatty acid metabolism	rno01212	3	57	2.00E-03	P15650, P14604	A0A8I6A2Z2
14	Ubiquinone and other terpenoid-quinone biosynthesis	rno00130	2	11	3.40E-03	A0A8L2Q085, A6KFS5	–
15	Biosynthesis of amino acids	rno01230	3	76	4.00E-03	P50137, P18757, A6JU24	–
16	Arginine biosynthesis	rno00220	2	18	7.30E-03	P10860, A6JU24	–
17	Pathways in cancer	rno05200	5	499	1.19E-02	B6DYP8, A0A8I6ALY9, A6KFS5, P04904, P04905	–
18	Propanoate metabolism	rno00640	2	30	1.58E-02	P14604	P04642
19	Alanine, aspartate, and glutamate metabolism	rno00250	2	34	1.90E-02	P10860, A6JU24	–
20	Fatty acid degradation	rno00071	2	40	2.35E-02	P15650, P14604	–
21	Valine, leucine, and isoleucine degradation	rno00280	2	49	3.31E-02	P14604, HA0A8I6AFS1	–
22	Legionellosis	rno05134	2	50	3.31E-02	–	P46462, A0A8I6AQI9
23	Retinol metabolism	rno00830	2	62	4.76E-02	P08541	A0A0U1RVK8

β-oxidation, and energy metabolism (Wang, Pan, et al., 2021). Following treatment of TE and TN, 3-hydroxyacyl-CoA dehydrogenase type 2 was found to be downregulated. This enzyme was previously suggested as a potential oncogene for colorectal cancer due to its role in promoting colon cancer proliferation by fatty acid oxidation (Fang et al., 2022). Thus, the downregulation of this enzyme may be linked to the anticancer effect of TE and TN by regulating fatty acid oxidation. Argininosuccinate synthase was also downregulated following treatment with both TE and TN. A study demonstrated the involvement of arginosuccinate synthase in regulating glycolytic and lipid metabolism in promoting colorectal cancer cells (Bateman et al., 2017). Therefore, the observed downregulation of this enzyme by TE and TN suggests a potential anti-cancer mechanism by disrupting crucial energy pathways within cancer cells. Interestingly, the mitochondrial enoyl-CoA hydratase exhibited significant downregulation ($p < .05$) relative to NC following treatment with TN alone. Although similar downregulatory trends were observed from the DC and TE group, it does not achieve statistical significance in comparison to the NC group. These enzymes were linked to fatty acid β-oxidation, which promotes colorectal cancer proliferation by maintaining energy supply (Zhao et al., 2015). Thus, the downregulation of enoyl-CoA hydratase has been linked to a poorer prognosis in colorectal cancer. Thus, the expression of

Table 4. Summary of protein expression trends, enriched pathways, and previous association with cancer among the treatment groups.

No.	Protein name	Protein ID	Expression trends in treatment groups	Enriched pathway	Previous findings on cancer	Ref.
	Differentially expressed protein					
1.	Cystathionine Gamma-Lyase	P18757	Downregulated in TE	• Metabolic pathways • Cysteine and methionine metabolism • Biosynthesis of amino acids	Upregulation by the Wnt signalling pathway promotes colorectal cancer proliferation	(Fan et al., 2014)
2.	Glutathione S-Transferase	B6DYP8	Downregulated in TE	• Metabolic pathways • Metabolism of xenobiotics by cytochrome P450 • Drug metabolism—cytochrome P450 • Glutathione metabolism • Fluid shear stress and atherosclerosis • Drug metabolism—other enzymes • Chemical carcinogenesis • Hepatocellular carcinoma • Platinum drug resistance • Pathways in cancer	Upregulated in colorectal cancer patients	(Nomani et al., 2005)
4.	Microsomal Glutathione S-Transferase 1	A0A8I6ALY9	Downregulated in TE	• Metabolic pathways • Metabolism of xenobiotics by cytochrome P450 • Drug metabolism—cytochrome P450 • Glutathione metabolism • Fluid shear stress and atherosclerosis • Drug metabolism—other enzymes • Chemical carcinogenesis • Hepatocellular carcinoma • Platinum drug resistance • Pathways in cancer	Upregulated in tumour and involved in chemotherapy resistance	(Johansson et al., 2007)
5.	Heat Shock Protein Family A (Hsp70) Member 8	A0A8I6A5N6	Downregulated in TE	No significant pathways identified	Upregulated in colon cancer and associated with poor prognosis	(Jiang et al., 2021)
7.	Transketolase	P50137	Downregulated in TE	• Metabolic pathways • Carbon metabolism • Biosynthesis of amino acids	Promotes colorectal cancer metastasis through AKT activation	(Li et al., 2022)
11.	Enoyl-Coa Hydratase Mitochondrial	P14604	Downregulated in TN	• Metabolic pathways • Carbon metabolism • Tryptophan metabolism • Fatty acid metabolism • Propanoate metabolism • Fatty acid degradation • Valine, leucine, and isoleucine degradation	Upregulated in colorectal cancer and involved in mitochondrial fatty acid β-oxidation pathway	(Zhao et al., 2015)
15.	Peroxiredoxin-6	A0A8I6A038	Downregulated in TE and TN	• Metabolic pathways • Glutathione metabolism	Oxidative stress marker and overexpressed in aggressive stage colon cancer	(Falidas et al., 2021)
16.	3-Hydroxyacyl-Coa Dehydrogenase Type-2	O70351	Downregulated in TE and TN	No pathways enriched	Promotes colon cancer proliferation	(Fang et al., 2022)
17.	Aldehyde Dehydrogenase 8 Family Member A1	D3ZXY4	Downregulated in TE and TN	• Metabolic pathways • Tryptophan metabolism	Associated with shortened overall survival	(Tunçer et al., 2019)

