PHARMACIA

Pharmacia 70(4): 905–919
DOI 10.3897/pharmacia.70.e103936

Research Article

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

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Received 23 March 2023 ♦ **Accepted** 1 September 2023 ♦ **Published** 02 October 2023

Citation: Al-Areer NW, Al Azzam KM, Al Omari RH, 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

Abstract

Objectives: To quantify quercetin, gallic acid, rutin, naringin, and caffeic acid in the rhizome of *Zingiber officinale* different extracts in seven different solvents (methanol, ethanol, ethyl acetate, water, dichloromethane, chloroform, and *n*-hexane), for the first time, using HPLC/UV. Also, to study the anticancer activity of *Zingiber officinale* different extracts by evaluating its *in vitro* toxicity on HT-29 colorectal cancer cell line.

Methods: The fresh and dried rhizomes were extracted using Soxhlet (SOX) and maceration (MAC) methods. Separation of compounds was conducted using HPLC. The cell line used for MTT cell proliferation assay antiproliferative; is HT-29 (HTB-38) colorectal adenocarcinoma.

Results: The MTT test indicated that powder ginger extracted by MAC or SOX showed high cytotoxicity activity (IC_{50}<50) against HT-29 cells, except water using SOX, which showed mild cytotoxicity activity. The fresh ginger extracted by MAC using dichloromethane and those extracted by SOX using ethyl acetate showed strong cytotoxicity activity (IC_{50} <50).

Conclusion: The phenolic and flavonoid contents of ginger can vary depending on the different extracts from ginger plant. Also, HPLC results revealed that quercetin was the highest in all extracts.

Keywords

Colorectal cancer, Soxhlet, *Zingiber officinale*, HPLC

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PENSOFT.

Introduction

Medicinal plants are a substantial source of health impact on the human body. Ginger is the most cultivated plant throughout the country because of its distinguished characteristics of the refreshing aroma and pungent taste as well as their essential constituents in worldwide cuisines and as a food additive in cooking (Ezez and Tefera 2021). In China and India Ginger has been used as a medicinal spice. It has been used for medicinal purposes in France and Germany since the ninth century, and in England since the tenth (Munda et al. 2018).

Ginger (*Zingiber officinale*) belongs to the Zingiberaceae family and the Zingiber genus. It is a plant that is extensively used and available all over the world as a culinary and herbal medicine (Mao et al. 2019). A variety of bioactive substances exist in plant parts, some of which include phenolic, flavonoids, and essential oils. It has various biological potency such as antioxidant, anti-inflammatory, anti-bacterial, anti-tumor, anti-aging, and other properties.

Zingiber officinale, particularly the ginger root, has long been used as a substitution herbal medicine to treat several ailments such as headaches, emesis, colds, and nausea. Furthermore, many studies reported that ginger prevents and controls a variety of illnesses, including, cardiovascular diseases, neurodegeneration diseases, obesity, diabetes mellitus, chemotherapy-induced nausea and vomiting, and respiratory illnesses (Mao et al. 2019). Furthermore, the anti-inflammation, antioxidative, anti-carcinogenic, and anti-mutagenic effects of ginger have lately received a lot of attention. Ginger includes several bioactive chemicals, such as gingerols, paradols, and shogaols, which have been associated with considerable flavoring and health advantages (Shao et al. 2010). Moreover, ginger is usually considered a pain killer for arthritis, muscle aches, chest pain, backache, and menstrual pain. In addition, *Zingiber officinale* was reported as an anti- inflammatory agent in curing cough, upper respiratory tract infections, and bronchitis. The bioactive constituent of ginger can be used as a purgative and gastric antacid ailment. Also, it shows a good source of warming effect by boosting circulation and reducing the blood pressure of the body (Prasad and Tyagi 2015).

Cancer poses a major threat to human existence. For the treatment of cancer, a variety of methods have been devised, including surgery, radiotherapy, chemotherapy, and targeted therapy. Because of all these treatments, the rate of malignancy has stabilized in women and has declined in males during the last ten years (2006–2015), and the percentage death of malignant has also declined over the same years (2007–2016) (Yang et al. 2020). Conventional cancer treatments, though, only provide the action on selected kinds of malignant tumors. Metastasis, recurrence, heterogeneity, resistance to chemotherapy as well as radiotherapy, and avoiding immunological detection are the main reasons for cancer treatment failure (Yang et al. 2020). One of the most typical malignancies is colorectal cancer (CRC). It is closely tied to the global mortality rate from cancer and is the fourth biggest cause of malignant tumor-related deaths globally (Huang et al. 2019). Chemotherapy and surgery are the two key components of the current clinical CRC treatment. Finding new and more potent medications for the treatment of CRC is urgently needed, nonetheless, due to the development of side effects and the emergence of drug resistance. Numerous studies have shown that numerous natural products have potent anti-CRC properties and could replace chemotherapeutic medications in the treatment of CRC (Huang et al. 2019).

