Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025154

RESEARCH ARTICLE

Anticancer and antioxidant activities of polyphenolic pomegranate peel extracts obtained by a novel hybrid ultrasound-microwave method: *In vitro* and *in vivo* studies in albino mice with HeLa, colon, and HepG2 cancerous cell lines

Lamaia R. Altarjami[1]*

[1]Department of Chemistry, College of Science and Arts, King Abdulaziz University, Rabigh, 21911, Saudi Arabia
*Corresponding author: Laltarjami@kau.edu.sa (Lamaia R. Altarjami)

ARTICLE HISTORY (25-132)

Received: February 15, 2025
Revised: March 29, 2025
Accepted: March 30, 2025
Published online: April 27, 2025

Key words:
Cancer
Colon
Mice models
Phenolic
Pomegranate
Waste

ABSTRACT

Cancer is a deadly disease and ranks as the second leading cause of death globally, which predominantly arises as a result of continuous exposure of human beings to carcinogenic agents and environmental contaminants. Natural compounds can potentially treat up to 60% of cancer cases. Therefore, this study investigated the anticancer and antioxidant activities of pomegranate peel extracts, which were prepared using a novel hybrid ultrasound-microwave assisted extraction (HUME) method. The HUME demonstrated significant improvements in extraction yield and polyphenol contents compared to conventional methods. The anticancer activity of pomegranate peel extract (PPE) was evaluated against HeLa, colon, and HepG2 cell lines and *in vivo* colon cancer in albino mice. The PPE demonstrated potent antioxidant activity, with DPPH and ABTS radical scavenging capacities of 91% and 95%, respectively. This potent activity was attributed to its high levels of phenolic acids (181 mg GAE/g) and flavonoids (35 mg QE/g). The secondary metabolites of PPE were detected by MS/MS using negative and positive ionization modes; the most abundant detected compounds were 4-hydroxycoumarin, p-coumaric, and gallic acid. A 40 Albino mice were randomly assigned to four experimental groups: a control group, a pathogen-induced cancer group, a 5-fluorouracil-treated group, and a group administered pomegranate peel extract (PPE). Albino mice treated with the PPE showed significant ($P<0.05$) tumor volume reduction and tumor growth inhibition compared to the diseased group. Pomegranate peel extract (PPE) demonstrated *in vivo* anticancer activity against bacterial pathogen-induced colon cancer. This was evident by significantly ($P<0.05$) downregulated B-cell leukemia/lymphoma 2 (BCL2) and Hypoxia-inducible factor 1-alpha (HIF1-α) level in the serum by 50% and 30%, respectively, in the PPE-treated group compared to the infected control. Concomitantly, PPE treatment improved histopathological features in the colon tissue, where the glandular structures remained intact and normal histological structure. These findings suggest that pomegranate peel extracts obtained by HUME possess potent anticancer and antioxidant activities. Further research is warranted to elucidate the underlying mechanisms and explore their potential as promising therapeutic agents for cancer treatment.

To Cite This Article: Altarjami LR, 2025. Anticancer and antioxidant activities of polyphenolic pomegranate peel extracts obtained by a novel hybrid ultrasound-microwave method: *In vitro* and *in vivo* studies in albino mice with HeLa, colon, and HepG2 cancerous cell lines. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2025.154

INTRODUCTION

Cancer is a significant public health issue and the leading cause of death globally (Ferlay *et al.*, 2020). It remains the second leading cause of morbidity and mortality after cardiovascular disease and microbial infections around the globe, and predominantly, all cancer cases have their roots in lifestyle and environment (Gaidai *et al.*, 2023; Guo *et al.*, 2024). Recognizing the high mortality and poor recovery rates linked to current chemotherapies, mainly because of their adverse effects (Anand *et al.*, 2023), there remains a critical need for innovative therapeutic approaches that can simultaneously improve treatment effectiveness and reduce toxicity (Sung *et al.*, 2021).

Conventional cancer treatments often fail to distinguish between malignant and healthy cells (Kaur *et al.,* 2023). Anticancer therapies should selectively target cancerous cells with minimal impact on surrounding healthy tissues (Liu *et al.,* 2024). This challenge has accelerated the search for new anticancer drugs, particularly those derived from plants, which tend to have fewer side effects when used to treat tumors (El-Saadony *et al.,* 2023; 2025). Around 35,000 phytochemicals from terrestrial and aquatic environments have been identified as potential complements to traditional cancer therapies (Lewandowska *et al.,* 2022).

Various plant-derived metabolites, including phenols, terpenoids, saponins, and alkaloids, have demonstrated chemoprotective effects against different types of cancer cells, with the capacity to trigger either cell cycle arrest or apoptosis (Mueed *et al.,* 2024; Alharbi *et al.,* 2024). These bioactive compounds work through multiple mechanisms, such as promoting apoptosis and reducing DNA damage induced by oxidative stress by disrupting cellular checkpoints or decreasing levels of antiapoptotic proteins (Al-Quwaie *et al.,* 2023). The therapeutic value of natural compounds has gained increasing recognition, especially the bioactive components found in *Punica granatum* (Fakudze *et al.,* 2022).

Pomegranate fruits *Punica granatum* L. is a family Lythraceae member grown in tropical and subtropical temperate zones on evergreen, deciduous small trees or shrubs (Das *et al.,* 2025). The pomegranate is an old plant cultivated in the Mediterranean, Middle East, and South Asia for several ages. Pomegranate peels, which constitute half the fruit weight, are often considered waste but are a rich source of phytochemicals (Fahmy and Farag, 2022). The pomegranate peel possesses a higher concentration of antioxidants than the juice, making it a valuable source of bioactive compounds. It contains proteins, polysaccharides, minerals, and phenolic substances (Teniente *et al.,* 2023). Furthermore, pomegranate peel extract (PPE) demonstrates diverse biological activities, encompassing anticancer, anti-inflammatory, neuroprotective, antiviral, and antibacterial effects (Xiang *et al.,* 2022).

Inflammation develops chronic diseases initiated by prolonged oxidative stress, which occurs when an imbalance between harmful reactive oxygen species (ROS) and the body's defense mechanisms (Leyane *et al.,* 2022). This oxidative stress can then activate the NF-κB pathway, a central controller of inflammation (Saaoud *et al.,* 2024). This activation leads to increased expression of cyclooxygenase-2 (COX-2), the enzyme involved in the production of proinflammatory prostaglandins, and the induction of inducible nitric oxide synthase (iNOS), which generates nitric oxide (NO), a signaling molecule with complex roles in inflammation (Coutinho *et al.,* 2024).

Furthermore, inflammation can arise from invading pathogens, i.e., *Fusobacterium nucleatum*, *Bacteroides fragilis*, and certain species of *Escherichia coli* (Quaglio *et al.,* 2022). These invaders trigger an immune response characterized by the upregulation of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-8 (IL-8) (Cao *et al.,* 2024). Simultaneously, the production of chemokines like CCL2 and CXCL8, which attract immune cells to the site of inflammation, is also increased. Conversely, the levels of anti-inflammatory cytokines, like interleukin-10 (IL-10), which help to dampen the inflammatory response, are often reduced (Song *et al.,* 2024).

Chronic inflammation (CI), a persistent and long-lasting inflammatory state, has been strongly implicated in the pathogenesis of a wide range of debilitating diseases (Wang *et al.,* 2021). These include atherosclerosis, the underlying cause of cardiovascular diseases (CVD); inflammatory bowel disease (IBD), a group of disorders affecting the digestive system; kidney disease; and diabetes Mellitus (Wu *et al.,* 2024). The global impact of CI is staggering, where recent studies suggested that it is a leading cause of death worldwide, with approximately half of all mortalities attributed to inflammation-related conditions and autoimmune diseases (Yin *et al.,* 2024).

Chronic inflammation can compromise the integrity of this crucial barrier, leading to increased permeability, a condition often referred to as "leaky gut" (Paray *et al.,* 2020). This breach allows macromolecules, including pathogens, exotoxins, and undigested fats, to cross from the intestinal lumen into the underlying tissues (Fasano, 2020). This increased permeability and the subsequent influx of foreign substances can contribute significantly to the development of colorectal cancer, particularly in individuals with pre-existing inflammatory bowel disease (Escalante *et al.,* 2024). Therefore, effective management of inflammation is paramount in preventing colorectal cancer, especially in the vulnerable IBD patient population.

While extensive research *in vitro* and *in vivo* has explored the potential therapeutic benefits of various compounds, including PPE, the specific *in vivo* cytotoxic effects of PPE on colon cancer and the precise molecular mechanisms by which it exerts its effects remain fully elucidated (Teniente *et al.,* 2023). Given the growing interest in utilizing industrial by-products such as pomegranate peel, this study uses advanced chromatographic techniques to examine its ethanolic extract's chemical composition and bioactivity. The study also evaluates the extract's antioxidant and cytotoxic effects on human cervical cancer cells (HeLa), colon cancer cells (Caco-2), and liver cancer cell (HepG2) lines. Then, the anticancer potential of pomegranate peel extract (PPE) on pathogen-induced colon cancer in albino mice will be tested by evaluating biochemical, metabolomics, and histological characteristics.

