

RESEARCH ARTICLE

Assessment of the anticancer potential of certain phenolic and flavonoid components in ginger capsules using colorectal cancer cell lines coupled with quantitative analysis

Khaldun M. Al Azzam¹  | Nadeen Waleed Al-Areer² | Rima H. Al Omari² |
Ibrahim Al-Deeb³  | Nadia Bounoua^{4,5} | El-Sayed Negim^{6,7} | Ali Al-Samydai²  |
Adam A. Aboalroub² | Rana Said²

¹Department of Chemistry, Faculty of Science, The University of Jordan, Amman, 11942, Jordan

²Department of Pharmaceutical Sciences, Pharmacological and Diagnostic Research Center (PDRC), Faculty of Pharmacy, Al-Ahliyya Amman University, Amman, 19328, Jordan

³Department of Biopharmaceutics and Clinical Pharmacy, Pharmacological and Diagnostic Research Center (PDRC), Faculty of Pharmacy, Al-Ahliyya Amman University, Amman, 19328, Jordan

⁴Laboratory of the Innovation Sponsorship and the Emerging Institution for Graduates of Higher Education of Sustainable Development and Dealing with Emerging Conditions, Department of Exact Sciences, Normal Higher School of Bechar, Bechar, 8000, Algeria

⁵Laboratory of Chemical and Environmental Science (LCSE), Bechar, Algeria, 8000

⁶School of Petroleum Engineering, Satpayev University, 22 Satpayev Street, Almaty, 050013, Kazakhstan

⁷School of Materials Science and Green Technologies, Kazakh-British Technical University, 59 Tole bi St., Almaty, 050000, Kazakhstan

Correspondence

Khaldun M. Al Azzam, Department of Chemistry, Faculty of Science, The University of Jordan, 11942 Amman, Jordan.

Emails:

Email: azzamkha@yahoo.com; k.alazzam@ju.edu.jo

Abstract

Colorectal cancer (CRC) is the fourth most common cause of malignant tumor death. The development of novel, more effective drugs is desperately needed to treat CRC. *Zingiber officinale* is believed to possess anticancer properties due to its flavonoids and phenols. Using Soxhlet (SOXT) and maceration (MACR) techniques, the present study aimed to evaluate the amounts of quercetin, gallic acid, rutin, naringin, and caffeic acid in ginger capsules of *Z. officinale*. High-performance liquid chromatography (HPLC)/ultraviolet was used for separation and quantitation. In vitro toxicity evaluation of ginger capsules on the CRC cell line HT-29 was also conducted to assess the anticancer activity of the supplement. The cell line HT-29 (HTB-38) colorectal adenocarcinoma was utilized for the antiproliferative effect of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide. Ginger herbal supplement extract at dosages of 200 and 100 µg had strong cytotoxic effects ($IC_{50} < 50 \mu\text{g/mL}$) on HT-29 CRC cells via MACR. This extract is comparable to the SOXT extract, which has an IC_{50} of less than 50 µg/mL. The anticancer effect of ginger herbal supplement formulations against CRC lines was investigated, and the results obtained from both the MACR and SOXT extraction procedures were noteworthy. The quercetin content was the highest of all the extracts according to the HPLC data.

KEYWORDS

cancer, ginger capsules, HPLC, HT-29 cells, HTB-38, Soxhlet, *Zingiber officinale*

1 | INTRODUCTION

Medicinal plants are the oldest ancient kind of treatment, and have been utilized in traditional medicine for thousands of years in many nations throughout the world. The empirical knowledge of their positive

benefits has been passed down via human culture over time (Marrelli, 2021). Natural products serve as an important source of pharmacological ingredients, and many contemporary medications originating from ancient herbal medicine are now employed in modern pharmacotherapy (Marrelli, 2021; Spence, 2023; Zagórska et al., 2023).

Ginger (*Zingiber officinale* Roscoe), a member of the Zingiberaceae family and genus, has been traditionally used as a spice and herbal medicine (Shahrajabian et al., 2019). Ginger root is used to relieve and treat a variety of common ailments, including headaches, colds, nausea, and emesis. Numerous bioactive components, including phenolic and terpene compounds, have been found in ginger. The primary phenolic chemicals are gingerols, shogaols, and paradols, which are responsible for the numerous bioactivities of ginger (Shaukat et al., 2023).

Ginger has recently been shown to have biological effects, such as antioxidant (Nam et al., 2024), anti-inflammatory (Zhang et al., 2016), antibacterial (Kumar et al., 2014), and anticancer (Chen et al., 2024) effects. Furthermore, accumulating research has shown that ginger has the potential to prevent and treat a variety of diseases, including neurodegenerative diseases (Arcusa et al., 2022), cardiovascular diseases (Akinyemi et al., 2015), obesity (Suk et al., 2017), diabetes mellitus (Wei et al., 2017), chemotherapy-induced nausea and emesis (Choi et al., 2022), and respiratory disorders (Ballester et al., 2022). In this review, we concentrate on the bioactive components and bioactivities of ginger, with a particular emphasis on its modes of action (Mao et al., 2019).

One of the world's greatest public health issue is cancer. Globally, its prevalence is increasing (El Orfi et al., 2022; Khazaei et al., 2019). According to the International Agency for Research on Cancer, there were 19.2 million new instances of cancer and 9.9 million cancer-related deaths in 2020. According to estimates, there will be 35.265 cancer-related fatalities and 59.370 new cases of the disease in Morocco in 2020 (Sung et al., 2020). Doctors employ a range of traditional medicines, such as immunotherapy, targeted therapies, chemotherapy, radiation, and surgery, to treat this illness (Loubna et al., 2019). Like any medication, these treatments are associated with a long list of adverse effects. Cancer patients use alternative medicines, such as medicinal plants, to treat these issues and ultimately recover from their condition (El Orfi et al., 2022).

Cancer treatment failure is mostly caused by metastasis, recurrence, heterogeneity, resistance to radiation and chemotherapy, and immune evasion. Colorectal cancer (CRC) is among the most common cancers. It is the fourth greatest cause of malignant tumor-related deaths globally and has a high correlation with the global cancer mortality rate (Yang et al., 2023).

Chemotherapy and surgery are currently the primary therapeutic options for CRC patients. However, given the increase in medication resistance and the emergence of adverse effects, it is critical to discover new and more effective treatments for CRC. Many natural ingredients have powerful anti-CRC properties, and research has shown that these compounds can be utilized in conjunction with chemical medications to treat CRC (Krasteva & Georgieva, 2022).

This study utilized high-performance liquid chromatography (HPLC) to analyze 6-gingerol levels in extracts from *Z. officinale*, revealing the highest concentration in ginger leaves (4.9 mg/g), followed by flowers (2.87 mg/g) and rhizomes (1.03 mg/g) (Tanweer et al., 2020).

Pawar et al. (2011) used reverse-phase HPLC to quantify 6-gingerol from ginger varieties in India. The study revealed that cultivars with the highest 6-gingerol content had strong free radical scavenging properties. The Rio de Janeiro rhizome cultivar was found to be the best source of gingerol and its compounds.

Furthermore, both extracts showed a significant degree of inhibitory efficacy with free radical scavenging activity in the antioxidant experiment. All of the tests revealed in strong and considerable in vitro antioxidant activity, which is consistent with the findings of numerous prior studies on radical scavenging (Pawar et al., 2011). These findings are supported by those of Ghasemzadeh et al., 2010, who demonstrated the significant scavenging activity of methanolic extracts in distinct sections of two kinds of *Z. officinale* (Ezez & Tefera, 2021; Pawar et al., 2011).