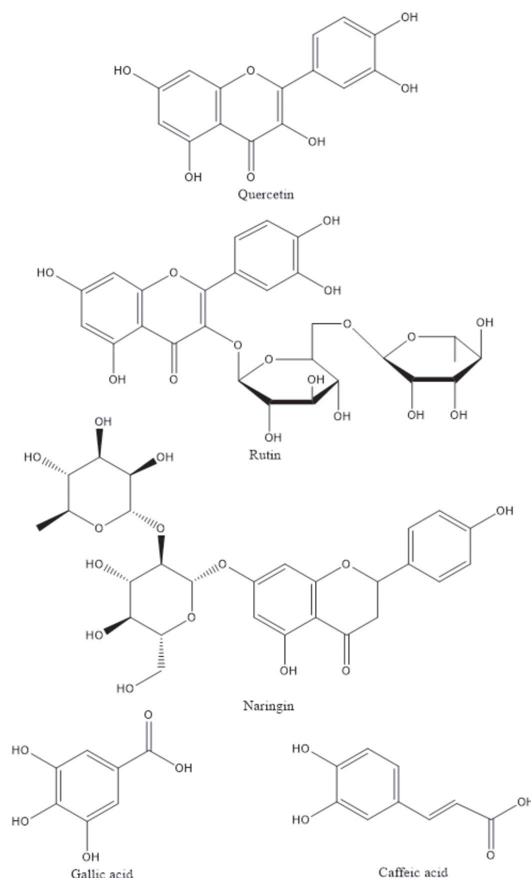

Figure 1. The chemical structures of the components studied in *Zingiber officinale* extracts.

Tanweer et al. carried out a comparison between the content of 6-gingerol in extracts of different parts of *Zingiber officinale* including flowers, leaves, and rhizome using HPLC and the findings proved that a maximum of 6-gingerol was present in ginger leaves (4.9 mg/g) tackled by ginger flowers (2.87 mg/g) and ginger rhizome (1.03 mg/g).

Moreover, Pawar et al., quantified 6-gingerol from different ginger cultivars using Reverse Phase HPLC. The examination was determined by two methods including antioxidant capacity using DPPH and FRAP (ferric-reducing antioxidant power) assays. Ginger rhizomes were collected from various regions of India. Acetonitrile and methanol were utilized in the HPLC analysis. The cultivars with high 6-gingerol content had the strongest free radical scavenging activities and this was also supported by the statistically highly significant correlation between the two.

Rio De Janeiro rhizome cultivars manifested a good origin for gingerol and its derivatives among twelve different ginger cultivars (Pawar et al. 2011). In addition, for the antioxidant assay both extracts showed a high level of inhibitory activity with free radical scavenging activity, findings also reported a high and significant in *vitro* antioxidant activity in all tests performed, which are all coherent with several other research, of the radical scavenging test (Pawar et al. 2011). The results discovered were consistent with those reported by Ghasemzadeh et al. who found a powerful action in scavenging the methanolic extracts in various parts of two kinds of *Z. officinal* (Pawar et al. 2011).

According to the findings revealed in the study of Ezez and Tefera, The type and polarity of the solvents used have a crucial impact on the quantity and quality of phenols extraction. High-polarity solvents are responsible for extracting the highest quantities of phenols. First of all, methanol showed greater extraction capacity for phenolics from the ginger rhizome (1183.813 mg GAE/100 g extract dry weight). Followed by ethanol extract of Ayikel specimen (1009.917 mg GAE/100 g extract dry weight). Similarly, the methanol extract for the Mandura specimen showed high phenolic content (1022.409 mg GAE/100 g extract dry weight), while the ethanol extract exhibited only 941.847 mg GAE/100 g extract dry weight. Therefore, compared to other solvents, methanol was the most effective at extracting phenols from *Zingiber officinale*. Moreover, regarding the antioxidant effects of ginger. High antioxidant activity is seen in methanolic, ethanolic, ethyl acetate, and acetone extracts, respectively (Ezez and Tefera 2021).

In Fernández et al. three CRC cell lines (Colorectal carcinoma cell line (ATCC CCL- 247) (HCT116), Human Colorectal Adenocarcinoma Cell Line (ATCC HTB-38) (HT-29) and Transplantable human carcinoma cell line derived from lung metastasis of colon carcinoma (T84)) were used to investigate five flavonoids for their potential as anticancer medications. These cell lines show three distinct tumor phases, including one that is metastatic. Even better than the therapeutic medicine 5-fluorouracil (5-FU), Xanthohumol demonstrated the strongest anticancer activity on the three cancer cell lines. Apigenin and luteolin, on the other hand, demonstrated relatively weaker anticancer activity on these cancer cell lines but, in the case of HCT116, exhibited a synergistic effect when combined with 5-FU, which may be of clinical significance Fernández et al. 2021).