MATERIALS AND METHODS

Plant material and extraction process: Mature *Punica granatum* fruits were obtained from local markets, then ground to prepare pomegranate peel powder. The peels were manually separated, dried at 40°C, and finely ground using an electric blender (LM1A0, Moulinex, France). The resulting fine powder was stored at -20°C in a freezer for future use. The extraction process involved mixing 10 g of the powder with 70 % ethanol and agitating it on an orbital shaker for 24h. The mixture was then filtered under vacuum and concentrated using a rotary evaporator. Subsequently, it was frozen and lyophilized using a lyophilizer at -80 °C under pressure for 72h (Khodadadi *et al.,* 2021).

Phytochemical analysis of bioactive compounds: The phenolic compounds in PPE were detected using High-Performance Liquid Chromatography with diode-array detection (HPLC-DAD). The separation process was conducted using an Inertsil ODS-3 guard column (150 mm × 4.0 mm, film thickness 4 µm) at a temperature of 35°C. The extracts were dissolved to create stock solutions (8 mg/mL) in a methanol and water mixture (80/20, v/v)). The samples were pre-filtered using an Agilent 0.45 µm PTFE filter. The mobile phase consisted of a solution containing 0.5 % acetic acid in aqueous (A) and methanolic (B) solvents. The gradient elution method varied the solvent composition as follows: 0 to 20 % B (0 - 0.01 min), 20 to 60 % B (0.01 - 0.02 min), 60 to 80 % B (0.02 - 15.2 min), and 100 % B (15.2 - 30 min). Between 30 and 35 min, the composition shifted from 100 % B to 10 % B, and from 35 to 40 min, it further changed from 10 % B to 0 % B. After injecting the sample, the phenolic compounds were identified using a diode array detector (DAD) set to a wavelength range of 200 - 600 nm (Tokul-Ölmez *et al.*, 2020).

Characterization of PPE using Tandem mass spectrometry (MS/MS): The pomegranate peel extract was characterized qualitatively using a Shimadzu 8040 ultra-high sensitivity system as previously described (Belguidoum *et al.*, 2024). The MS/MS analysis was performed with electrospray ionization (ESI) under specific parameters.

Determination of antioxidant activity: The antioxidant activity of PPE was tested against DPPH (2,2- diphenyl-1-picrylhydrazyl, 24 µM) and ABTS (2,2'-azino- bis(3-ethylbenzothiazoline-6-sulfonic acid) radicals methods using JENEWAY spectrophotometer (UK) with Amari *et al.*, (2014); Altarawneh *et al.*, (2022). In the DPPH assay, a 50 µL sample was mixed with a 0.004 % DPPH solution and incubated in the dark for 30 min, after which absorbance was measured at 517 nm. A working solution was prepared for the ABTS assay by reacting 7 mM ABTS with 2.45 mM potassium persulfate and adjusting it with ethanol. A 20 µL sample was then combined with 2 mL of this solution and incubated in the dark at room temperature for 6 min. Absorbance was measured at 517 nm for DPPH and 734 nm for ABTS. Trolox was used as a reference standard. The radical scavenging activity (S) was calculated using Eq 1.

$$\% \, Antioxidant \, activity = \frac{Abs \, control - Abs \, sample}{Abs \, control} x100$$

Where Abs sample is the absorbance of the solution with the sample, and Abs control is the absorbance of the solution in the absence of the sample.

Cytotoxic assay: The cytotoxicity of *P. granatum* peel extract was tested on human HeLa, Caco-2, and HepG2 cell lines (ATCC, USA). The cancerous cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Oxiod, UK) supplemented with 20 ng/mL epidermal growth factor, 500 ng/mL hydrocortisone, 0.01 ng/mL insulin, 5% FBS and 1% Pen/Strep. The cells were maintained in standard conditions (humidified atmosphere with 5% CO_2 and 37 °C). The MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide) was used to measure the cell viability by measuring the absorbance of the reaction mixture on 590 nm after 24 h of treatment with varying PPE extract concentrations (Zhang *et al.*, 2023).

Animal design model: Albino/c male mice were selected for this study. BALB/c mice were albino and had pink eyes and white hair. The "c" was added at F26 by Snell in 1932; it refers to coat color (Wang *et al.*, 2015). Forty BALB/c mice (6-8 weeks old) were bought and housed under 12h day/night cycles. All mice in the four groups had free access to water and a high-fat diet throughout the experimental period. These mice were maintained at 23°C with relative humidity (50%) and moisture (10 %) (Ludgero-Correia *et al.*, 2012). The mice were separated into four groups (ten per group): group one was orally administered 0.5 mL saline as a control. Induction of colon tumor using bacterial pathogens supported with protein and lipid-rich saturated fatty acid diet for groups 2, 3 and 4. Pathogenic bacteria, *Escherichia coli* and *Klebsiella* sp., were used at 10^6 CFU (colony-forming unit) in a single dose as the starting point of infection. Treatment was carried out for groups 3 & 4 with standard drug and PPE, respectively. Treatment of group 3 was performed via intraperitoneal administration of 5-fluorouracil (25 mg/kg) every 3 days for 2 weeks. Group 4 administered PPE (100 mg/kg).

Biochemical analysis of mice serum: At the end of the experiments, the animals were slaughtered, and blood samples were collected and centrifuged (3000 rpm for 10 min). Sera were isolated and then stored at -20°C until analysis. Using ELISA kits, the content of apoptosis key regulators, i.e., B cell lymphoma gene 2 (BCL2) and hypoxia-inducible factor 1 -α (HIF1-α) proteins (Biosource International Inc., California, USA) were investigated following the manufacturer's instructions (Gür *et al.*, 2011).

Histopathological examination: In parallel, animal colons were collected immediately from all mice after euthanasia using light ether. After being cleaned with a saline solution, the colon was first fixed in 10%; then, paraffin sections of 5 µm thickness were prepared and stained with hematoxylin and eosin (H & E). Paraffin slices of 5 µm thickness were prepared for microscopic examination and stained with H & E (Bastaki *et al.*, 2016).

Serum metabolomics analysis using GC-MS analysis: The serum metabolites of the studied groups were profiled using gas chromatography (Thermo Scientific Corp., USA) coupled with a thermal mass spectrometer detector. Metabolites separation were achieved under conditions described in previous work (Hassan *et al.*, 2020; Ammar *et al.*, 2021). The gas chromatography-mass spectrometry (GC-MS) data were cleaned, deconvoluted, and aligned using the MS-DIAL interface (Zhang *et al.*, 2020).

Statistical analysis: All experiments were done in triplicate and analyzed with a *T*-test and one-way ANOVA (Dunnett) using GraphPad Prism (Version 9) and Microsoft Excel spreadsheet. *P*-value ≤ 0.05 was considered significant.

RESULTS

Active compounds profile of PPE: The analysis of *P. granatum* peels revealed a significant concentration of phenolic compounds. Pyrocatechol was identified as the predominant compound, with a concentration of 19.5 mg/g (Table 1), followed by ellagic acid (5.2 mg/g), epicatechin, and fisetin at 2.2 mg/g and 1.9 mg/g, respectively.

Table 1: Phenolic composition of PPE by HPLC-DAD (mg/g)

Phenolic compound	Retention time (min)	PPE (mg/g)
Fumaric acid	14.00	0.065±0.001
Protocatechic acid	24.5	0.01±0.001
4-oh-benzoic acid	30.9	0.09±0.01
Vanillic acid	34.5	0.32±0.02
Caffeic acid	35.3	0.02±0.01
Ellagic acid	50.11	5.2±0.09
p-Coumaric acid	40.77	0.09±0.002
Pyrocatechol	24.6	19.5±0.05
Theophylline	29.5	0.02±0.00
Vanillin	36.90	0.01±0.001
Hesperidin	47.31	0.02±0.05
Rutin	47.6	0.61±0.02
Fisetin	51.33	1.9±0.04
Quercetin	55.23	0.01±0.02
Curcumin	72.71	0.23±0.01
2,4-dihydroxybenz	41.21	0.01±0.001
Prophylgallate	46.92	0.02±0.002

n=3, data are presented mean±SE.

Characterization of PPE Using LC-MS/MS: Table 2 provides detailed information on the compounds. Three phenolic acids were identified: 4-hydroxycoumarin, p-coumaric, and gallic acids. Additionally, the analysis detected several phytoconstituents, such as flavonoids (naringenin, myricetin, quercetin, and rutin) and other phenolic compounds (vanillin, beta-carotene, folic acid, and maleic acid). Most of the compounds were detected in positive ionization modes.

Table 2: The compounds identified in the PPE using LC/MS-MS results

Compound	Ionization mode (m/z)	Precursor ion	Product ion	PPE
Phenolic acids				
p-Coumaric acid	(MH)+	166.2	58.9	+
4-Hydroxy coumarin acid	(MH)-	161.1	117.6	+
Gallic acid	(MH)-	169.2	124.8	+
Folic acid	(MH)+	443.3	59.2, 25.6, 324.1	+
Maleic acid	(MH)+	118.3	85.9	+
Flavonoids				
Naringenin	(MH)+	271.22	192.1, 231.5	+
Myricetin	(MH)+	335.9	45.6, 71.6, 239.2	+
Quercetin	(MH)+	305.22	84.9	+
Rutin	(MH)+	610.55	72.8, 281.9	+
Vanillin	(MH)+	152.36	93.6	+
Beta-carotene	(MH)+	541.33	24.1, 201.33	+

Antioxidant potential of PPE: Table 3 shows the scavenging activity of PPE against DPPH and ABTS free radicals, where PPE (200 µg/ml) scavenged 91% and 95% of free radicals, respectively. Furthermore, The IC50 of PPE was 120 and 85 µg/ml against DPPH and ABTS free radicals because of the considerable content of phenolic and flavonoids (181 and 35 mg/g).