Mian et al. (2019) analyzed the physicochemical properties of ginger (*Z. officinale* Rosc.) rhizome along with its thin-layer chromatography (TLC), HPLC, and HPTLC profiles. The HPLC pattern shows 29 peaks, and peak nos. 2, 7, and 5 are major peaks with area concentrations and retention times of 9.64% at 2.66 min, 10.12% at 3.25 min, and 15.22% at 3.61 min, respectively.

To investigate the potential of five flavonoids as anticancer drugs, Fernández et al. (2021) used three CRC cell lines: the transplantable human carcinoma cell line derived from lung metastasis of colon carcinoma (T84), a human colorectal adenocarcinoma cell line (ATCC HTB-38) (HT-29), and a colonorectal carcinoma cell line (ATCC® CCL-247TM) (HCT116). These cell lines exhibit three distinct tumor stages, including one that is metastatic. On the three cancer cell lines, xanthohumol had the greatest anticancer activity, surpassing the efficacy of the medication 5-fluorouracil (5-FU). In contrast, luteolin and protegenin had somewhat less of an anticancer effect on these cancer cell lines; however, when combined with 5-FU, they had synergistic effects on HCT116 cells, which may have practical ramifications (Fernández et al., 2021).

The antiproliferative activity of ginger extract (GE) was found to be significant, as indicated by its activation of both intrinsic and extrinsic apoptotic pathways, with an IC_{50} of 12.5 g/mL. GE outperformed cisplatin in mitigating 1,2-dimethylhydrazine-induced CRC, reversing side effects without causing hepatotoxicity or nephrotoxicity. Unlike cisplatin, GE has a stronger anticancer impact and is less hazardous than cisplatin, causing apoptosis (Abdel-Rasol et al., 2022).

According to Mahomoodally et al. (2021), ginger and its bioactive derivatives are cytotoxic to colon cancer cell lines, including Dukes C, HCT116, and HT29. They induce apoptosis by upregulating cyclin D1 and suppressing the G1 phase of the cell cycle, controlling proliferation.

In this work, the maceration (MACR) and Soxhlet (SOXT) techniques were used to identify and quantify the compounds quercetin, gallic acid, rutin, naringin, and caffeic acid that have been found in various *Z. officinale* extracts. Moreover, quercetin, gallic acid, rutin, naringin, and caffeic acid were all measured in single-run analysis using an HPLC/ultraviolet (UV) detector (Figure 1). Finally, in order to assess the in vitro toxicity of *Z. officinale* extracts on the CRC cell line HT-29, the anticancer qualities of ginger capsules were investigated.

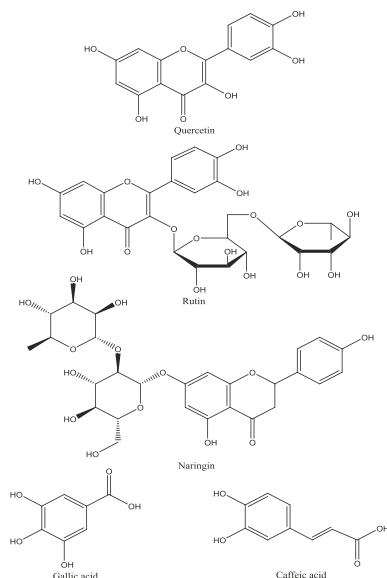


FIGURE 1 The chemical structures of the components examined in the extracts of ginger capsules (ChemDraw).

2 | MATERIALS AND METHODS

2.1 | Ginger capsules

Ginger root extract (550 mg per capsule) was obtained from a local pharmacy in Jordan.

2.2 | Chemicals and reagents

Quercetin, caffeic acid, gallic acid, rutin, and naringin hydrate were purchased from Geochem World (Mumbai, India). Formic acid and methanol were purchased from Carlo Erba Reagents (Milan, Italy). All reagents were of analytical reagent grade.

2.3 | Equipment and instruments

A digital balance (ADAM equipment PGW 453i, P. R. China) was used to weigh the ginger species. Using a Buchi Rotavapor R-300 (Flawil, Switzerland), MACR and SOXT extraction were carried out. The absorbance was measured via an HPLC/UV spectrophotometer, stirrer, sonicator, and water bath (Thermo Scientific) using a UV–visible (VIS) spectrophotometer (UV-1800; Shimadzu, Japan).

2.4 | Preparation of ginger capsule root extract

The MACR and SOXT extraction techniques, both of which use methanol as a solvent, were used in this investigation to extract ginger herbal supplements. Following the extraction of the ginger herbal supplement, the products were concentrated using a rotating evaporator (Rota-vap)

at the necessary temperature and vacuum pressure. After that, the crude extracts were weighed, dried, and stored at -4°C until needed.

2.5 | Extraction procedures

Extraction is the initial stage in the process of obtaining vital natural extracts from raw materials. To summarize, the MACR extraction process involved weighing 100 g of the ginger capsule and soaking it in 1 L of methanol in an aluminum foil-covered container for 72 h at room temperature (RT) in the dark with sporadic shaking. After being filtered in a conical flask and evaporated in a rotary evaporator, the mixture was then dried and weighed for the final sample preparation process (Al-Areer et al., 2023).

Al-Areer et al. (2023) claim that the SOXT extraction process is a high-efficiency automated continuous extraction technology that uses less solvent and time than alternative approaches. Briefly, the thimble was placed into the SOXT apparatus after 40 g of the ginger capsule had been filled with it and 200 mL (1:5) of the extraction solvent was added to the flask with a flat bottom. Heat was then applied in accordance with the solvent extract. The solvents were extracted for nine hours at 90°C using a magnetic stirrer that was stirred at 400 rpm. Ultimately, the extract was collected in a round-bottom flask and transferred to a rotary evaporator.

2.6 | Optimization of extraction methods

Using methanol, two procedures, MACR and SOXT, were used to extract the active components from ginger capsule extract. To calculate the extraction yield, the following formula was utilized:

$$\% \text{ Extractive Value of Solvent} = (\text{Weight of the extract} / \text{Weight of the sample}) \times 100.$$

2.7 | Total phenolic content (TPC) quantification

To determine the TPC, Wolfe et al. (2003) and Al-Areer et al. used the Folin–Ciocalteu technique to calculate the TPC. Each plant extract was first diluted with methanol to a concentration of 10 mg/mL. Next, the mixture was vortexed for 20 s. Subsequently, 4 mL of 7.5% sodium carbonate was added, the mixture was swirled for 20 s, and the mixture was allowed to develop a blue color in a water bath at 40°C for 30 min. The last method used to estimate the phenolic content was calorimetric measurements at 765 nm. The gallic acid equivalent (GAE) (mg/g) was utilized to calculate the TPC using an equation derived from the calibration curve.

2.8 | Total flavonoid content (TFC) quantification

The total amount of flavonoids was measured using the method of Al-Areer et al., 2023. In summary, 10 mL of a volumetric flask

containing 0.1 mg/mL extracted ginger capsules was diluted with 4 mL of water. First, 0.3 mL of a 5% NaNO₂ solution was added to each volumetric flask and allowed to stand for five minutes. After that, a 10% AlCl₃ (w/w) solution was added for 6 min. Next, the volumetric flask was filled with 2.4 mL of distilled water (DW) and 2 mL of NaOH (1.0 M), and the mixture was thoroughly mixed in the reaction flask. At 430 nm, the absorbance of the reaction mixture was measured. The quercetin content/g dry weight was calculated according to Ghasemzadeh et al. (2010).