Additionally, a review of the literature revealed that these flavonoids exhibit highly intriguing palliative effects on clinical symptoms including diarrhea, mucositis, neuropathic pain, and others frequently connected to the chemotherapy treatment of CRC. By simultaneously reducing significant 5-FU chemotherapy side effects and amplifying the anticancer effects of the drug, flavonoids may offer a dual benefit for combination therapy (Fernández et al. 2021).

To assess the anticancer effects of ethanolic Ginger Extract (GE) on HCT-116 colon cells and colorectal cancers brought on by dimethylhydrazine (DMH), Abdel-Rasol et al. used MTT assay to quantify the antiproliferative activity, and quantitative reverse transcription polymerase chain reaction (q-RTPCR) was used to evaluate the gene expression. Rats were randomly assigned to one of five groups for the antitumor study; control, group two received 300 mg/kg of GE orally for 21 weeks, group three received an s/c injection of DMH for 9 weeks, and groups four and five received DMH and then received cisplatin (2.5 mg/kg) or GE, respectively, for 21 weeks (Abdel-Rasol et al. 2022). Results showed that GE had an IC_{50} of 12.5 g/mL, therefore, GE demonstrated significant antiproliferative action by activating both intrinsic and extrinsic apoptotic pathways.

In terms of its ameliorative benefits, GE was superior to cisplatin and was able to reverse all of DMH's prior negative effects without causing the hepatotoxicity or nephrotoxicity that were observed in the group receiving DMH and cisplatin (Abdel-Rasol et al. 2022). The findings of this study demonstrated that GE has a greater anticancer effect against DMH-induced-CRC than cisplatin. In addition to being less harmful than cisplatin, GE did not cause hepatotoxicity or nephrotoxicity. GE possesses carcinostatic action and induces apoptosis (Abdel-Rasol et al. 2022).

Mahomoodally et al. discovered that certain colon cancer cell lines, including the cells isolated from the large intestine of a male Dukes C colorectal cancer patient (HCT15), Colorectal carcinoma cell line HCT116 (ATCC CCL-247), and Human Colorectal Adenocarcinoma Cell Line HT29 (ATCC HTB-38) cells, are cytotoxic to ginger and its bioactive derivatives. In colorectal cancer cells, 6-gingerol was found to induce apoptosis by enhancing the regulation of NAG-1 and suppressing the G1 cell cycle which is identified as the primary phase of the four phases in the cell cycle, through the down regulation of cyclin D1 that plays an important role in the regulation of proliferation. The activity of 6-gingerol appears to be mediated by several processes, including protein breakdown. Through the suppression of MAPK/AP-1 signaling, 6-gingerol also promoted caspase-dependent apoptosis and stopped phorbol myristate acetate-induced growth in colon cancer cells. In addition, it was shown that the Bcl-2 family may function as a key regulator since 6-shogaol triggered apoptosis largely through the mitochondrial pathway (Abubakar and Haque 2020).

The current research aims to separate and quantify the following compounds: quercetin, gallic acid, rutin, naringin, and caffeic acid (Fig. 1) that might be present in rhizome of *Zingiber officinale* different extracts using seven different solvents namely, ethanol, methanol, ethyl acetate, water, dichloromethane, chloroform and *n*-hexane using maceration and Soxhlet methods. The total phenolic compounds and total flavonoid compounds will be quantitatively determined. Additionally, each compound of quercetin, gallic acid, rutin, naringin, and caffeic acid will be quantitatively determined in a single-run analysis using HPLC/UV detector. Lastly, to study the anticancer activity of *Zingiber officinale* different extracts (28 extracts) by evaluating its *in vitro* toxicity on HT-29 colorectal cancer cell line.

Materials and methods

Plant material

Rhizomes of fresh *Zingiber officinale* and crude ginger extracts were purchased from a local market and herbalist in Jordan.

Chemicals and reagents

Gallic acid, quercetin, caffeic acid, rutin, and naringin hydrate were purchased from Geochem World (Mumbai, India). Reagents Include sodium nitrate ($NaNO_2$), and aluminum chloride anhydrous ($AlCl_3$) from Alpha Chemika (Mumbai, India). Sodium hydroxide pellets (NaOH) were supplied by Loba Chemie, (Milan, Italy), and the Folin-Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). The solvents ethanol, methanol, ethyl acetate, dichloromethane, and *n*-hexane were purchased from Carlo Erba Reagents (Milan, Italy), and chloroform from Alpha Chemika (Mumbai, India). All reagents are of analytical reagent grade.

Equipment and instruments

Digital balances (ADAM equipment PGW 453i, P.R China) were used for weighing purposes before and after the extraction of ginger different extracts. Buchi Rotavapor R-300 (Flawil, Switzerland) was used for both methods; maceration, and Soxhlet extraction methods. UV-visible spectrophotometer UV-1800 (Shimadzu, Japan) was used for the determination of absorbance, HPLC/UV, Stirrer, Sonicator, as well as water bath (Thermo Scientific) for the determination of TPC, and SYKAM.