Anticancer activity of PPE: Figure 1A-F shows the potent cytotoxic activity of PPE against three cancer cell lines (Hela, liver, and colon cancer cell lines). As the concentration of the extract is increased, there is a significant inhibition in cell viability, particularly at doses of 75 and 100 µg/mL. The IC_{50} value against all cancer cells was 75 µg/mL, which indicates the extract's effectiveness in reducing the cancer cell population by 50 %. PPE at 100 µg/mL significantly ($P<0.05$) reduced the cancer cell viability by 23, 17, and 20 % against Hela, liver, and colon cancer cell lines, respectively (Figure 1G).

Table 3: Antioxidant potential of PPE

Antioxidant poteintal	Value
Total phenolic (mg/g)	181±2.3
Total flavaniods (mg/g)	35±0.2
DPPH scavenging (%)	91±1.1
ABTS scavenging (%)	95±1.8
IC_{50} against DPPH (µg/ml)	120±5.2
IC_{50} against ABTS (µg/ml)	85±1.6

n=3, data are presented mean±SE

Effects of PPE on BCL2 and HIF1-α in mice sera with colon cancer: Induction of colon cancer by pathogenic bacteria caused significantly elevated levels of BCL2 and HIF1- α compared to the control group, as depicted in Figure 2 (A & B). The PPE treatment significantly downregulated the levels of BCL2 and HIF1-α to nearly normal levels, as illustrated in Figure 2 (A & B).

Histological studies on mice colon cancer tissues: Figure 3 shows the histopathological analysis demonstrated the protective effects of PPE against bacterial infection-induced colon cancer in mice (H&E, 100x), whereas Figure 3A showed the control group with normal colon tissue structure, including intact mucosa with glandular structures, submucosa, muscularis, and serosa. Figure 3B, bacterial infection-induced colon cancer showed desquamation of the mucosal epithelium, significant inflammatory cell infiltration in the lamina propria of the mucosa and submucosa, and anaplasia of the glandular epithelium exhibiting malignant characteristics such as disorganization, hyperchromasia, polarity loss, and pleomorphism, along with surrounding inflammatory cell infiltration. 5-fluorouracil-treated group after infection showed medium to mild lymphoid follicle hyperplasia in the submucosa, extending to the mucosa, accompanied by inflammatory cell infiltration (Figure 3C), and PPE post-treated: the glandular structures remained intact and normal histological structure was maintained (Figure 3D).

Oxidative stress markers in colon tissues: Analysis of colon tissue revealed that pathogen induction of colon cancer significantly ($p\leq0.05$) decreased glucose, calcium, and lactate dehydrogenase (LDH) levels compared to controls. PPE treatment significantly ($p\leq0.05$) increased glucose levels in all treated groups compared to the pathogen group, restoring them to normal in the PPE-treated group. Calcium levels were significantly ($p\leq0.05$) increased by the extract in all treated groups, achieving normal levels.

Furthermore, bacterial infection significantly ($p\leq0.05$) reduced total antioxidant capacity (TAC) and the activity of catalase (CAT) and glutathione peroxidase (GPx) enzymes while significantly ($p\leq0.05$) increasing lipid peroxidation (LPO) and total phenolic content (TPC) compared to controls. PPE treatment significantly

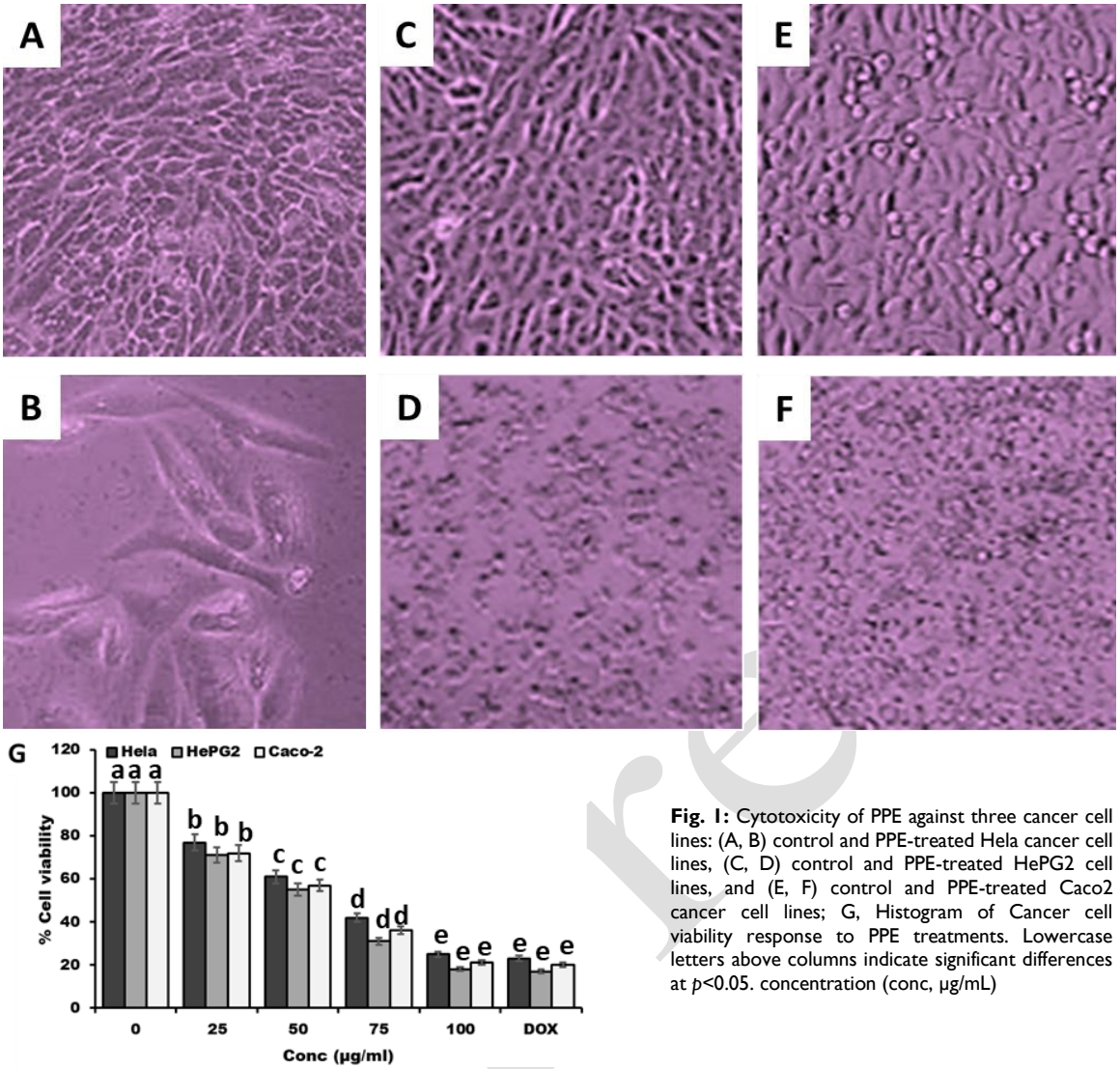

Fig. 1: Cytotoxicity of PPE against three cancer cell lines: (A, B) control and PPE-treated Hela cancer cell lines, (C, D) control and PPE-treated HePG2 cell lines, and (E, F) control and PPE-treated Caco2 cancer cell lines; G, Histogram of Cancer cell viability response to PPE treatments. Lowercase letters above columns indicate significant differences at $p<0.05$. concentration (conc, µg/mL)

($P \leq 0.05$) increased TAC, CAT, and GPx while decreasing LPO and TPC in all treated groups compared to the infected group, with complete restoration to normal levels observed in the PPE-treated group.

GC-MS based metabolomics: In the present study, the GC-MS-based metabolomics approach has been applied to study the effect of PPE on the serum metabolic levels of BALB/c mice with pathogen-induced colon cancer, as shown in Figure 4. The score plot of this model shows complete segregation between the untreated colon cancer group (G2) and mice treated with PPE (G4), as illustrated in (Figure 4). The ratio between G2/G4 detected the fold change.

Table 4: Impact of PPE on cellular components and oxidative stress markers in the colon tissues of albino mice with bacterial-induced colon cancer

Markers	C	T1	T2	T3
Glucose	3.77±0.1	1.2±0.2	2.69±0.2	3.71±0.6
LDH	1750±6.3	1045±11.2	1425±10.3	1552±9.8
Ca	0.19±0.02	0.2±0.01	0.18±0.03	0.17±0.02
TAC	1.5±0.1	0.5±0.02	0.91±0.02	1.66±0.1
CAT	44.9±0.98	11.9±0.5	43.69±1.2	44.45±1.6
POD	20.98±1.2	11.5±0.4	20.55±1.5	21.41±1.1
LPO	44.3±1.1	145.63±3.5	56.33±2.1	45.32±1.4
TPC	11.36±0.9	19.36±0.6	10.69±0.5	11.22±1.1

n=3, data are presented mean±SE. lactate dehydrogenase (LDH), total antioxidant capacity (TAC), catalase (CAT), peroxidase (POD), lipid peroxidation (LPO), and total phenolic content (TPC).