2.9 | HPLC analysis

2.9.1 | HPLC chromatographic conditions

HPLC analysis was performed using the method described by Al-Areer et al., 2023. A gradient Shimadzu HPLC system (Prominence-i LC-2030C plus, Kyoto, Japan) was used to analyze the ginger capsule extract and reference compounds. A UV-VIS Plus detector, a DGU-20A degasser, SIL-20A autosampler, and a solvent delivery system pump were equipped with the HPLC unit. The Chrom Quest program version 4.2.34 was utilized to record the signals on an LC-solution version 1.25 (2009–2010) workstation (Shimadzu, Japan) running Microsoft Windows XP.

An optimized mobile phase comprising 0.2% formic acid aqueous solution as mobile phase B and methanol as mobile phase A was used to accomplish chromatographic separation at 40°C using an analytical EC HPLC column (NUCLEODUR 100–5 C18 ec, 5 µm, 250 mm × 4.6 mm; MACHEREY-NAGEL, Germany). Elution was performed using the gradient approach. The following steps were used: flow rate of 1.0 mL/min, constant column temperature of 40°C, and injection volume of 4 µL. Using an automated sampler, an HPLC UV detector with a wavelength of 283 nm was employed for detection.

2.9.2 | Preparation of the standard solution

A total of 25.0 mg of each standard (naringin, quercetin, gallic acid, caffeic acid, and rutin) was weighed using an analytical balance. The samples were then diluted in methanol in a 25-mL volumetric flask and kept for later use at 4°C. Additionally, standard curves were created by diluting the standard solution to various concentrations (0.1, 0.5, 5, 10, 25, and 50 µg/mL for gallic acid; 0.1, 0.5, 5, 10, 25, and 50 µg/mL for caffeic acid; 0.1, 0.5, 5, 10, 25, and 50 µg/mL for naringin; 0.1, 0.5, 5, 10, 25, and 50 µg/mL for rutin; and 1, 5, 10, 25, 50, and 100 µg/mL for quercetin). The detection was carried out using the same chromatographic parameters as mentioned earlier.

2.9.3 | Preparation of the sample solution

Each extract (20.0 mg) was weighed using an analytical balance, diluted with methanol in a 10-mL volumetric flask, sonicated, filtered

through a 0.2-µm syringe filter, and then injected into the HPLC system. The combination was stored at 4°C in a refrigerator for no more than 2 days.

2.10 | Cytotoxicity assay

2.10.1 | Cell culture

The colorectal adenocarcinoma cell line utilized in this investigation was HT-29 (HTB-38), which was obtained from the American Type Culture Collection (ATCC; USA) and maintained in accordance with ATCC guidelines.

2.10.2 | Antiproliferative effect of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) on cell proliferation

The basis for this colorimetric test is the reduction of MTT dye, a yellow tetrazole, to purple formazan in living viable cells. This reduction process only occurs in the mitochondria of living cells; it does not occur in cells that are the target of certain medications or treatments intended to kill cancer cells.

To count a portion of the trypsinized cells for the MTT experiment, an automated cell counter (QuadCount, Accuris Instruments, USA) was utilized. An automated counter was used to count the number of suspended cells in a sample of known volume, and trypan blue dye was added at a 1:1 ratio.

In brief, a coated flat bottom 96-well microplate was used, and 5000 cells/100 L were seeded into each well. The cells were then incubated for 24 h to allow full cell adherence to the surface. Subsequently, each well received a progressive addition of 200, 100, and 50 g/mL of the 28 freshly extracted and powdered GEs that were previously obtained, which were made from the main stock (2000 g/mL).

Two hundred microliters of each GE was applied to the cells in triplicate. After 48 h of incubation, the plate was incubated for 4 h at 37°C, after which 20 µL of MTT reagent (Genochem World, Spain) was added to each well. Five milligrams of MTT powder was dissolved in 1 mL of phosphate-buffered saline to alter the MTT concentration. After the medium and MTT were removed, 150 µL of DMSO was added to each well to dissolve the formazan crystals that had formed. Sixty minutes later, the absorbance of the dissolved crystals in DMSO was measured at 590 nm using a 96-well microplate reader.

Three replicate wells were used as blanks; no treatments or cells were added. The same 0.5% DMSO concentration used in the wells treated with GEs was also applied to duplicate wells that were seeded with cells as a control. Microsoft Excel was used to evaluate the results of the MTT cell proliferation experiments to determine the “inhibitory concentration (IC₅₀),” that is, the concentration at which, in comparison to untreated control cells, 50% of the cells remained viable after treatment.

The average absorbance in the blank wells was measured before calculating the average absorbance for each concentration. To determine the percentage of cells that survived at each concentration, the average absorbance of the drug-treated wells was calculated by dividing the average absorbance of the control wells and multiplying the result by 100%. Using Microsoft Excel, the concentration at which 50% of the cells were alive and the IC_{50} values were displayed against the survival (%) data.

3 | RESULTS AND DISCUSSION

In our investigation, we examined the optimal 9 h of extraction in a SOXT apparatus for extracting ginger capsules. Extraction using MACR was conducted for 72 h. The SOXT extraction method takes less time than the MACR method, which helps us reduce the time needed for the preparation of extracts. This extraction method has the advantage of obtaining highly active components while reducing the extraction time.

These results indicated that the SOXT method, which yielded extraction yields of 8.1% and 6.7% using SOXT and MACR, respectively, is the most efficient for separating the active ingredients from ginger capsule extract when employing distinct methods (MACR and SOXT). In addition, the SOXT technique yielded higher yields of ginger capsules than methanolic extracts from MACR.

This finding can be explained by the different temperatures used in the two techniques: SOXT operates at a higher temperature (90°C) because the solvent must boil. By altering the saturation equilibrium constant in favor of extraction, increasing the temperature contributes to increased process efficiency (Zhang et al., 2018). The data suggest that in terms of time consumption, SOXT is a better extraction method than MAC. Utilizing SOXT equipment also has the benefit of providing the best extraction yield.

The MACR method is a method that is easy to do, simple, and cheap so it is often used by researchers but requires a long extraction time. The SOXT method requires a faster extraction time in just a few hours compared to the MACR method which extracts for days. Moreover, SOXT method is generally carried out on a laboratory scale because it uses tools that are usually found in a laboratory. Based on the yield obtained by the two methods (MACR & SOXT), the highest extraction yield is obtained by SOXT (8.1%) (Tambun et al., 2021).

According to Arawande et al., solvents are a measure of the potential extraction rate that may be produced from a particular material (Arawande et al., 2018). It is believed that bioactive compounds may be effectively extracted using high extractive solvents such as methanol, but distinct bioactive compounds can be identified more easily using low extractive solvent findings.

Similar results were obtained by Ezez and Tefera in their study with methanol as the solvent. They observed that the kind and polarity of the solvents used greatly affect the amount and quality of phenol extraction. The greatest amounts of phenols are extracted using high-polarity solvents such as methanol. Additionally, Al-Areer et al., 2023, methanol showed greater extraction capacity for

phenolics from the ginger rhizome (1183.813 mg GAE/100 g extract dry weight). In addition, the Mandura specimen's methanol extract had a significant phenolic content (1022.409 mg GAE/100 g extract dry weight), but the ethanol extract only displayed 941.847 mg GAE/100 g extract dry weight. Consequently, methanol proved to be the most successful solvent in extracting phenols from *Zingiber officinale* when compared to other solvents (Al-Areer et al., 2023; Ezez & Tefera, 2021).