Preparation of plant extracts

Fresh *Zingiber officinale* were washed thoroughly to remove any trace amount of soil, then cut into pieces before grinding it in a blender. After that, stored in the refrigerator at -4 °C until required for extraction. The crude powdered ginger extract was bought from a local herbalist for the further extraction process. In this study, we use two methods for the extraction which are maceration and Soxhlet extraction methods using seven different solvents (ethanol, methanol, ethyl acetate, water, chloroform, dichloromethane, and *n*-hexane). After obtaining the extracts, the resulting solution was concentrated using a rotary evaporator (rota-vap) under the required conditions for each solvent at a certain vacuum pressure and temperature. Finally, the obtained crude extract was dried, weighed, and stored at -4 °C until use.

Extraction methods

It's well known that extraction is the primary step for the separation of the required natural extracts from raw materials. The plant extraction method is to prepare the rhizome of *Zingiber officinale* different extracts and different crude extracts of ginger using the Soxhlet and maceration extraction methods. In brief, the maceration extraction method was conducted by weighing 100 g and then soaked in 1 L of each solvent separately (ethanol, methanol, ethyl acetate, water, chloroform, dichloromethane, and *n*-hexane) in a container covered with aluminum foil for 72 hours with occasional shaking at room temperature (RT) in a dark condition. After that, the whole mixture was filtered in a conical flask and vaporized in a rota-vap, then dried and weighed for the final sample preparation (Abubakar and Haque 2020).

Soxhlet extraction method which is considered a high-efficiency automatic continuous extraction technology that takes less time and uses less solvent than other methods (Zhang et al. 2018). In brief, 40 g of fresh rhizome of *Zingiber officinale* was placed into the thimble and 200 mL (1:5) of the solvent to be used for the extraction was placed in the round bottom flask before fixing the thimble into the Soxhlet apparatus, then the heat is applied depending on the solvent extract. The temperature was adjusted to 90 °C for 9 hours of extraction for the solvents with agitation utilizing the magnetic stirrer at 400 rounds per minute (rpm). Lastly, the extraction is collected in the round bottom flask and applied into the rota-vap to remove any remaining solvent from the extraction. The same protocol was performed for the crude ginger extract with 30 g of crude ginger extract and 150 mL of solvents (1:5).

Optimization of the extraction methods

Two techniques, Maceration (MAC) and Soxhlet (SOX) apparatus were utilized to extract the active ingredients from fresh and powdered *Zingiber officinale* utilizing seven solvents including (ethanol, methanol, water, ethyl acetate, chloroform, dichloromethane, and *n*-hexane). Therefore, to select the best extraction yield of *Zingiber officinale* using different solvents, the following equation was used:

% Extractive Value of Solvent = (Weight of the extract / Weigh of the sample) × 100

Determination of total phenolic content

Total phenol content (TPC) was measured using the Folin-Ciocalteu method by Wolfe et al. First, a dilution for each plant extract was done (10 mg/mL) with its corresponding solvent. Then, 4 mL of 7.5% sodium carbonate was added, mixed for 15 seconds in a vortex, and allowed to develop its blue color in the water bath at 40 °C for 30 minutes. Finally, calorimetric analysis at 765 nm was used to determine the phenolic concentration. The TPC was expressed as a gallic acid equivalent (GAE) (mg/g) using equation based on the calibration curve.

Determination of total flavonoid content

The amount of total flavonoids was determined using the Saima et al. method. In brief, 10 mL volumetric flask, 0.1 mg/mL plant material extracts from each plant were di-

luted with 4 mL of water. Each volumetric flask was first filled with a 5% $NaNO_2$ solution of 0.3 mL for five minutes, followed by 10% $AlCl_3$ (w/w) for six minutes. Next, the addition of 2 mL of NaOH (1.0 M) to the volumetric flask and 2.4 mL of distilled water (DW) was followed, then thoroughly mixed in the reaction flask. At 430 nm, the reaction mixture's absorbance was measured. The results were calculated in mg quercetin/g dry weight (Ghasemzadeh et al. 2010).

HPLC analysis

HPLC chromatographic system and conditions

The HPLC analysis was done using the procedure established by Xue et al. with some modifications. HPLC analysis of ginger different extracts, along with reference compounds was performed using a gradient HPLC system named; Prominence-i LC-2030C plus HPLC system consisting of a solvent delivery system pump, DGU-20A degasser, SIL- 20A auto-sampler, UV-VIS Plus detector and a CBM-20A communication bus module (All from Shimadzu, Kyoto, Japan).