DISCUSSION

Colon cancer is the 3rd most common cancer and the 2nd leading cause of cancer-related deaths globally (Morgan *et al.*, 2023). Significant advances in the treatment of colon cancer were found either with resectable or metastatic tumors (Loree and Kopetz, 2017). Recent research demonstrated that specific pathogenic bacteria can significantly contribute to colon carcinogenesis through multiple mechanisms (Avril and DePaolo, 2021). Experimental studies utilizing rat models have been particularly valuable in elucidating these microbial influences, providing crucial insights into the complex relationship between gut microbiota and cancer development (Lindell *et al.*, 2022). These animal studies revealed that pathogenic bacteria promote tumorigenesis through distinct and interrelated pathways, including disrupting microbial homeostasis, induction of chronic inflammation, direct DNA damage, and modulation of host immune responses (Li *et al.*, 2022).

The process begins with microbial dysbiosis, where an imbalance in gut microbiota composition creates a pro-tumorigenic environment (Mignini *et al.*, 2023). Research in rat models has shown that antibiotic-mediated depletion of gut microbiota can reduce tumor incidence

while

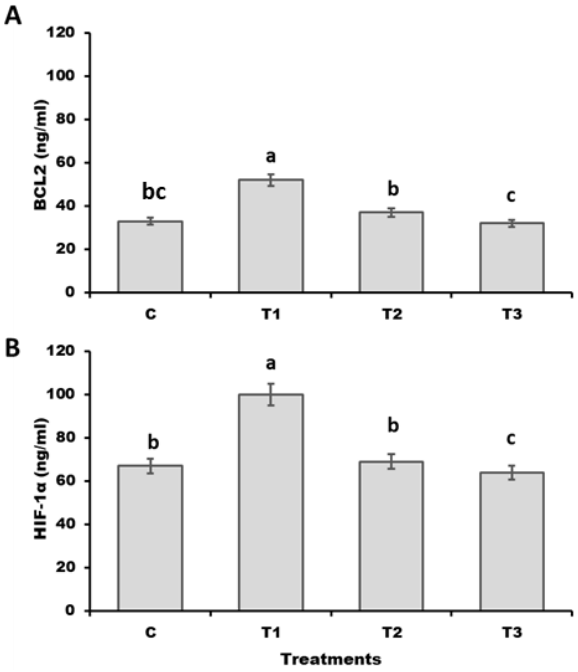

Fig. 2: Serum levels of BCL2 (A), and HIF1-α (B) in various studied groups. Data were expressed as mean ± SD (n=8 for each group). Different letters indicate significant differences at P< 0.05.

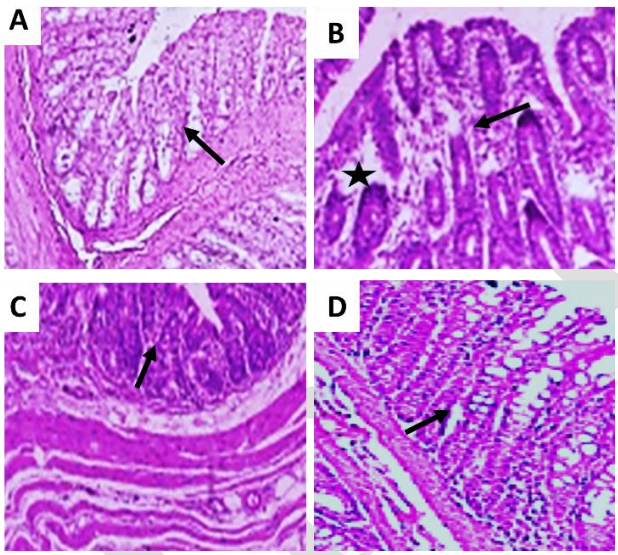

Fig. 3: Histopathological analysis demonstrated the protective effects of PPE against bacterial infection-induced colon cancer in rats (H&E, 100x). A) control colon tissues; (B) bacterial infection-induced colon cancer group; (C) 5-fluorouracil-treated group after infection; (D) PPE-treated group after infection.

deliberate colonization with pathogenic bacteria accelerates tumor formation (Paudel *et al.*, 2024). Particular attention focused on *Fusobacterium nucleatum*, which was consistently identified in human CRC patients and experimental rat models (Liu *et al.*, 2024). This pathogen promotes tumor progression through its ability to suppress host immune responses, creating favorable conditions for malignant growth (Wu *et al.*, 2022).

The inflammatory cascade represents another critical pathway, with enterotoxigenic *Bacteroides fragilis* (ETBF) as a prime example (Zhuang, 2022). ETBF secretes the *B. fragilis* toxin (BFT) that damages the intestinal

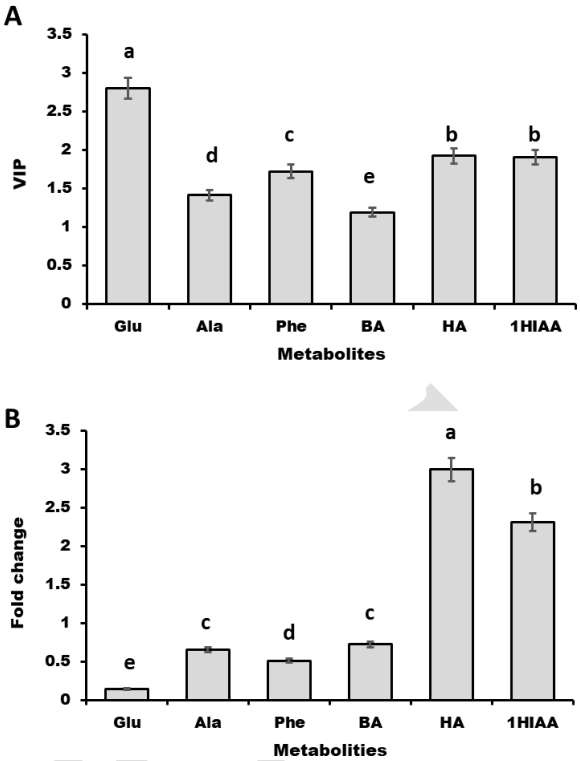

Fig. 4: Differential metabolite biomarkers as revealed for the multivariate OPLS-DA and univariate analysis of the studied groups *i.e.,* untreated mice with pathogen-induced colon cancer group (G2) vs mice treated with PPE group (G4). The VIP values of significant biomarkers (A) and fold change in biomarkers between untreated and PPE-treated groups (B).

epithelial cells, triggering chronic inflammation (Yang *et al.*, 2024). This persistent inflammation was stated to increase reactive oxygen species (ROS) production and proinflammatory cytokines such as IL-6 and TNF-α, promoting DNA damage and uncontrolled cellular proliferation (Antar *et al.*, 2023). Rat studies demonstrated that ETBF infection induces T helper 17 (Th17) immune responses strongly associated with CRC progression (Wu et al., 2022).

Perhaps more directly concerning is the genotoxic potential of certain bacterial species. Some pathogens produce toxins that damaged host DNA (Martin and Frisan, 2020). Notably, specific strains of *Escherichia coli* (particularly those carrying the pks genomic island) produce colibactin, a potent genotoxin that induces double-strand DNA breaks and chromosomal instability (Auvray *et al.*, 2021). Experimental evidence from rat models shows that exposure to colibactin-producing *E. coli* significantly increases the frequency of colonic adenocarcinomas (Wang and Fu, 2023). Similarly, *Salmonella Typhimurium* infection has been shown to promote CRC development in rats by activating the β-catenin signaling pathway, a key regulator of cell proliferation and tumorigenesis (Al-Qarraawi and Al-Awade, 2024). These findings highlight the diverse mechanisms pathogenic bacteria can initiate and promote colorectal carcinogenesis.

The immune system's role in this process is equally critical, as pathogenic bacteria developed sophisticated strategies to evade host defenses (Thakur *et al.*, 2019). Multiple studies have demonstrated that these microbes

can suppress anti-tumor immunity by modulating regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) (Haist *et al.,* 2021). *Fusobacterium nucleatum* inhibited natural killer (NK) cell activity, enabling tumor cells to evade immune surveillance (Pignatelli *et al.*, 2023). Rat models further revealed that bacterial infections increase immunosuppressive cytokines such as IL-10 and TGF-β production, creating a microenvironment that favors tumor growth and progression (Mir *et al.*, 2023).

The azoxymethane (AOM)-treated rat model has been particularly informative in studying these processes (Dzhalilova *et al.,* 2023). Research using this model has shown that ETBF colonization results in hyperproliferative crypts and tumor development at significantly higher rates than controls (Zhuang, 2022). Mechanistic studies have revealed that the BFT toxin activates NF-κB and STAT3 signaling pathways, which are known drivers of inflammation and tumor growth (Wu *et al.,* 2022). Similarly, rats infected with colibactin-producing *E. coli* exhibit increased aberrant crypt foci (ACF), recognized precursors to CRC (Wang *et al.*, 2021). The impact of *Fusobacterium nucleatum* has also been demonstrated, with infected rats developing larger and more invasive tumors than controls. This effect appears mediated through the bacterium's ability to enhance Wnt/β-catenin signaling, a pathway fundamental to CRC progression (Li *et al.*, 2021).