3.1 | TPC determination

It is well recognized that phenolics are necessary for a regular diet. Furthermore, because they can improve health, Tanweer et al. (2020) noted that their antioxidant capabilities are advantageous. A calibration curve for gallic acid was created by serially diluting the stock solution (from 10 to 200 mg/mL) and dissolving 250 mg of the stock solution in 25 mL of methanol. The TPC was expressed as a GAE (mg/g) using the following equation, which was derived from the calibration curve: $y = 0.0027x + 0.0259$, $R^2 = 0.9933$, where X is the concentration and Y is the absorbance.

Each working test tube was filled with the following mixture: 4 mL of 7.5% sodium carbonate, 5 mL of Folin-Ciocalteu 10% reagent diluted with DW 1:10 v/v, and 0.15 mL of extract solution. After 15 s, the mixture was vortexed. Then, the mixture was placed in a 40°C water bath for 30 min, or until the mixture became blue. The amount of phenolic compounds was then determined using a calorimetric technique at 765 nm. The linear regression equation derived from the calibration curve of standard gallic acid was used to calculate the TPC for each extract as expressed in terms of GAE. The TPC of the ginger capsule extracts was determined using MACR and SOXT and was 102.32 and 89.43 (mg/g), respectively. The TPC was expressed as the GAE (mg/g).

By comparing the results of the current study to those of Al-Areer et al., 2023, it was observed that the TPC for powder ginger, using methanol as a solvent, were 110.41 and 99.58 mg/g for the MACR and SOXT techniques, respectively. According to Al-Areer et al. (2023), the same behavior was observed for both the powdered ginger and ginger capsules (present study). The total phenol levels decreased with SOXT, most likely due to the temperature effect. The main issue with this technique is its use of thermolabile compounds. Ezez and Tefera (2021), on the other hand, investigated the outcomes of a comparison of the solvents employed to extract ginger rhizomes. When using thermal procedures such as SOXT instead of nonthermal methods such as MACR, the TPC decreases.

The total phenolic and flavonoid contents of ginger increased as the processing temperatures decreased, as shown in the present study. These findings agreed with the report of Offei-Oknye et al., 2015. The TPC varies from place to place because, as Dias et al. demonstrated, phenolic compounds are not directly linked to the growth functions and development of plant tissue. Rather, they are produced as a defense against pathogen attack or in response to a stressful environment.

TABLE 1 Linear regression data for the calibration curves of gallic acid, caffeic acid, naringin, rutin, and quercetin.

Parameters	Gallic acid	Caffeic acid	Naringin	Rutin	Quercetin
Linearity range ($\mu\text{g/mL}$)	0.1–50	0.1–50	0.1–50	1.0–50	1.0–100
Regression equation	$y = 11.593x - 3890.3$	$y = 14.395x - 3280.2$	$y = 7220.1x - 1502.7$	$y = 2040.3x - 1585.2$	$y = 4173.7x - 19,791$
Correlation coefficient (R^2)	0.9972	0.9977	0.9979	0.9945	0.9920
Slope	11.593	14.395	7220.1	2040.3	4173.7
Intercept	3890.3	3280.2	1502.7	1585.2	19,791
LOD ($\mu\text{g/mL}$)	0.04	0.05	0.06	0.05	0.07
LOQ ($\mu\text{g/mL}$)	0.15	0.13	0.17	0.21	0.24

Abbreviations: LOD, limit of detection; LOQ, limit of quantification.

According to the study's findings, the total phenolic and flavonoid contents of ginger increased as the processing temperature decreased. The results of the study by Offei-Oknye et al. (2015) were in accordance with these findings. Additionally, Dias et al. (Dias et al., 2016) demonstrated that the production of phenolic compounds as a defense against pathogen attack or in response to a stressful environment is what causes the TPC to vary from one location to another and has nothing to do with the growth functions and development of plant tissue. Furthermore, the results indicate that extracts with the highest concentration of phenolic compounds were produced by high-polarity solvents such as methanol. Since polyphenolic chemicals are known to have antioxidant properties, it is probable that these properties are connected to the activity of the extracts.

3.2 | TFC determination

Flavonoid compounds are substances that are classified as phenolic or polyphenol compounds. Among all the polyphenols studied, most related research has been performed on flavonoids. Maulana et al. (2019) reported that the basic structure of this group is an oxygenated heterocycle composed of two aromatic rings connected by three carbon atoms.

Five hundred milligrams of quercetin was dissolved in 50 mL of ethanol. A series of concentration dilutions ranging from 10 to 600 mg/mL were then made. A UV-visible device was used to detect the absorbance at 430 nm, and a calibration curve was constructed using the standard quercetin. The following calibration curve equation, $y = 0.0010x + 0.077$, $R^2 = 0.9918$, was used to calculate the TFC of the ginger capsules as mg of quercetin equivalents/mL. Y represents the absorbance, and X represents the concentration.

First, the flavonoid contents of the *Z. officinale* extracts were determined. A 10-mL volumetric flask was filled with 1 mL of solvent extract diluted with 4 mL of DW. After introducing 0.3 mL of a 5% NaNO_2 solution for 5 min, 10% AlCl_3 (w/w) was added for 6 min before adding 1.0 M NaOH (2 mL). After adding 2.4 mL of DW, the absorbance of the reaction mixture was measured at 430 nm. The results are presented in mg quercetin/mL in contrast to the quercetin standard curve.

TABLE 2 HPLC analysis of phenolic and flavonoid compounds extracted from ginger capsules in methanol using the maceration (MACR) and Soxhlet (SOXT) methods. The results are expressed in $\mu\text{g/mL}$. All analyses are presented as the mean value of triplicate (measurements $\pm$ standard deviation).

Components	Methanol (MACR)	Methanol (SOXT)
Gallic acid	0.60 ± 2.33	0.83 ± 0.94
Caffeic acid	0.43 ± 1.71	0.77 ± 1.07
Naringin	0.31 ± 1.71	0.42 ± 2.10
Rutin	ND	ND
Quercetin	11.26 ± 1.04	10.81 ± 2.21

Abbreviations: HPLC, high-performance liquid chromatography; MACR, maceration; ND, not detected; SOXT, Soxhlet.

The TFC of the ginger capsule extracts was determined using MACR and SOXT and was 393.6 and 305.33 (mg/g), respectively. The TFC was quantified as mg/g quercetin. Because a particular class of flavonoid compounds was not heat resistant or readily oxidized according to the SOX method, the overall flavonoid concentrations were greater in the MACR. These results might be related to the polarity index and solvent strength, which affect the solubility of bioactive compounds in the plant matrix. These findings agree with the work of Al-Areer et al. (2023) (TFC content: 440 and 296.67 mg/g using the MACR and SOXT techniques, respectively). Flavonoids are known to have varying solubilities in different solvents, and their polarity often influences the extraction solvent used. Plant materials have inherent diversity, which may account for variations in flavonoid content. Given that flavonoids are now a crucial component of many different nutraceutical, pharmacological, therapeutic, and cosmetic uses, a high flavonoid concentration indicates the advantages of the plant material.