The signals were captured using LC-solution version 1.25 (2009–2010) workstation (Shimadzu, Japan) operating under Microsoft Windows XP, and the software used was Chrom Quest software 4.2.34. Chromatographic separation was achieved on EC HPLC column (analytical), NUCLEODUR 100-5 C18 ec, 5 µm, 250 mm × 4.6 mm (MACHEREY-NAGEL, Germany) column at 40 °C using an optimized mobile phase consisting of methanol as mobile phase A, and 0.2% formic acid aqueous solution as mobile phase B and using the gradient method for elution. The column temperature was maintained at 40 °C, the injected volume was 4 µL, and the flow rate was 1.0 mL/min. HPLC ultraviolet detector was used for detection using the wavelength of 283 nm and an automatic sampler.

Standard solution preparation

A 15.0 mg of each standard (gallic acid, caffeic acid, naringin, rutin, and quercetin) was weighed using an electronic analytical balance, dissolved in methanol in a 25 mL volumetric flask, and stored at 4 °C for later use. Furthermore, the standards solution was diluted to different concentrations to produce standard curves ((gallic acid (0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 100, 200, and 400 µg/mL)), caffeic acid (0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 100, 200, and 400 µg/mL)), naringin (0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 100, 200, and 400 µg/mL)), rutin (1, 5, 10, 25, 50, and 100 µg/mL)), and quercetin (0.05, 0.1, 0.5, 1, 5, 10, 25, 50, 100, 200, and 400 µg/mL))). The detection was carried out under the same chromatographic conditions as previously described.

Sample solution preparation

20.0 mg of each extract (maceration or Soxhlet) was weighed using an electronic analytical balance, dissolved in methanol in a 10 mL volumetric flask, sonicated, filtered using a syringe filter (0.2 µm), and then injected into the HPLC system. It was stored at 4 °C in the refrigerator for not more than two days.

Cytotoxicity assay

Cell culture

The cell line used in our research is HT-29 (HTB-38) colorectal adenocarcinoma; which was obtained from the American Type Culture Collection (ATCC; USA) and preserved according to the instructions of the ATCC.

MTT cell proliferation assay antiproliferative

This colorimetric test, following Mossman 1983 method with modifications, is based on the reduction of 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) dye, a yellow tetrazole, to purple formazan in living viable cells, a process that occurs in the mitochondria of viable cells only and not is the cells targeted by certain treatments or drugs proposed to terminate cancer cells.

Part of the trypsinized cells were counted to be used for MTT assay using an automated cell counter (QuadCount, Accuris Instruments, USA). Trypan blue dye was used to stain a sample of a known volume of the suspended cells using a 1:1 ratio before being counted using the automated counter.

In summary, 5000 cells/100 µL were seeded into each well of a coated flat bottom 96-well microplate and incubated for 24 hours to allow full adherence of cells to the surface. Later, gradual incremental concentrations of the 28 previously prepared fresh and powdered ginger extracts prepared from the main stock (2000µg/ 1 mL) were added to each well with varying concentrations (200, 100, and 50) µg/mL.

Cells were treated with 200 µL of each ginger extract in triplicates. After 48 hours of incubation, 20 µL of MTT reagent (Genochem World, Spain) was added to each well and the plate was incubated at 37 °C for 4 hours. MTT concentration was prepared by dissolving 5mg MTT powder in 1 mL PBS. At last, media and MTT were aspirated and discarded, and 150 µL DMSO was added to each well in order to dissolve the formed formazan crystals. The absorbance of the dissolved crystals in DMSO was recorded 60 minutes later at 590 nm wavelength using a 96-well microplate reader. As blank, triplicate wells without cells neither any treatments were set. As a control, triplicate wells seeded with cells were treated with complete growth media and 0.5% DMSO, which is the same DMSO concentration used in ginger extracts-treated wells. MTT cell proliferation assay results were analyzed using Microsoft Excel to obtain what so-called "Inhibitory concentration (IC_{50})" which is the drug concentration value at which 50% of cells are viable after drug treatment when compared to non-treated control cells. First, the average of the absorbance readings of the blank wells was subtracted from the average of the absorbance readings relevant to each concentration. Survival of cells at each point of concentration

was calculated by dividing the average of the absorbance for drug-treated wells by the average of the absorbance of the control wells, multiplied by 100%. The concentrations vs. Survival % values were plotted using Microsoft Excel, and the IC_{50} value is the concentration at which 50% of cells are viable.

Results and discussion

Optimization of the extraction

In the experiment, we investigated the ideal 9 hours of extraction in SOX apparatus, the extraction of active substances from ginger was higher than MAC for 72 hours for both fresh and powdered ginger. Additionally, SOX extraction time is shorter than the MAC technique which gives us a positive response in reducing the time consumption in the preparation process. The benefit of this extraction technique is to shorten the extraction time and achieve high active ingredients (Figs 2, 3).