These findings have important therapeutic implications, suggesting multiple avenues for intervention. Microbiome-based strategies such as probiotics and fecal microbiota transplantation (FMT) promise to restore healthy microbial balance and potentially reduce CRC risk (Sahle *et al.,* 2024). Targeted approaches, including antibiotic therapies and specific anti-toxin agents (such as colibactin inhibitors), could help prevent tumor initiation in high-risk individuals. Additionally, immunomodulatory therapies designed to enhance antibacterial immune responses may help counteract the inflammation-driven aspects of carcinogenesis (Terveer, 2020).

Nevertheless, these treatments have many serious side effects. Therapeutic modalities using plant sources have been increasingly popular recently as safer options with fewer side effects than traditional anticancer drugs (Huang *et al.*, 2021). Pomegranates have been used in the treatment of several diseases. Moreover, pomegranate showed promising chemo-preventive activity against different types of cancer (Rauf *et al.,* 2025), such as breast, prostate, and lung cancers in cells, animal models, and humans (Sharma *et al.*, 2017).

Medicinal plants have historically been utilized for their diverse pharmacological properties to prevent and treat various human diseases. This therapeutic potential is mainly due to their rich array of primary and secondary metabolites, including flavonoids, phenolic compounds, alkaloids, and tannins (Selim *et al*., 2022; El-Saadony *et al.,* 2024a,b). Pomegranates, in particular, have demonstrated several medicinal benefits and are effective in managing diabetes, erectile dysfunction, obesity, reproductive disorders, and arthritis (Mueed *et al*., 2023; Rauf *et al.,* 2025).

In this context, extensive research conducted over the past two decades (Singh *et al.,* 2023) underscored the multifaceted pharmacological benefits of *P. granatum* L., commonly known as pomegranate. These benefits include anticancer, anti-inflammatory, antioxidant, and antimicrobial properties (Alsubhi *et al*., 2022). With the growing production of pomegranate products, peel extract (PPE) has emerged as a valuable by-product (El Hosry *et al.,* 2023). PPE is notably rich in primary bioactive compounds, often in higher concentrations than those found in the fruit's edible parts, and is abundant in antioxidants and phytochemicals (Yassin *et al.,* 2021).

The investigation utilized HPLC-DAD and MS/MS techniques to analyze the pomegranate peel extract, revealing a wide range of secondary metabolites through phytochemical profiling (Saad *et al*., 2021; Ruan et al., 2022). The PPE contains a wide range of phenolic compounds, which vary depending on environmental factors, cultivar differences, and ripening stages (Kharchoufi *et al.,* 2018). *P. granatum* extract had a similar antioxidant capacity as Trolox. The lower IC50 values suggest that the pomegranate peel extract may exhibit substantial antioxidant effects at lower doses than Trolox, highlighting its impressive antioxidant potential (Sihag *et al.,* 2022). Furthermore, a significant correlation was observed between polyphenol concentration and antioxidant efficacy, reinforcing that polyphenols neutralize free radicals and protect essential biomolecules from oxidative damage (Benchagra *et al.,* 2021).

Research indicates that PPE is rich in gallic acid, ellagic acid, and punicalagin derivatives, critical components of its phenolic profile (Feng *et al.,* 2022). Pomegranate peel is exceptionally high in phenolic acids, flavonoids, and ellagitannins, contributing to its antioxidant activity and health benefits (Kharchoufi *et al.,* 2018). The pomegranate peel demonstrates greater antioxidant activity and a higher concentration of phenolic compounds than other parts of the fruit. The accumulation of phenolic compounds in the peel likely reflects its role as a protective barrier for the fruit (Derakhshan *et al.,* 2018).

Most chemo-preventive agents are antioxidant in nature. Fruits high in polyphenols are considered antioxidant and chemo-preventive (Imran *et al.,* 2023). Pomegranate has been shown to exert anticancer and antioxidant activity, which is generally attributed to its high content of polyphenols due to its effects of neutralizing free radicals (Turrini *et al.,* 2015). In this study, PPE showed high antioxidant capacity, which may be attributed to the excessive polyphenolic contents, represented by flavonoids and phenolic acids as free radical scavengers. The high antioxidant capacity may be attributed to excessive polyphenolic content, represented by flavonoids and phenolic acids (Sihag *et al.,* 2022; Rauf *et al.,* 2025).

One of the most distinguishing characteristics of cancer cells is their resistance to apoptosis. Oncogenes and tumor suppressor genes are well-established apoptosis regulators (Xia *et al.,* 2022). Regarding oncogenes, they can regulate apoptosis by producing antiapoptotic proteins, conferring cancer cells a survival benefit over normal cells (Wang *et al.,* 2023). So, these proteins are overexpressed in cancer cells and have a concurrent lower

level in normal cells (Li *et al.*, 2012). Thus, disrupting the function of these antiapoptotic proteins is one of the approaches used to eradicate malignant cells with minimal effect in the surrounding normal cells (Radha and Raghavan, 2017).

Antiapoptotic protein BCL2 is encoded by the BCL2 (B cell lymphoma gene 2) gene family that produces either antiapoptotic proteins such as BCL2 or proapoptotic proteins such as Bcl-2 associated protein X (Bax) and Bcl-2 homologous antagonist/killer (Bak) (Poincloux *et al.*, 2009). Thus, the overexpression of BCL2 protein protects the malignant cells from apoptosis and triggers their propagation. BCL2 overexpression was first noted in B-cell follicular lymphoma (Miyaoka *et al.*, 2018). Subsequently, BCL2 overexpression was reported in several cancers, such as breast, lung, thyroid, nasopharyngeal, prostate, liver, ovarian, leukemia, neuroblastoma, and colorectal cancers (Credendino *et al.*, 2019; Zhou *et al.*, 2019; Jin *et al.*, 2021). The observed overexpression of BCL2 in many cancers confirms its vital role in cancer and makes it an ideal target for cancer therapy.

In the current study, we assessed the reducing effect of PPE on BCL2 serum levels in the bacterial pathogen-induced colon cancer mice group compared to the negative control group. In line with our findings, Larrosa *et al.*, (2006) stated that ellagitannins (pomegranate Punicalagin) and its metabolite ellagic acid provoke apoptosis in human colon adenocarcinoma Caco-2 cells without affecting the normal colon cells, suggesting their anticancer effect of dietary ellagitannins in colon cancer which support our results. Recently and similar to our findings, Ganjouzadeh *et al.* (2022) reported the cytotoxicity activity of PPE on breast cancer cells via increasing Bax/Bcl-2 ratio and the intracellular ROS. Moreover, our results agreed with that of Cheshomi *et al.* (2022), who demonstrated a significant anticancer activity of ellagic acid of pomegranate extract on gastric cancer cell via reduction of BCL2 and stimulation of apoptosis in addition to inhibition of tumor growth in immunocompromised mice, supporting our study outcomes.

Interestingly, PPE showed an important anticancer activity against prostate cancer cells via reduction of BCL2 expression, inducing apoptosis, and impairs metastasis (Farooqi, 2021). Also, pomegranate extract showed an antiproliferative effect against oral cancer cells through the induction of mitochondrial dysfunction and apoptosis and the Bax/BCL2 ratio (Peng *et al.*, 2021), which agreed with our results. Most solid tumors, including colon cancer, are permanently or transiently exposed to hypoxia due to a deficient blood supply and aberrant vascularization (Matuszewska *et al.*, 2021). The hypoxia-inducible factors (HIFs) mediate the cellular response to hypoxia, thus encouraging modifications associated with cancer progression and metastasis (Lappano *et al.*, 2020). The transcription factor hypoxia-inducible factor 1 (HIF-1) is a member of the HIF family, consisting of an O2-regulated HIF-1α subunit assembled with a constitutively produced HIF-1b subunit (Albadari *et al.*, 2019).

Hypoxia controls HIF-1α activation via post-translational modifications. Oxygen leads to the post-translational hydroxylation of HIF-1α and promotes its degradation (Daly *et al.*, 2021). In contrast, the absence of oxygen stabilizes HIF-1α, allowing its binding to hypoxia-response elements in the nucleus, thus activating many HIF-target genes involved in cancer growth, metastasis, and anaerobic metabolism (Albanese *et al.*, 2020). Furthermore, HIF-1α disrupts DNA repair and, more importantly, suppresses apoptosis by altering the ratio between proapoptotic and antiapoptotic BCL-2 family members via triggering antiapoptotic proteins such as BCL2 and BCL-xl (Wang *et al.*, 2022). Thus, HIF-1α protein is overexpressed in several human cancers (Iovine *et al.*, 2016).

In the present study, we evaluated the serum level of HIF-1α in bacterial pathogen-induced colon cancer mice treated with the PPE group compared with the negative control group; consistent with our findings, the inhibition of HIF-1α and autophagy suppress colon cancer growth and proliferation (Albanese *et al.*, 2020). Similarly, suppressing HIF-1α by L-carnosine dipeptide prevents colon cancer cells' resistance to 5- 5-fluorouracil, promoting its anticancer activity and stimulating apoptosis of colon cancer cells (Husari *et al.*, 2017).