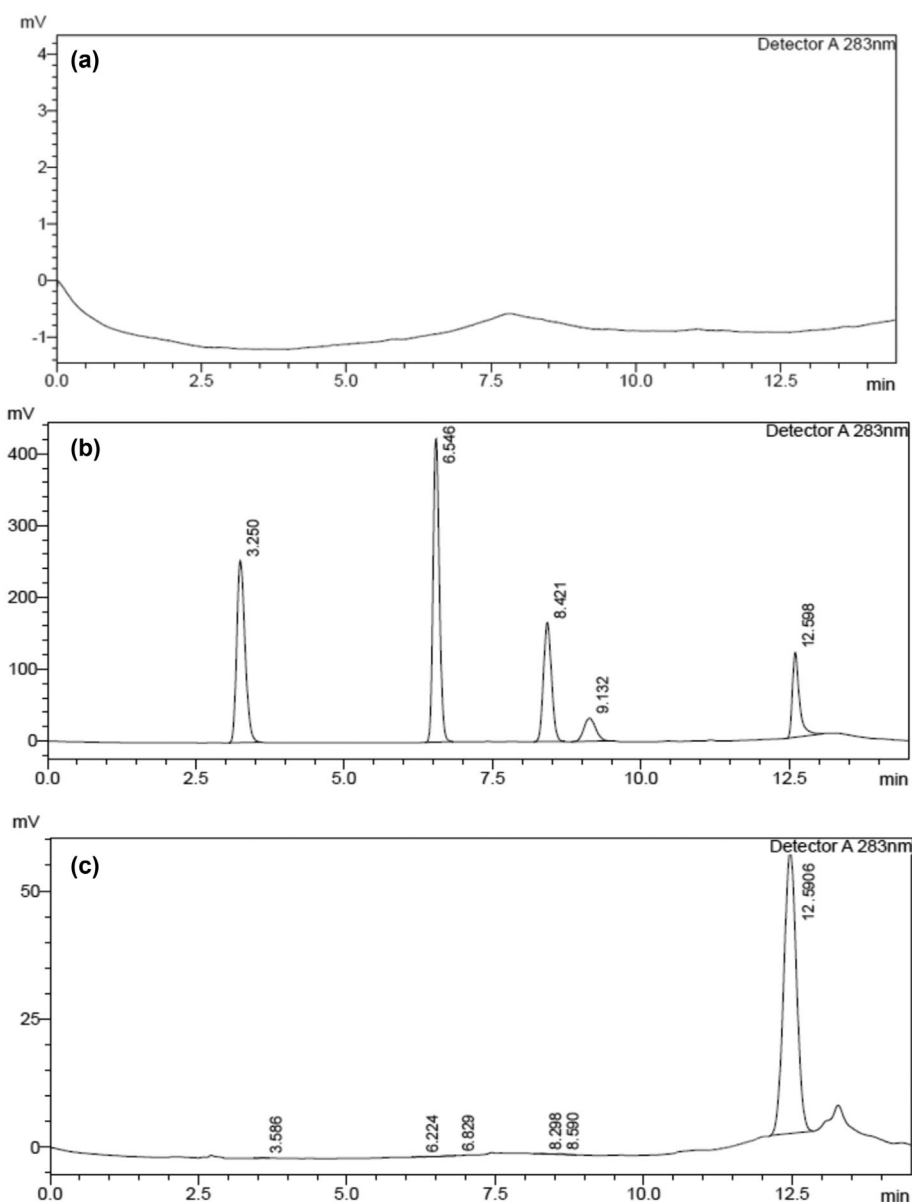
Flavonoids successfully scavenge the vast majority of oxidizing substances, including singlet oxygen and other free radicals. The type of functional group and how it is organized around the nuclear structure determine the antioxidant capacity of flavonoids (Akullo et al., 2023). This description highlights the importance of investigating how MACR and SOXT extraction procedures alter the quantity of flavonoids in ginger capsule extracts when a methanol solvent is used.

3.3 | HPLC analysis

3.3.1 | Calibration curves

By comparing the extracts' retention times to those of standards, it was possible to identify the five compounds that were present. The concentrations of caffeic acid, gallic acid, naringin, rutin, and quercetin

FIGURE 2 A representative chromatogram of (a) the blank injection, (b) the standard mixture of phenolic and flavonoid compounds (200 µg/mL), and (c) the sample injection of ginger capsule extract using the methanol solvent and SOXT method via HPLC separation. Gallic acid, caffeic acid, naringin, rutin, and quercetin were measured using HPLC. Peak identification was based on the following standard injections: gallic acid (retention time [RT]: 3.250 min), caffeic acid (RT: 6.546 min), naringin (RT: 8.421 min), rutin (RT: 9.132 min), and quercetin (RT: 12.598 min). HPLC, high-performance liquid chromatography.



were determined with the aid of standard calibration curves. The calibration curves for gallic acid, caffeic acid, naringin, rutin, and quercetin were constructed using the following equations: $y = 11,593x - 3890.3$, $y = 14,395x - 3280.2$, $y = 7220.1x - 1502.7$, $y = 2040.3x - 1585.2$, and $y = 4173.7x - 19,791$, respectively. The corresponding correlation coefficients (R^2) were 0.9972, 0.9977, 0.9979, 0.9945, and 0.9920 for caffeic acid, gallic acid, naringin, rutin, and quercetin, respectively.

The linearity range, equations, slope, intercept, correlation coefficients (R^2), limit of quantification (LOQ), and limit of detection (LOD) are shown for each in Table 1. These data suggest that the proposed method is sensitive enough to measure the aforementioned compounds simultaneously. The validation procedure was conducted in accordance with the linearity range, LOD, and LOQ guidelines published by the International Conference on Harmonization (ICH) in 2005. Based on the standard deviation (SD) and slope (S) of the calibration curves, the formulae for calculating the LOD and LOQ were $\text{LOD} = 3.3 (\text{SD}/S)$ and $\text{LOQ} = 10 (\text{SD}/S)$, respectively (Abdul Rahim et al., 2018).

3.3.2 | HPLC analysis of selected flavonoids and phenolic compounds

Reversed-phase high-performance liquid chromatography (RP-HPLC) was used for separation. The primary principal points of the optimization approach were the separation temperature, composition of the mobile phase, and analytical column. Positive results were obtained from the C18 analytical column, which also demonstrated good stability across a wide pH range. Thus, a NUCLEODUR 100-5 C18 ec, 5 μm , 250 mm $\times$ 4.6 mm (MACHEREY-NAGEL, Germany) was chosen for the study. Mixed standards that eluted near one another (close retention time) could not be separated; however, the analysis time was sufficient when longer columns (250 mm) were used.

With an injection volume of 4 μL and a fixed temperature of 40°C, the flow rate was maintained at 1 mL/min. After 15 min, all the compounds were separated under these circumstances, and the resolution between the analyte and matrix peaks was good.

The chromatographic column's stationary phase was affected if the formic acid content in the mobile phase was very high. Using 0.2% formic acid and methanol as the mobile phase, the gradient elution approach, as reported in the work of Al-Areer et al. (2023) and Xue et al. (2021), was utilized to separate the target chemicals. After less than 15 min, all the compounds were separated with excellent resolution between the analyte and matrix peaks.

Table 2 provides the results from the RP-HPLC analysis of certain flavonoids and phenolic acids. Figure 2(a) displays a blank injection chromatogram, while Figure 2(b) shows the standard mixture injection. However, a typical injection from the extract is also shown in Figure 2(c), and it can be inferred that there was no interference, demonstrating the selectivity of the method used. According to the

data acquired, quercetin, a common flavonoid, had the maximum concentration among the extracts utilized, regardless of whether the SOXT method or MACR was used (Table 2).