According to the results, the Soxhlet method is the best technique for isolating active ingredients from fresh ginger rhizomes and ginger powder extract by using different solvents. In addition, the results of fresh ginger shown in Fig. 2 for the MAC method in the ethanolic extract is low compared to the SOX method, and other shows some conflicting results, this could be an indication of the presence of water molecules in fresh ginger so this factor could affect on the extraction yield of the active substance in ginger. For instance, dichloromethane, chloroform, and *n*-hexane reveal significant variations, as demonstrated in Fig. 2, with MAC yielding more than SOX.

The results in powdered ginger extract in Fig. 3 show that the maceration method exhibits minimal extraction of the active ingredient, except for dichloromethane solvent the results are in the range of 3.3 in MAC and 3.2 in SOX. In this study, we used both types of ginger fresh and powdered, the high active components are achieved in powdered ginger rather than fresh ginger.

According to the results in Table 1, SOX shows the best extraction technique rather than MAC when compared

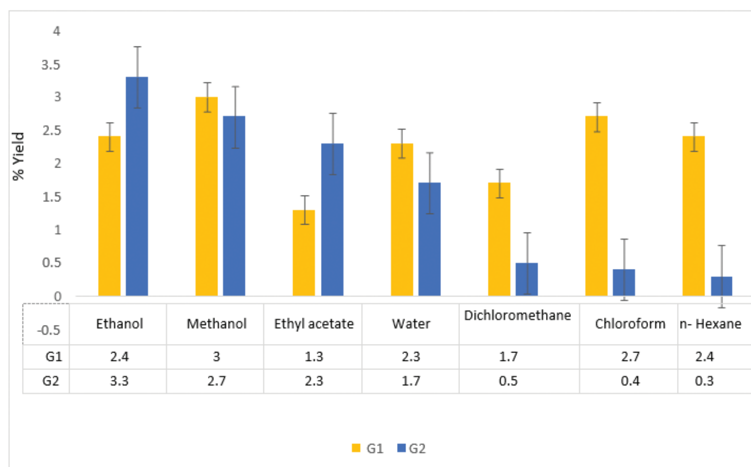

Figure 2. % Yield of extracts obtained from fresh ginger rhizome using different solvents and methods of extraction, G1: Maceration, G2: Soxhlet.

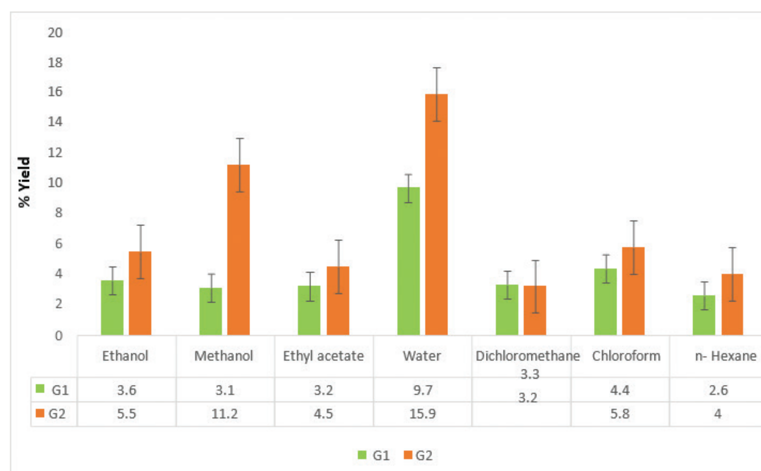

Figure 3. % Yield of extracts obtained from ginger powder using different solvents and methods of extraction, G1: Maceration, G2: Soxhlet.

Table 1. The extraction yield (%) for different solvents in fresh and powdered *Zingiber officinale* extracts. The extraction yield of each solvent was obtained. MAC: maceration, SOX: Soxhlet.

Solvent	MAC (extraction yield %)		SOX (extraction yield %)	
	Fresh extract of *Zingiber officinale*	Powdered extract of *Zingiber officinale*	Fresh extract of *Zingiber officinale*	Powdered extract of *Zingiber officinale*
Ethanol	2.4	3.6	3.3	5.5
Methanol	3.0	3.1	2.7	11.2
ethyl acetate	1.3	3.2	2.3	4.5
Water	2.3	9.7	1.7	15.9
Dichloromethane	1.7	3.3	0.5	3.2
Chloroform	2.7	4.4	0.4	5.8
n-hexane	2.4	2.6	0.3	4.0

with the time consumption. Moreover, the privilege of the usage of SOX apparatus is that it offers the best extraction yield for both types of fresh and powder ginger according to the result obtained.

According to Arawande et al., solvents are a measure of the potential extractive rate that could be obtained from a given sample; high extractive solvents are estimated to be effective in extracting bioactive compounds, while low extractive solvent results are estimated to have less impact on the identification of various bioactive compounds.