In line with our findings, Husari *et al.*, (2017) reported pomegranate concentrate has chemotherapeutic activity against cigarette smoke-induced lung cancer in an animal model via inhibition of HIF-1α expression. In addition, treatment of lung cancer cells and tumor-bearing mice with ellagitanins significantly inhibited tumor growth via increased AMP-activated protein kinase and suppressed HIF-1α, suggesting that ellagitanins might be a promising anticancer agent (Duan *et al.*, 2020).

Using a based metabolomics approach to analyze the relationship between different metabolites and diseases is of great value (Galal *et al.*, 2022). Thus, it could support us with valuable knowledge about disease diagnosis, prognosis, and pathogenesis (Piras *et al.*, 2022). In the current study, numerous metabolites related to bacterial pathogen-induced colon cancer were observed via GC-MS-based metabolomics approach as 1H-indole-3-acetic acid, heptanoic acid, benzoic acid, alanine, phenylalanine, and glucose. This study showed a significant reduction of 1H- indole-3-acetic acid and heptanoic acid in mice treated with the PPE group compared with the untreated mice with the pathogen-induced colon cancer group.

In contrast, the PPE-treated mice group significantly increased benzoic acid, alanine, phenylalanine, and glucose. Indole derivatives are a type of serum metabolite, including indole-3-acetic acid, indole-3-propionic acid, serotonin, and other compounds. All these metabolites resulted from tryptophan metabolism (Konopelski and Mogilnicka, 2022). These protect the gastrointestinal tract from stress-induced illnesses such as cancers. These are produced mainly by the intestinal transformation of *Escherichia coli* (Xiao *et al.*, 2024). Recently and supporting our results, gut microbiota-derived tryptophan metabolites such as indole-3-acetic acid maintain gut and systemic homeostasis (Su *et al.*, 2022).

Conclusions: The novel hybrid ultrasound-microwave extraction method proved to be an efficient technique for obtaining polyphenolic-rich pomegranate peel extracts with significant anticancer and antioxidant properties. In

vitro studies demonstrated potent cytotoxic effects against HeLa, colon, and HepG2 cancer cell lines, suggesting the potential of these extracts as natural chemotherapeutic agents. Additionally, *in vivo* studies in albino mice confirmed their antioxidant capacity, reducing oxidative stress markers while exhibiting minimal toxicity to normal cells. These findings highlight the therapeutic potential of pomegranate peel polyphenols as complementary agents in cancer treatment, offering a promising alternative to synthetic drugs with fewer side effects. Further research is warranted to elucidate the precise molecular mechanisms and optimize dosage formulations for potential clinical applications.

CRediT authorship contribution statement: LRA (Lamaia R. Altarjami): Conceptualization, project administration, funding acquisition, writing the original draft, writing – review, and editing.

Declaration of competing of interest: The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Albadari N, Deng S, Li W, 2019. The transcriptional factors HIF-1 and HIF-2 and their novel inhibitors in cancer therapy. Expert Opin Drug Discov 14:667-682.

Albanese A, Daly LA, Mennerich D, *et al.,* 2020. The role of hypoxia-inducible factor post-translational modifications in regulating its localisation, stability, and activity. Int J Mol Sci 22(1):268.

Alharbi YM, Elzahar KM, Qahl SH, *et al.,* 2024. Nutritional significance, antimicrobial, antioxidants, anticancer, and antiviral activities of lemongrass leaves extract and its application as hepatoprotective agent against CCl_4-Induced hepatic injury in rats. An Acad Bras Ciênc 96(3): e20230646.

Alsubhi NH, Al-Quwaie DA, Alrefaei GI, *et al*., 2022. Pomegranate pomace extract with antioxidant, anticancer, antimicrobial, and antiviral activity enhances the quality of strawberry-yogurt smoothie. Bioengineering 9(12): 735.

Al-Qarraawi R, Al-Awade H, 2024. Improved Histo-logical effect of *Salmonella* Typhimurium ghost vaccine used in the treatment of colon cancer in-duced in male albino rats. Cent Asian J Med Sci 5:172-185.

Al-Quwaie DA, Allohibi A, Aljadani M, *et al.,* 2023. Characterization of Portulaca oleracea whole plant: Evaluating antioxidant, anticancer, antibacterial, and antiviral activities and application as quality enhancer in yogurt. Molecules 28(15): 5859.

Altarawneh RM, Al-Jaafreh AM, Qaralleh H, *et al.,* 2022. Chemical profiling of Punica granatum peels from Jordan using LC–MS/MS and study on their biological activities. International Journal of Food Science and Technology 57:5256-5267.

Amari NO, Bouzouina M, Berkani A, *et al.,* 2014. Phytochemical screening and antioxidant capacity of the aerial parts of *Thymelaea hirsuta* L. Asian Pac J Trop Dis 4:104-109.

Ammar NM, Hassan HA, Mohammed MA, *et al.,* 2021. Metabolomic profiling to reveal the therapeutic potency of Posidonia oceanica nanoparticles in diabetic rats. RSC Adv 11:8398-8410.

Anand U, Dey A, Chandel AKS, *et al.,* 2023. Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics. Genes Dis *10*(4): 1367-1401.

Antar SA, Ashour NA, Marawan ME, *et al.,* 2023. Fibrosis: types, effects, markers, mechanisms for disease progression, and its relation with oxidative stress, immunity, and inflammation. Int J Mol Sci 24:4004.

Auvray F, Perrat A, Arimizu Y, *et al.,* 2021. Insights into the acquisition of the pks island and production of colibactin in the *Escherichia coli* population. Microb Genom 7:000579.

Avril M, DePaolo RW. 2021. "Driver-passenger" bacteria and their metabolites in the pathogenesis of colorectal cancer. Gut Microb 13:1941710.

Bastaki SM, Al Ahmed MM, Al Zaabi A, *et al.,* 2016. Effect of turmeric on colon histology, body weight, ulcer, IL-23, MPO and glutathione in acetic-acid-induced inflammatory bowel disease in rats. BMC Complement Altern Med 16: 1-14.

Belguidoum M, Harchaoui L, Khattabi L, *et al.,* 2024. *Teucrium pseudochamaepitys* L.: chemical composition, acute toxicity, antioxidant, anti-inflammatory, and analgesic properties. Chem Pap 78:1989-2003.

Benchagra L, Berrougui H, Islam MO, *et al.,* 2021. Antioxidant effect of moroccan pomegranate (*Punica granatum* L. sefri variety) extracts rich in punicalagin against the oxidative stress process. Foods 10(9): 2219

Cao Y, Xia H, Tan X, *et al.,* 2024. Intratumoural microbiota: a new frontier in cancer development and therapy. Signal Transduct Target Ther 9(1):15.

Cheshomi H, Bahrami AR, Rafatpanah H, *et al.,* 2022. The effects of ellagic acid and other pomegranate (*Punica granatum* L.) derivatives on human gastric cancer AGS cells. Hum Exp Toxicol 41:09603271211064534.

Coutinho LL, Femino EL, Gonzalez AL, *et al.,* 2024. NOS2 and COX-2 Co-Expression promotes cancer progression: a potential target for developing agents to prevent or treat highly aggressive breast cancer. Int J Mol Sci 25:6103.

Credendino SC, Bellone ML, Lewin N, *et al.,* 2019. A ceRNA circuitry involving the long noncoding RNA Klhl14-AS, Pax8, and Bcl2 drives thyroid carcinogenesis. Cancer Res 79:5746-5757.

Daly LA, Brownridge PJ, Batie M, *et al.,* 2021. Oxygen-dependent changes in binding partners and post-translational modifications regulate the abundance and activity of HIF-1α/2α. Sci Signal 14(692): eabf6685.

Das P, Al Rashid H, Kundu A. 2025. Pharmacological activities of a perennial shrub pomegranate (*Punica granatum* L.): A Review. Chapter, Medicinal Spices and Herbs from India, Apple Academic Press 207-232.

Derakhshan Z, Ferrante M, Tadi M, *et al.,* 2018. Antioxidant activity and total phenolic content of ethanolic extract of pomegranate peels, juice and seeds. Food Chem Toxicol 114:108-111.

Duan J, Li Y, Gao H, *et al.,* 2020. Phenolic compound ellagic acid inhibits mitochondrial respiration and tumor growth in lung cancer. Food & Function 11:6332-6339.

Dzhalilova D, Zolotova N, Fokichev N, *et al.,* 2023. Murine models of colorectal cancer: The azoxymethane (AOM)/dextran sulfate sodium (DSS) model of colitis-associated cancer. PeerJ 11:e16159.

El Hosry L, Bou-Mitri C, Dargham MB, *et al.,* 2023. Phytochemical composition, biological activities and antioxidant potential of pomegranate fruit, juice and molasses: A review. Food Biosci 55: 103034.

El-Saadony MT, Saad AM, Korma SA, *et al.,* 2024a. Garlic bioactive substances and their therapeutic applications for improving human health: A comprehensive review. Front Immunol 15: 1277074.

El-Saadony MT, Saad AM, Mohammed DDM. 2025. Medicinal plants: Bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits-A comprehensive review. Front Immunol 16: 1491777.