Furthermore, naringin had the lowest concentration of all the compounds analyzed using the MACR or SOXT extraction methods. Rutin was the compound with the lowest concentration that was not detected using the SOXT or MACR extraction methods.

Overall, the HPLC analysis of the flavonoid components in ginger capsules revealed that quercetin was the most concentrated extract of all the extracts, regardless of whether they were made using the SOXT or MACR method. Naringin and caffeic acid were the second most prevalent compounds. Gallic acid came in third.

The antitumor effectiveness of quercetin derivatives differs from that of quercetin depending on the subunit position and chain length. The anticancer activity of quercetin varies from that of its various derivatives. Lotfi et al. (2023) reported that adding a phenolic hydroxyl group by etherification (O-alkylation) greatly reduced cancer cell proliferation. Moreover, a number of nanoparticles, including chemotherapeutic medications such as adriamycin (ADR) and mitoxantrone (MTX), have been applied on the surface of quercetin. This modified quercetin may have greater synergistic anticancer benefits while having fewer side effects and reducing drug resistance from chemotherapy drugs.

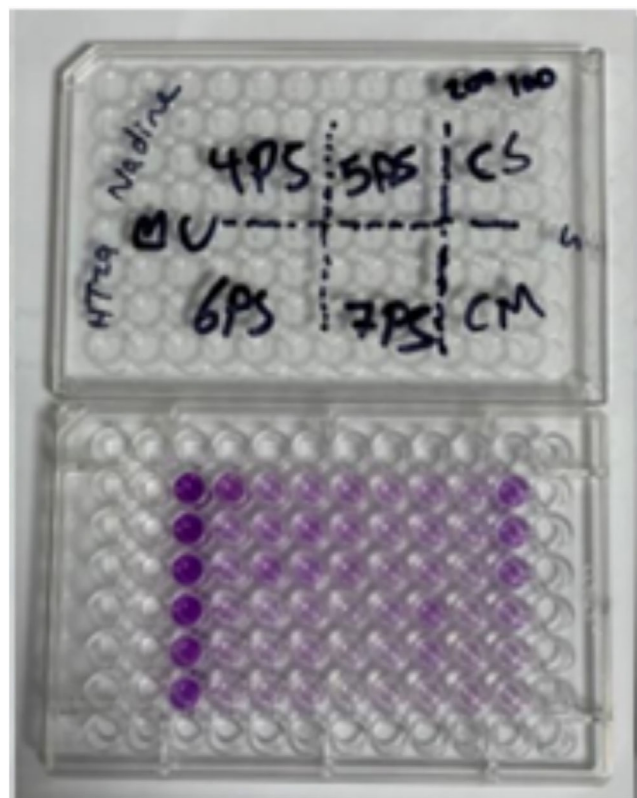


FIGURE 3 MTT cell proliferation assay. Methanol in ginger capsules obtained by the Soxhlet (SOXT) method. Methanol in ginger capsules by the maceration (MACR) method. MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

TABLE 3 MTT cell proliferation assay results, % cell survival, and IC₅₀ of two extraction methods for ginger herbal supplement extract.

Solvent	% survival in ginger herbal supplement/MACR different concentration			% survival in ginger herbal supplement/SOXT different concentration		
	200 µg/mL	100 µg/mL	IC ₅₀ (µg/mL)	200 µg/mL	100 µg/mL	IC ₅₀ (µg/mL)
Methanol	10.37	13.36	<50	12.00	32.45	<50

Abbreviations: MACR, maceration; SOXT, Soxhlet; IC₅₀, half maximal inhibitory concentration.

3.3.3 | Antiproliferative effects of MTT

As a means of evaluating cellular metabolic activity, the MTT assay can be used to measure the cytotoxicity, proliferation, and viability of cells (Figure 3). The IC₅₀ values for the extracts from the ginger herbal supplement were determined, and the concentration versus survival data are displayed. Table 3 provides a summary of the results.

MACR had strong cytotoxic effects on HT-29 CRC cells (IC₅₀ < 50 µg/mL) when the ginger herbal supplement extract was combined with MACR at concentrations of 200 and 100 µg. As with. The SOXT extract had an IC₅₀ of less than 50 µg/mL. Both the MACR and SOXT extraction methods yielded notable findings when we tested the anticancer impact of ginger herbal supplement formulations against CRC lines in this work.

These data agree with other studies showing that HT-29 CRC cells are more sensitive to the anticancer action of dried GE, with an IC₅₀ (455 µg/mL) much lower than that of HCT-116 cells (496 µg/mL) (Zadorozhna & Mangieri, 2021). The cytotoxic impact is most likely caused by gingerol, which is known to inhibit the growth of liver HepG2 cancer cells and HCT116 CRC cells.

These findings are consistent with previous research, which revealed that HT-29 CRC cells were more susceptible to the anticancer effect of dried GE, with an IC₅₀ (455 µg/mL) substantially lower than that of HCT-116 cells (496 µg/mL) (Ezez & Tefera, 2021). The presence of gingerol, which is known to suppress the proliferation of HCT116 CRC cells and liver HepG2 cancer cells, is most likely responsible for the cytotoxic effect.

Similarly, ginger was shown to have the lowest IC₅₀ of any plant studied by Al-Tamimi et al., (Al-Tamimi et al., 2016) with values of 40 µL/mL for HT-29 cells that secreted mucus and 60 µL/mL for cells that did not secrete mucus. The findings of this study suggest that *Z. officinale* extract has clinically meaningful, safe anticancer activity that is therapeutically promising for patients with few adverse effects.

The key advantages of the current study identify bioactive compounds responsible for anticancer activities, aiding in the development of targeted therapies, accurate quantification determines effective doses without toxicity. Moreover, preliminary data on efficacy and safety in cell lines assess potential risks before animal or human trials. This multidisciplinary approach promotes comprehensive findings by integrating pharmacology, oncology, biochemistry, and analytical chemistry.

4 | CONCLUSIONS

After less than 15 min, the five phenolic and flavonoid compounds (quercetin, rutin, naringin, and gallic acid) and their extracts were effectively separated and quantified by HPLC analysis. This study showed that the extraction technique employed affected the phenolic and flavonoid contents of in ginger plants. Significant differences between the SOXT and MACR extraction techniques were observed in the HPLC data.

HPLC analysis of ginger capsule flavonoid components revealed quercetin as the most concentrated extract, followed by naringin, caffeic acid, and gallic acid. Naringin and rutin were found to have the lowest concentrations among the compounds analyzed using MACR or SOXT extraction methods. Moreover, the HPLC analysis method successfully separated and quantified five phenolic and flavonoid compounds standards (gallic acid, caffeic acid, rutin, naringin, and quercetin) and their extract in less than 13 min.

Finally, the proposed technique/procedure may be applied to rapidly and precisely identify certain phenols in a variety of plant extracts without necessitating further sample preparation, offering vital details on these important substances with possible health benefits.