As a result, we have used seven solvents for the two types of ginger rhizome; fresh and powdered. Additionally, we used two extraction techniques (MAC and SOX), each of which has a unique extractive value. As demonstrated in Tables 1, 2, we can conclude from this experiment that SOX delivers the greatest outcome for ginger extraction for both types.

Comparison research of extraction yield by different extraction methods by Nourbakhsh Amiria et al., including MAC, SOX, subcritical water extraction (SWE), and further methods were utilized. The best yield was in SOX than MAC, and the operation time was 8 hours instead of 14 hours for the MAC method. In this study, a greater extraction yield for fresh ginger was obtained in methanol than chloroform in MAC, while in SOX the highest yield was developed in ethanol followed by methanol (Table 1). For powdered ginger in the MAC technique, the best result was shown in water followed by chloroform, as well as in the SOX technique also water then methanol (Table 2), respectively.

Determination of total phenolic content (TPC)

Phenolics have been acknowledged as a necessary component of a daily diet. In addition, their antioxidant properties because of their useful capacity to improve health (Tanweer et al. 2020). The calibration curve of gallic acid was constructed by mixing 500 mg in 50 mL of methanol as a stock solution with a concentration of 10 mg/mL, The stock solution was serially diluted (25 to 250 mg/mL). Based on the calibration curve, TPC was expressed as a gallic acid equivalent (GAE) (mg/g) using the following equation $y = 0.0026x + 0.0317$, $R^2 = 0.9974$, where Y is the absorbance and X axis is the concentration.

In each working test tube, 0.15 mL of plant solution was mixed with 5 mL Folin-Ciocalteu 10% reagent diluted with DW 1:10 v/v, and 4 mL of 7.5% sodium carbonate for 15 seconds in a vortex mixer, in the water bath at 40 °C for 30 minutes until color turns to blue. Then, the phenolic contents were determined calorimetrically at 765 nm. TPC for each extract was determined as GAE according to the linear regression equation obtained from the calibration curve of standard gallic acid.

Based on the research that has been done, the highest TPC for both types of *Zingiber officinal* fresh and powdered extraction are shown in Table 2, where by MAC starting from the fresh ginger was ethyl acetate, followed by *n*-hexane. As a result, in powdered ginger the highest phenolic content was found in ethanol, followed by methanol. Moreover, the highest TPC by SOX beginning with the fresh ginger was ethyl acetate followed by methanol. In addition, in powdered ginger the highest TPC was found in ethyl acetate-tracked methanol.

The findings of the current investigation are shown in Table 2. In fresh ginger the content varied within the range of 20 to 252.08 mg of GAE values in the MAC and SOX method, the highest TPC was found in ethyl acetate for the MAC and SOX were 252.08 and 131.6 mg/g in mean GAE values. Followed by *n*-hexane in the MAC technique where the value was 103.6 mg/g.

On the other hand, Ezez and Tefera studied a comparison result of solvents used to extract the ginger rhizome. In addition, the outcomes show that the high polarity solvents yielded extracts with the largest concentration of phenolic compounds, while methanol extract demonstrated a stronger ability to extract phenolic compounds from the ginger rhizome.

In the research work, a new trend was discovered, which indicates that fresh ginger has the second-highest TPC for low-polarity *n*-hexane. In the SOX technique, the second-highest phenolic content was in methanol the mean GAE values were 128.3 mg/g, respectively. Table 3 lists more results.

In powdered ginger among the solvents used, the highest TPC was found in ethanol using the MAC technique, with a value of 142.91 mg/g. Additionally, for the SOX technique, the highest TPC was discovered in ethyl acetate at a value of 110.83 mg/g in mean GAE values. Therefore, different intrinsic and extrinsic factors, including culti-

Table 2. Determination of total phenolic content (TPC) mg/g for fresh and powdered ginger extracts using two methods maceration: MAC and Soxhlet: SOX. TPC was expressed as a gallic acid equivalent: GAE (mg/g).

Solvent	TPC using MAC (mg/g)		TPC using SOX (mg/g)	
	Fresh ginger	Powdered ginger	Fresh ginger	Powdered ginger
Ethanol	42.08	142.91	40.41	96.67
Methanol	71.25	110.41	128.3	99.58
Water	37.91	25.0	20.0	22.5
Ethyl acetate	252.08	60.41	131.6	110.83
Chloroform	37.5	41.66	73.33	11.67
Dichloromethane	80.83	35.0	69.16	46.67
n-hexane	103.6	50.83	69.17	90.0

Table 3. Determination of total flavonoid content (TFC) mg/g for fresh and powdered ginger using two methods maceration: MAC and Soxhlet: SOX. TFC was expressed as a mg quercetin/g.