El-Saadony MT, Yang T, Saad AM, *et al.,* 2024b. Polyphenols: Chemistry, bioavailability, bioactivity, nutritional aspects and human health benefits: A review. Int J Biol Macromol 277(3): 134223.

El-Saadony MT, Zabermawi NM, Zabermawi NM, *et al.,* 2023. Nutritional aspects and health benefits of bioactive plant compounds against infectious diseases: a review. Food Rev Inte 39(4): 2138-2160.

Escalante J, Artaiz O, Diwakarla S, *et al.,* 2024. Leaky gut in systemic inflammation: exploring the link between gastrointestinal disorders and age-related diseases. GeroSci 16:1-22.

Fahmy HA, Farag MA. 2022. Ongoing and potential novel trends of pomegranate fruit peel; a comprehensive review of its health benefits and future perspectives as nutraceutical. J Food Biochem 46(1): e14024.

Fakudze NT, Aniogo EC, George BP, *et al.,* 2022. The therapeutic efficacy of Punica granatum and its bioactive constituents with special reference to photodynamic therapy. Plants 11:2820.

Farooqi AA, 2021. Regulation of deregulated cell signaling pathways by pomegranate in different cancers: Re-interpretation of knowledge gaps. In: Seminars in Cancer Biology: Elsevier. p 294-301.

Fasano A. 2020. All disease begins in the (leaky) gut: role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases. *F1000Research 9*: F1000.

Feng Y, Lin J, He G, *et al.,* 2022. Compositions and biological activities of pomegranate peel polyphenols extracted by different solvents. Molecules 27(15): 4796.

Ferlay J, Ervik M, Lam F, *et al.,* 2020. Global cancer observatory: cancer tomorrow. International Agency for Research on Cancer, Lyon. Available from: https://gco.iarc.fr/tomorrow. Accessed 10 Jan 2022

Gaidai O, Cao Y, Loginov S. 2023. Global cardiovascular diseases death rate prediction. Curr Probl Cardiol 8(5): 101622.

Galal A, Talal M, Moustafa A. 2022. Applications of machine learning in metabolomics: Disease modeling and classification. Front Genet 13: 1017340.

Ganjouzadeh F, Khorrami S, Gharbi S, 2022. Controlled cytotoxicity of Ag-GO nanocomposite biosynthesized using black peel pomegranate extract against MCF-7 cell line. J Drug Deliv Technol 71:103340.

Guo Q, Zhu X, Beeraka NM, *et al.,* 2024. Projected epidemiological trends and burden of liver cancer by 2040 based on GBD, CI5plus, and WHO data. Sci Rep 14:28131.

Gür T, Demir H, Kotan MÇ. 2011. Tumor markers and biochemical parameters in colon cancer patients before and after chemotherapy. Asian Pac J Cancer Prev 12(11): 3147-50.

Haist M, Stege H, Grabbe S, *et al.,* 2021. The functional crosstalk between myeloid-derived suppressor cells and regulatory T cells within the immunosuppressive tumor microenvironment. Cancers 13:210.

Hassan HA, Ammar NM, Serag A, *et al.,* 2020. Metabolomics driven analysis of obesity-linked colorectal cancer patients via GC-MS and chemometrics: A pilot study. Microchem J 155:104742.

Huang M, Lu J-J, Ding J, 2021. Natural products in cancer therapy: Past, present and future. Nat Prod Bioprospect 11:5-13.

Husari A, Hashem Y, Zaatari G, *et al.,* 2017. Pomegranate juice prevents the formation of lung nodules secondary to chronic cigarette smoke exposure in an animal model. Oxid Med Cell Longev 2017:6063201.

Imran M, Insaf A, Hasan N, *et al.,* 2023. Exploring the remarkable chemotherapeutic potential of polyphenolic antioxidants in battling various forms of cancer. Molecules 28(8): 3475.

Iovine B, Guardia F, Irace C, *et al.,* 2016. l-carnosine dipeptide overcomes acquired resistance to 5-fluorouracil in HT29 human colon cancer cells via downregulation of HIF1-alpha and induction of apoptosis. Biochimie 127:196-204.

Jin L, Chen Y, Cheng D, *et al.,* 2021. YAP inhibits autophagy and promotes progression of colorectal cancer via upregulating Bcl-2 expression. Cell Death Dis 12:457.

Kaur R, Bhardwaj A, Gupta S. 2023. Cancer treatment therapies: traditional to modern approaches to combat cancers. Mol Biol Rep 50(11): 9663-9676.

Kharchoufi S, Licciardello F, Siracusa L, *et al.,* 2018. Antimicrobial and antioxidant features of 'Gabsi' pomegranate peel extracts. Ind Crops Prod 111:345-352.

Khodadadi S, Mahdinezhad N, Fazeli-Nasab B, *et al.,* 2021. Investigating the possibility of green synthesis of silver nanoparticles using vaccinium arctostaphlyos extract and evaluating its antibacterial properties. BioMed Res Int 2021:5572252.

Konopelski P, Mogilnicka I. 2022. Biological effects of indole-3-propionic acid, a gut microbiota-derived metabolite, and its precursor tryptophan in mammals' health and disease. Int J Mol Sci 23(3): 1222.

Lappano R, Talia M, Cirillo F, *et al.,* 2020. The IL1β-IL1R signaling is involved in the stimulatory effects triggered by hypoxia in breast cancer cells and cancer-associated fibroblasts (CAFs). J Exp Clin Cancer Res 39:1-22.

Larrosa M, Tomás-Barberán FA, Espín JC, 2006. The dietary hydrolysable tannin punicalagin releases ellagic acid that induces apoptosis in human colon adenocarcinoma Caco-2 cells by using the mitochondrial pathway. J Nutr Biochem 17:611-625.

Lewandowska A, Rudzki G, Lewandowski T, *et al.,* 2022. Risk factors for the diagnosis of colorectal cancer. Cancer Con 29:10732748211056692.

Leyane TS, Jere SW, Houreld NN. 2022. Oxidative stress in ageing and chronic degenerative pathologies: molecular mechanisms involved in counteracting oxidative stress and chronic inflammation. Int J Mol Sci 23(13): 7273.

Li J, Chen F, Cona MM, *et al.,* 2012. A review on various targeted anticancer therapies. Target Oncol 7:69-85.

Li S, Liu J, Zheng X, *et al.,* 2022. Tumorigenic bacteria in colorectal cancer: mechanisms and treatments. Cancer Biol Med 19:147-162.

Li X, Huang J, Yu T, *et al.,* 2021. *Fusobacterium nucleatum* promotes the progression of colorectal cancer through Cdk5-activated Wnt/β-catenin signaling. Front Microbiol 11:545251.

Lindell AE, Zimmermann-Kogadeeva M, Patil KR. 2022. Multimodal interactions of drugs, natural compounds and pollutants with the gut microbiota. Nat Rev Microbiol 20:431-443.

Liu B, Zhou H, Tan L, *et al.,* 2024. Exploring treatment options in cancer: tumor treatment strategies. Signal Transduct Target Ther 9(1): 175.

Liu H, Yu Y, Dong A, Elsabahy M, *et al.,* 2024. Emerging strategies for combating *Fusobacterium nucleatum* in colorectal cancer treatment: Systematic review, improvements and future challenges. In: Exploration: Wiley Online Library. p 20230092.

Loree JM, Kopetz S. 2017. Recent developments in the treatment of metastatic colorectal cancer. Ther Adv Med Oncol 9(8): 551-564.

Ludgero-Correia Jr A, Aguila MB, Mandarim-de-Lacerda CA, *et al.,* 2012. Effects of high-fat diet on plasma lipids, adiposity, and inflammatory markers in ovariectomized C57BL/6 mice. Nutrition 28(3): 316-323.

Martin OC, Frisan T. 2020. Bacterial genotoxin-induced DNA damage and modulation of the host immune microenvironment. Toxins 12:63.

Matuszewska K, Pereira M, Petrik D, *et al.,* 2021. Normalizing tumor vasculature to reduce hypoxia, enhance perfusion, and optimize therapy uptake. Cancers 13(17): 4444.

Mignini I, Ainora ME, Di Francesco S, *et al.,* 2023. Tumorigenesis in inflammatory bowel disease: microbiota-environment interconnections. Cancers 15:3200.

Mir MA, Rashid M, Jan N. 2023. Cytokines and Chemokines in Tumor Growth and Progression. In: Cytokine and Chemokine Networks in Cancer: Springer. p 33-77.

Miyaoka M, Kikuti YY, Carreras J, *et al.,* 2018. Clinicopathological and genomic analysis of double-hit follicular lymphoma: comparison with high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements. Modern Pathol 31:313-326.

Morgan E, Arnold M, Gini A, *et al.,* 2023. Global burden of colorectal cancer in 2020 and 2040: incidence and mortality estimates from Globocan. Gut 72:338-344.

Mueed A, Aljahdali SM, Albalawi M, *et al.,* 2024. Phenolic compounds and biological activities of berberis fruit: Enhancing role on physiochemical and antioxidant properties of yogurt. LWT-Food Sci Technol 211: 116834.