This study focused on CRC and emphasized the cytotoxic properties of ginger. Using MACR and SOXT, we assessed the anticancer potential of ginger capsule extracts against CRC lines in this investigation. When used against HT-29 cells, the extracts showed promise. Conversely, the HT-29 cells exposed to the crude capsule extracts showed more powerful cytotoxic effects (IC₅₀ < 50 µg/mL), suggesting that these extracts might be useful as anticancer drugs for the treatment of CRC. More cytotoxicity studies are required to assess the efficacy of these agents and ensure the right safe dose for patient compliance.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Khalidun M. Al Azzam  <https://orcid.org/0000-0003-4097-6991>

Ibrahim Al-Deeb  <https://orcid.org/0000-0002-7387-0598>

Ali Al-Samyda  <https://orcid.org/0000-0003-0093-2310>

REFERENCES

- Abdel-Rasol, M. A., El-Beih, N. M., Yahya, S. S., & El-Sayed, W. M. (2022). The antitumor activity of ginger against colorectal cancer induced by

- dimethylhydrazine in rats. *Anti-Cancer Agents in Medicinal Chemistry*, 22(8), 1601–1610. <https://doi.org/10.2174/1871520621666210903112813>
- Abdul Rahim, R., Ahmad, N. H., Al Azzam, K. M., & Mat, I. (2018). Determination and quantification of the vinblastine content in purple, red, and white *Catharanthus roseus* leaves using RP-HPLC method. *Advanced Pharmaceutical Bulletin*, 8(1), 157–161. <https://doi.org/10.15171/apb.2018.019>
- Akinyemi, A. J., Thome, G. R., Morsch, V. M., Stefanello, N., Goularte, J. F., Bello-Klein, A., Oboh, G., & Chitolina, S. M. R. E. (2015). Dietary supplementation of ginger and turmeric rhizomes on angiotensin-1 converting enzyme (ACE) and arginase activities in L-NAME induced hypertensive rats. *Journal of Functional Foods*, 17, 792–801. <https://doi.org/10.1002/ptr.5621>
- Akullo, J. O., Kiage-Mokua, B. N., Nakimbugwe, D., Ng'ang'a, J., & Kinyuru, J. (2023). Phytochemical profile and antioxidant activity of various solvent extracts of two varieties of ginger and garlic. *Heliyon*, 9(8), e18806. <https://doi.org/10.1016/j.heliyon.2023.e18806>
- Al-Areer, N. W., Al Azzam, K. M., Al Omari, R. H., Al-Deeb, I., Bekbayeva, L., & Negim, E.-S. (2023). Quantitative analysis of total phenolic and flavonoid compounds in different extracts from ginger plant (*Zingiber officinale*) and evaluation of their anticancer effect against colorectal cancer cell lines. *Pharmacia*, 70(4), 905–919. <https://doi.org/10.3897/pharmacia.70.e103936>
- Al-Tamimi, M. A., Rastall, B., & Abu-Reidah, I. M. (2016). Chemical composition, cytotoxic, apoptotic and antioxidant activities of main commercial essential oils in Palestine: A comparative study. *Medicine*, 3(4), 1–11. <https://doi.org/10.3390/medicines3040027>
- Arawande, J. O., Akinnusotu, A., & Alademeyin, J. O. (2018). Extractive value and phytochemical screening of ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) using different solvents. *International Journal of Traditional and Natural Medicines*, 8(1), 13–22. <https://oer.unimed.edu.ng/JOURNALS/5/3/Dr-Arawande-Extractive-Value-and-Phytochemical-Screening-of-Ginger-zingiber-officinale-and-Turmeric-OER1419599.pdf>
- Arcusa, R., Villano, D., Marhuenda, J., Cano, M., Cerdà, B., & Zafrilla, P. (2022). Potential role of ginger (*Zingiber officinale* roscoe) in the prevention of neurodegenerative diseases. *Frontiers in Nutrition*, 9, 809621. <https://doi.org/10.3389/fnut.2022.809621>
- Ballester, P., Cerdà, B., Arcusa, R., Marhuenda, J., Yamedjeu, K., & Zafrilla, P. (2022). Effect of ginger on inflammatory diseases. *Molecules*, 27(21), 7223. <https://doi.org/10.3390/molecules27217223>
- Chen, M., Lin, E., Xiao, R., Li, Z., Liu, B., & Wang, J. (2024). Structural characteristic, strong antioxidant, and anti-gastric cancer investigations on an oleoresin from ginger (*Zingiber officinale* var. *roscoe*). *Food*, 13(10), 1498. <https://doi.org/10.3390/foods13101498>
- Choi, J., Lee, J., Kim, K., Choi, H.-K., Lee, S.-A., & Lee, H.-J. (2022). Effects of ginger intake on chemotherapy-induced nausea and vomiting: A systematic review of randomized clinical trials. *Nutrients*, 14(23), 4982. <https://doi.org/10.3390/nu14234982>
- Dias, M. I., Sousa, M. J., Alves, R. C., & Ferreira, I. C. F. R. (2016). Exploring plant tissue culture to improve the production of phenolic compounds: A review. *Industrial Crops and Products*, 82, 9–12. <https://doi.org/10.1016/j.indcrop.2015.12.016>
- El Orfi, N., Boutayeb, S., Haddou, R. B., & Errihani, H. (2022). Use of medicinal plants by cancer patients under chemotherapy in the northwest of Morocco (Rabat area): Cross-sectional study. *Journal of evidence-based. Integrative Medicine*, 27, 2515690X2211280. <https://doi.org/10.1177/2515690X221128036>
- Ezez, D., & Tefera, M. (2021). Effects of solvents on total phenolic content and antioxidant activity of ginger extracts. *Journal of Chemistry*, 2021, 1–5. <https://doi.org/10.1155/2021/6635199>
- Fernández, J., Silván, B., Entralgo-Cadierno, R., Villar, C. J., Capasso, R., Uranga, J. A., Lombó, F., & Abalo, R. (2021). Antiproliferative and palliative activity of flavonoids in colorectal cancer. *Biomedicine and Pharmacotherapy*, 143, 112241. <https://doi.org/10.1016/j.biopha.2021.112241>
- Ghasemzadeh, A., Jaafar, H. Z. E., & Rahmat, A. (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* roscoe). *Molecules*, 15(6), 4324–4333. <https://doi.org/10.3390/molecules15064324>
- Khazaei, Z., Jarrahi, A. M., Momenabadi, V., et al. (2019). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide stomach cancers and their relationship with the human development index (HDI). *WCRJ*, 6, e1257. <https://www.wcrj.net/wp-content/uploads/sites/5/2019/04/e1257-Global-cancer-statistics-2018-GLOBOCAN-estimates-of-incidence-and-mortality-worldwide-stomach-cancers-and-their-relationship-with-the-Human-Development-Index-HDI.pdf>
- Krasteva, N., & Georgieva, M. (2022). Promising therapeutic strategies for colorectal cancer treatment based on nanomaterials. *Pharmaceutics*, 14(6), 1–38. <https://doi.org/10.3390/pharmaceutics14061213>
- Kumar, N. V., Murthy, P. S., Manjunatha, J. R., & Bettadaiah, B. K. (2014). Synthesis and quorum sensing inhibitory activity of key phenolic compounds of ginger and their derivatives. *Food Chemistry*, 159, 451–457. <https://doi.org/10.1016/j.foodchem.2014.03.039>
- Lotfi, N., Yousefi, Z., Golabi, M., Khalilian, P., Ghezelbash, B., Montazeri, M., Shams, M. H., Baghbadorani, P. Z., & Eskandari, N. (2023). The potential anticancer effects of quercetin on blood, prostate and lung cancers: An update. *Frontiers in Immunology*, 14, 1077531. <https://doi.org/10.3389/fimmu.2023.1077531>
- Loubna, E. F., Bito, V., Zaid, A., & Alaoui, T. M. (2019). Complimentary herbal treatments used in Meknes-tafilet region (Morocco) to manage cancer. *American Journal of Plant Sciences*, 10(5), 796–812. <https://doi.org/10.4236/ajps.2019.105058>
- Mahomoodally, M. F., Aumeeruddy, M. Z., Rengasamy, K. R. R., Roshan, S., Hammad, S., Pandohee, J., Hu, X., & Zengin, G. (2021). Ginger and its active compounds in cancer therapy: From folk uses to nanotherapeutic applications. *Seminars in Cancer Biology*, 69, 140–149. <https://doi.org/10.1016/j.semcancer.2019.08.009>
- Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Beta, T., & Li, H. B. (2019). Bioactive compounds and bioactivities of ginger (*Zingiber officinale* roscoe). *Food*, 8(6), 185. <https://doi.org/10.3390/foods8060185>
- Marrelli, M. (2021). Medicinal Plants. *Medicinal Plants. Plants.*, 10, 1355. <https://doi.org/10.3390/plants10071355>
- Maulana, T. I., Falah, S., & Andrianto, D. (2019). Total phenolic content, total flavonoid content, and antioxidant activity of water and ethanol extract from Surian (*Toona sinensis*) leaves. *IOP Conf Ser: Earth and Environmental Science*, 299(1), 1–10. <https://doi.org/10.1088/1755-1315/299/1/01202>
- Mian, S. S., Upadhyay, S., & Tajuddin, S. N. N. (2019). Physicochemical analysis of ginger (*Zingiber officinale* Rosc.) rhizome along with its TLC, HPLC and HPTLC profile. *Pharmaceutical Methods*, 10, 31–36. <https://doi.org/10.5530/phm.2019.1.6>
- Nam, D.-G., Kim, M., Choi, A.-J., & Choe, J.-S. (2024). Health benefits of antioxidant bioactive compounds in ginger (*Zingiber officinale*) leaves by network pharmacology analysis combined with experimental validation. *Antioxidants*, 13(6), 652. <https://doi.org/10.3390/antiox13060652>
- Offei-Oknye, R., Patterson, J., Walker, L. T., & Verghese, M. (2015). Processing effects on phytochemical content and antioxidative potential of ginger *Zingiber officinale*. *Food Science and Nutrition*, 6(5), 445–451. <https://doi.org/10.4236/fns.2015.65046>
- Pawar, N., Pai, S., Nimbalkar, M., & Dixit, G. (2011). RP-HPLC analysis of phenolic antioxidant compound 6-gingerol from different ginger cultivars. *Food Chemistry*, 126(3), 1330–1336. <https://doi.org/10.1016/j.foodchem.2010.11.090>
- Shahrajabian, M. H., Sun, W., & Cheng, Q. (2019). Clinical aspects and health benefits of ginger (*Zingiber officinale*) in both traditional Chinese medicine and modern industry. In *Acta Agriculturae*