(Continued)

Table 4. Continued.

No.	Protein name	Protein ID	Expression trends in treatment groups	Enriched pathway	Previous findings on cancer	Ref.
18.	Argininosuccinate Synthase	A6JU24	Downregulated in TE and TN	• Metabolic pathways • Fluid shear stress and atherosclerosis • Biosynthesis of amino acids • Arginine biosynthesis • Alanine, aspartate, and glutamate metabolism	Promotes cancer cell metabolism and proliferation	(Bateman et al., 2017)
19.	Hydroxyacyl-Coenzyme A Dehydrogenase Type II	B0BMW2	Downregulated in TE and TN	No pathways enriched	Promotes colon cancer proliferation	(Fang et al., 2022)
20.	Hydroxysteroid (17-Beta) Dehydrogenase 10	A0A8I6AFS1	Downregulated in TE and TN	• Metabolic pathways • Valine, leucine, and isoleucine degradation	Low expression is associated with poor prognosis	(Amberger et al., 2016)
	Uniquely expressed proteins					
1.	Transitional endoplasmic reticulum ATPase	P46462	Expressed in TE only	• Legionellosis	Upregulated among colorectal cancer patients	(Kim et al., 2012)
2.	Cytosolic 10-formyltetrahydrofolate dehydrogenase	P28037	Expressed in TE only	No pathways enriched	Downregulated in human cancers and involved in folate metabolism	(Krupenko & Krupenko, 2019)
3.	Diazepam binding inhibitor acyl-CoA binding protein	A0A8I5ZTD4	Expressed in TE only	No pathways enriched	Increases risk of cancer by inhibiting autophagy	(Montégut et al., 2024)
6.	L-lactate dehydrogenase A chain	P04642	Expressed in TE only	• Metabolic pathways • Cysteine and methionine metabolism • Propanoate metabolism	Promotes tumorigenesis	(Wang, Pan, et al., 2021)
8.	Elongation factor 1-alpha	A0A8I6AQI9	Expressed in TE only	• Legionellosis	Upregulation is associated with poor prognostic in colon cancer	(Joung et al., 2019)
10.	Acyl-CoA-binding protein	P11030	Expressed in TE only	No pathways enriched	Increases risk of cancer by inhibiting autophagy	(Montégut et al., 2024)
11.	Phosphatidylethanolamine binding protein 1	A0A8I5ZLC1	Expressed in TN only	No pathways enriched	Tumour suppressor by inhibiting tumorigenesis and cell invasion	(Nie et al., 2015)
12.	Delta-1-pyrroline-5-carboxylate dehydrogenase mitochondrial	P0C2X9	Expressed in TN only	No pathways enriched	Overexpressed in wide variation of cancers	(Burke et al., 2020)
14.	Phosphatidylethanolamine-binding protein 1	P31044	Expressed in TN only	No pathways enriched	Tumour suppressor by inhibiting tumorigenesis and cell invasion *via* MAPK pathway	(Nie et al., 2015)
16.	Fatty acid synthase	A0A8I6A2Z2	Expressed in TN only	• Metabolic pathways • Fatty acid metabolism	Promotes cancer survival by energy homeostasis	(Zaytseva et al., 2015)

Note. TE = tea extract; TN = tea nanoparticle.

mitochondrial enoyl-CoA hydratase was significantly downregulated by TN treatment, likely due to its ability to modulate cancer cell energy metabolism through the enhanced formulation of TN.