Mueed A, Shibli S, Al-Quwaie DA, *et al.,* 2023. Extraction, characterization of polyphenols from certain medicinal plants and evaluation of their antioxidant, antitumor, antidiabetic, antimicrobial properties, and potential use in human nutrition. Front Nutr 10: 1125106.

Paray BA, Albeshr MF, Jan AT, *et al.,* 2020. Leaky gut and autoimmunity: An intricate balance in individuals health and the diseased state. Int J Mol Sci 21(24): 9770.

Paudel D, Nair DV, Tian S, *et al.,* 2024. Dietary fiber guar gum-induced shift in gut microbiota metabolism and intestinal immune activity enhances susceptibility to colonic inflammation. Gut microbes 16:2341457.

Peng S-Y, Lin L-C, Chen S-R, *et al.,* 2021. Pomegranate extract (POMx) induces mitochondrial dysfunction and apoptosis of oral cancer cells. Antioxidants 10:1117.

Pignatelli P, Nuccio F, Piattelli A, *et al.,* 2023. The role of *Fusobacterium nucleatum* in oral and colorectal carcinogenesis. Microorganisms 11:2358.

Piras C, Pibiri M, Conte S, *et al.,* 2022. Metabolomics analysis of plasma samples of patients with fibromyalgia and electromagnetic sensitivity using GC–MS technique. Sci Rep 12:21923.

Poincloux L, Durando X, Seitz JF, *et al.,* 2009. Loss of Bcl-2 expression in colon cancer: a prognostic factor for recurrence in stage II colon cancer. Surg Oncol 18:357-365.

Quaglio AEV, Grillo TG, De Oliveira ECS, *et al.,* 2022. Gut microbiota, inflammatory bowel disease and colorectal cancer. World J Gastroenterol 28(30): 4053.

Radha G, Raghavan SC. 2017. BCL2: A promising cancer therapeutic target. Biochim Biophys Acta - Rev Cancer 1868:309-314.

Rauf A, Olatunde A, Akram Z, *et al.,* 2025. The role of pomegranate (*Punica granatum*) in cancer prevention and treatment: Modulating signaling pathways from inflammation to metastasis. Food Sci Nutr 13(2): e4674.

Ruan JH, Li J, Adili G, *et al.,* 2022. Phenolic compounds and bioactivities from pomegranate (*Punica granatum* L.) peels. J Agric Food Chem 70(12): 3678-3686.

Saad AM, El-Saadony MT, El-Tahan AM, *et al.,* 2021. Polyphenolic extracts from pomegranate and watermelon wastes as substrate to fabricate sustainable silver nanoparticles with larvicidal effect against *Spodoptera littoralis*. Saudi J Biol Sci 28(10): 5674-5683.

Saaoud F, Lu Y, Xu K, *et al.,* 2024. Protein-rich foods, sea foods, and gut microbiota amplify immune responses in chronic diseases and cancers–Targeting PERK as a novel therapeutic strategy for chronic inflammatory diseases, neurodegenerative disorders, and cancer. Pharmacol Ther 255:108604.

Sahle Z, Engidaye G, Shenkute Gebreyes D, *et al.,* 2024. Fecal microbiota transplantation and next-generation therapies: A review on targeting dysbiosis in metabolic disorders and beyond. SAGE Open Med 12:20503121241257486.

Selim S, Albqmi M, Al-Sanea MM, *et al.,* 2022. Valorizing the usage of olive leaves, bioactive compounds, biological activities, and food applications: A comprehensive review. Front Nutr 9: 1008349.

Sharma P, McClees SF, Afaq F, 2017. Pomegranate for prevention and treatment of cancer: an update. Molecules 22:177.

Sihag S, Pal A, Saharan V. 2022. Antioxidant properties and free radicals scavenging activities of pomegranate (*Punica granatum* L.) peels: An *in-vitro* study. Biocatal Agric Biotechnol 42: 102368.

Singh J, Kaur HP, Verma A, *et al.,* 2023. Pomegranate peel phytochemistry, pharmacological properties, methods of extraction, and its application: a comprehensive review. ACS omega 8:35452-35469.

Song N, Cui K, Zeng L, *et al.,* 2024. Advance in the role of chemokines/chemokine receptors in carcinogenesis: Focus on pancreatic cancer. Eur J Pharmacol 967:176357.

Su X, Gao Y, Yang R, 2022. Gut microbiota-derived tryptophan metabolites maintain gut and systemic homeostasis. Cells 11:2296.

Sung H, Ferlay J, Siegel RL, *et al.,* 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71:209-249.

Teniente SL, Flores-Gallegos AC, Esparza-González SC, *et al.,* 2023. Anticancer effect of pomegranate peel polyphenols against cervical cancer. Antioxidants 12(1): 127.

Teniente SL, Flores-Gallegos AC, Esparza-González SC, *et al.,* 2023. Anticancer effect of pomegranate peel polyphenols against cervical cancer. Antioxidants 12:127.

Terveer E. 2020. Exploring the role of the microbiota: in defence against *Clostridioides difficile* and. Emerg Microbes Infect 9:508-516.

Thakur A, Mikkelsen H, Jungersen G. 2019. Intracellular pathogens: host immunity and microbial persistence strategies. J Immunol Res 2019(1): 1356540.

Tokul-Ölmez Ö, Şahin B, Çakır C, *et al.,* 2020. Rapid and easy method for simultaneous measurement of widespread 27 compounds in natural products and foods. J Chem Metrol 14:1-11.

Turrini E, Ferruzzi L, Fimognari C, 2015. Potential effects of pomegranate polyphenols in cancer prevention and therapy. Oxid Med Cell Longev 2015:938475.

Wang H, Guo M, Wei H, *et al.,* 2023. Targeting p53 pathways: mechanisms, structures, and advances in therapy. Signal Transduct Target Ther 8:92.

Wang H, Zhang J, X, Li S, *et al.,* 2015. An animal model of trichloroethylene-induced skin sensitization in BALB/c mice. Int J Toxicol 34(5): 442-453.

Wang RX, Zhou M, Ma HL, *et al.,* 2021. The role of chronic inflammation in various diseases and anti-inflammatory therapies containing natural products. ChemMedChem 16(10): 1576-1592.

Wang X, Undi RB, Ali N, *et al.,* 2021. It takes a village: microbiota, parainflammation, paligenosis and bystander effects in colorectal cancer initiation. Dis Models Mech 14:048793.

Wang X, Wei L, Li Q, *et al.,* 2022. HIF-1α protects osteoblasts from ROS-induced apoptosis. Free Radic Res 56:143-153.

Wang Y, Fu K. 2023. Genotoxins: the mechanistic links between *Escherichia coli* and colorectal cancer. Cancers 15:1152.

Wu N, Feng Y-Q, Lyu N, *et al.,* 2022. *Fusobacterium nucleatum* promotes colon cancer progression by changing the mucosal microbiota and colon transcriptome in a mouse model. World J Gastroenterol 28:1981.

Wu W, Tang J, Bao W, *et al.,* 2024. Thiols-rich peptide from water buffalo horn keratin alleviates oxidative stress and inflammation through co-regulating Nrf2/Hmox-1 and NF-κB signaling pathway. Free Radic Biol Med 223:131-143.

Xia Z, Kon N, Gu AP, *et al.,* 2022. Deciphering the acetylation code of p53 in transcription regulation and tumor suppression. Oncogene 41:3039-3050.

Xiang Q, Li M, Wen J, *et al.,* 2022. The bioactivity and applications of pomegranate peel extract: A review. Journal of Food Biochemistry 46:e14105.

Xiao Y, Yang D, Zhang H, *et al.,* 2024. Theabrownin as a potential prebiotic compound, regulates lipid metabolism via the gut microbiota, microbiota-derived metabolites, and hepatic FoxO/PPAR signaling pathways. J Agric Food Chem 72:8506-8520.

Yang J, Wang X, Hu T, *et al.,* 2024. Entero-toxigenic Bacteroides fragilis contributes to intestinal barrier injury and colorectal cancer progression by mediating the BFT/STAT3/ZEB2 pathway. Cell Cycle 23:70-82.

Yassin MT, Mostafa AAF, Al Askar AA, 2021. *In vitro* evaluation of biological activities and phytochemical analysis of different solvent extracts of *Punica granatum* L.(Pomegranate) peels. Plants 10(12): 2742.

Yin X, Zhang Y, Zou J, *et al.,* 2024. Association of the systemic immune-inflammation index with all-cause and cardiovascular mortality in individuals with rheumatoid arthritis. Sci Rep 14:15129.

Zhang N, Baran A, Valioglu F, *et al.,* 2023. Antioxidant, AChE inhibitory, and anticancer effects of Verbascum thapsus extract. Mol Cell Biol 69(14):211-216.

Zhang YY, Zhang Q, Zhang YM, *et al.,* 2020. A comprehensive automatic data analysis strategy for gas chromatography-mass spectrometry based untargeted metabolomics. J Chromatogr A 1616: 460787.

Zhou J-d, Zhang T-j, Xu Z-j, *et al.,* 2019. BCL2 overexpression: clinical implication and biological insights in acute myeloid leukemia. Diagn Pathol 14:1-10.

Zhuang Y. 2022. Understanding the Genetic Basis of Immune Responses to Enterotoxigenic Bacteroides fragilis Infection. Thesis, In: Johns Hopkins University.