- Scandinavica, Section B – Soil & Plant Science, 69(6), 546–556. <https://doi.org/10.1080/09064710.2019.1606930>
- Shaukat, M. N., Nazir, A., & Fallico, B. (2023). Ginger bioactives: A comprehensive review of health benefits and potential food applications. *Antioxidants*, 12(11), 2015. <https://doi.org/10.3390/antiox12112015>
- Spence, C. (2023). Ginger: The pungent spice. *International Journal of Gastronomy and Food Science*, 33, 100793. <https://doi.org/10.1016/j.ijgfs.2023.100793>
- Suk, S., Kwon, G. T., Lee, E., Jang, W. J., Yang, H., Kim, J. H., Thimmegowda, N. R., Chung, M., Kwon, J. Y., Yang, S., et al. (2017). Gingerenone a, a polyphenol present in ginger, suppresses obesity and adipose tissue inflammation in high-fat diet-fed mice. *Molecular Nutrition and Food Research*, 61, 1700139. <https://doi.org/10.1002/mnfr.201700139>
- Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2020). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA a Cancer Journal for Clinicians*, 71(3), 209–249. <https://doi.org/10.3322/caac.21660>
- Tambun, R., Alexander, V., & Ginting, Y. (2021). Performance comparison of maceration method, soxhletation method, and microwave-assisted extraction in extracting active compounds from soursop leaves (*Annona muricata*): A review. In *IOP Conference Series: Materials Science and Engineering*, 1122(1), 012095. <https://doi.org/10.1088/1757-899x/1122/1/012095>
- Tanweer, S., Mehmood, T., Zainab, S., Ahmad, Z., & Shehzad, A. (2020). Comparison and HPLC quantification of antioxidant profiling of ginger rhizome, leaves and flower extracts. *Clinical Phytoscience*, 6, 12. <https://doi.org/10.1186/s40816-020-00158-z>
- Wei, C., Tsai, Y., Korinek, M., Hung, P., El-Shazly, M., Cheng, Y., Wu, Y., Hsieh, T., & Chang, F. (2017). 6-Paradol and 6-shogaol, the pungent compounds of ginger, promote glucose utilization in adipocytes and myotubes, and 6-paradol reduces blood glucose in high-fat diet-fed mice. *International Journal of Molecular Sciences*, 18, 168. <https://doi.org/10.3390/ijms18010168>
- Wolfe, K., Wu, X., & Liu, R. H. (2003). Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry*, 51(3), 609–614. <https://doi.org/10.1021/jf020782a>
- Xue, X., Zhao, A., Wang, Y., Ren, H., Du, J., Li, D., & Li, Y. (2021). Composition and content of phenolic acids and flavonoids among the different varieties, development stages, and tissues of Chinese jujube (*Ziziphus jujuba* mill.). *PLoS ONE*, 16(10), e0254058. <https://doi.org/10.1371/journal.pone.0254058>
- Yang, W. J., Zhao, H. P., Yu, Y., Wang, J. H., Guo, L., Liu, J. Y., Pu, J., & Lv, J. (2023). Updates on global epidemiology, risk and prognostic factors of gastric cancer. *World Journal of Gastroenterology*, 29(16), 2452–2468. <https://doi.org/10.3748/wjg.v29.i16.2452>
- Zadorozhna, M., & Mangieri, D. (2021). Mechanisms of chemopreventive and therapeutic proprieties of ginger extracts in cancer. *International Journal of Molecular Sciences*, 22(12), 1–18. <https://doi.org/10.3390/ijms22126599>
- Zagórska, J., Czernicka-Boś, L., Kukula-Koch, W., Iłowiecka, K., & Koch, W. (2023). Impact of thermal processing on the selected biological activities of ginger rhizome—A review. *Molecules*, 28(1), 1–18. <https://doi.org/10.3390/molecules28010412>
- Zhang, M., Viennois, E., Prasad, M., Zhang, Y., Wang, L., Zhang, Z., Han, M. K., Xiao, B., Xu, C., Srinivasan, S., & Merlin, D. (2016). Edible ginger-derived nanoparticles: A novel therapeutic approach for the prevention and treatment of inflammatory bowel disease and colitis-associated cancer. *Biomaterials*, 101, 321–340. <https://doi.org/10.1016/j.biomaterials.2016.06.018>
- Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review. *Chinese Medicine*, 13(20), 1–26. <https://doi.org/10.1186/s13020-018-0177-x>

How to cite this article: Al Azzam, K. M., Al-Areer, N. W., Al Omari, R. H., Al-Deeb, I., Bounoua, N., Negim, E.-S., Al-Samydai, A., Aboalroub, A. A., & Said, R. (2024). Assessment of the anticancer potential of certain phenolic and flavonoid components in ginger capsules using colorectal cancer cell lines coupled with quantitative analysis. *Biomedical Chromatography*, 38(10), e5993. <https://doi.org/10.1002/bmc.5993>