One out of six uniquely expressed proteins in TN was selected as a potential biomarker for its previously reported anticancer effect. The identified protein was Phosphatidylethanolamine-binding protein 1, also called Raf-1 kinase inhibitor protein. Previous studies have demonstrated that the upregulation of Phosphatidylethanolamine-binding protein 1 is a potential tumour suppressor protein, as it is associated with the inhibition of the MAPK signalling pathway, which subsequently reduces cell proliferation and metastasis (Nie et al., 2015). This finding is further supported by evidence indicating that the inhibition of MAPK signalling is a known anticancer mechanism of tea polyphenols (Ishak et al., 2025). Therefore, Phosphatidylethanolamine-binding protein 1 is a promising candidate biomarker for the anticancer mechanism of TN, as it inhibits the MAPK signalling pathway.

Nevertheless, this study has several key limitations. The scope of this study was limited to proteomic profiling, which helps identify broad patterns of protein expression but does not confirm the direct functional roles of these proteins in the observed effects. Validation tests, such as western blotting or gene expression studies, were not conducted, which limits the confirmation of protein expression in specific pathways. Additionally, the study was conducted on a rat model, which may not be fully representative of human colorectal cancer. Future research should address these limitations by conducting validation studies to confirm the expression and roles of the identified proteins. Employing targeted analyses, such as immunohistochemistry or western blotting, will help establish the functional significance of these proteins in antioxidant defence, detoxification, and metabolic regulation. To confirm these findings in clinical applications, future studies should also incorporate proteomic profiling using human clinical samples, together with comprehensive evaluations of nanoparticle safety and biodistribution, to better understand the implications for patients with colorectal cancer.

Conclusion

This study revealed 20 significantly downregulated liver proteins following treatment with TE and TN in an AOM-induced colorectal cancer rat model. The downregulated proteins were enriched in pathways involved in antioxidant defences, detoxification, and metabolic regulation, thus suggesting that both treatments modulate key biological processes associated with cancer progression. Notably, TN demonstrated a greater downregulation effect on Peroxiredoxin-6 (A0A8I6A038) and enoyl-CoA Hydratase Mitochondrial (P14604 and A6HXG6). This suggests that TN enhanced its therapeutic potential by modulating antioxidant defences and energy metabolism. The unique expression of phosphatidylethanolamine-binding protein 1 in the TN group indicates a possible involvement of the MAPK pathway modulation, a key regulator of cell proliferation and metastasis in colorectal cancer. These findings contribute new proteomic evidence supporting the potential of nanoparticle-based delivery to enhance the biological activity of tea polyphenols. However, this study is limited to proteomic profiling using an animal model; thus, further validation using targeted molecular assays and clinical validation using human studies are necessary to support these proteomic observations and elucidate TN potential as a novel therapeutic or complementary strategy for colorectal cancer.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Mohammad A. Fadzil (Conceptualisation, Formal analysis, Writing—review & editing [equal], Data curation, Methodology, Visualisation, Writing—original draft [lead]), Nurul Husna Shafie (Conceptualisation, Writing—review & editing [equal], Funding acquisition, Project administration, Supervision [lead]), Haziyah Amirah (Investigation, Methodology, Resources [equal]), Safaa Al-Awawdeh (Investigation, Methodology, Resources [equal]), Mohd Naeem Mohd Nawi (Formal analysis, Methodology, Writing—review & editing [equal]), and Aswir Abd Rashed (Supervision [supporting], Writing—review & editing [equal])

Funding

The authors express their gratitude to the Ministry of Education, Malaysia, for providing project funding through the Fundamental Research Grant Scheme (FRGS/1/2018/SKK10/UPM/02/5).

Conflicts of interest

None declared.

Acknowledgements

The authors would like to acknowledge BOH Plantation Sdn. Bhd. (Malaysia) for kindly providing the tea leaves used in this research project. The authors thank the Director General of Health Malaysia and the Director of the Institute for Medical Research (IMR), Malaysia, for permitting the publication of this article.

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International Journal of Food Science and Technology, 2026, **61(1)**, vvaf265
https://doi.org/10.1093/ijfood/vvaf265
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