Solvent	TFC using MAC (mg/g)		TFC using SOX (mg/g)	
	Fresh ginger	Powdered ginger	Fresh ginger	Powdered ginger
Ethanol	42.2	700	100.0	513.3
Methanol	96.67	440	52.2	296.67
Water	26.67	30	10.0	25.67
Ethyl acetate	507.78	504.4	406.67	546.67
Chloroform	398.89	153.3	194.4	512.2
Dichloromethane	530.0	164.4	227.78	530
n-hexane	428.89	496.6	174.4	451.1

vars, type of soil and growing circumstances, maturation state, and harvest conditions, may be responsible for the variation in the amount of TPC (Ezez and Tefera 2021).

In Figs 4, 5 a summarized chart for the two types of *Zingiber officinale*, MAC is a higher result than SOX in two sources of ginger fresh and powdered with some exceptional solvents. As well as a literature review reported that the highest polarity of the selected solvent manifested a greater power to extract phenolics from ginger (Ezez and Tefera 2021). In this experiment, we have obtained a high value of *n*-hexane in fresh ginger, as we know that *n*-hexane has a low polarity. In addition, other literature data Eberle et al., studied two different extraction techniques MAC and SOX using dry and fresh ginger for ethanol solvent and the result was high in SOX rather than MAC, unlike our recent study of ethanol for fresh and powdered ginger were higher in MAC with a value of 42.08 and 142.91 mg/g.

Polyphenolic compounds are known to have antioxidant activity, and the extracts' activity is likely related to these compounds. This activity is thought to be mostly owing to their redox characteristics, which are significant in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, and degrading peroxides (Ghasemzadeh et al. 2010). Antioxidants like polyphenols are indeed present in high concentrations in many medicinal plants. Quercetin, for example, has anticancer properties and has been demonstrated in prior research to be able to stop the growth of cancer cells (Ghasemzadeh et al. 2010). Gallic acid has been shown to act as a free radical scavenger, stimulate differentiation, and trigger apoptosis in leukemia, lung cancer, and colon adenocarcinoma, as well as in healthy lymphocyte cells (Sohi et al. 2003). Gallic acid, like quercetin, is thought to be crucial in preventing cancer development and malignant transformation. Therefore, the findings of this study demonstrated that flavonoids are significant parts of this plant, and some of its pharmacological actions could be linked to the presence of these valuable constituents. These results are supported by the high cytotoxicity activity (IC_{50}<50 μg/mL) against HT-29 colorectal adenocarcinoma obtained (Table 9). These findings are in line with the literature, where Shailah et al. demonstrated that HT 29 CRC cells were more susceptible to the anticancer activity of the dried ginger extract, with an IC_{50} (455 μg/mL) significantly lower than HCT 116 cells (496 μg/mL).

Determination of total flavonoid content (TFC)

One of the substances that fall within the category of phenolic or polyphenol compounds is flavonoid compounds. Of all polyphenols, flavonoids have undergone the greatest research. This group consists of basic structure of two aromatic rings that are connected by three carbon atoms to create an oxygenated heterocycle (Maulana et al. 2019).

About 800 mg of quercetin was dissolved in 100 mL of ethanol. After that, serial dilution of concentration from 15 to 700 mg/mL was applied. The UV-visible device was used to measure the absorbance at 430 nm and the calibration curve was plotted using standard quercetin. The TFC of fresh and powdered ginger was calculated as mg of quercetin equivalents/mL using the following calibration curve equation $y = 0.0009x + 0.023$, $R^2 = 0.9949$, where Y is the absorbance and X axis is the concentration.

The flavonoid contents of *Zingiber officinale* extracts were quantified as a starting point. 1 mL of solvent extracts was diluted with 4 mL of DW and added to a 10 mL volumetric

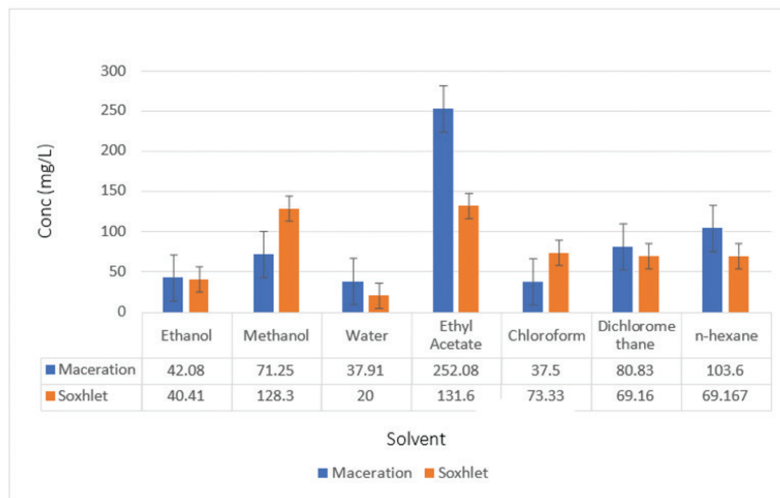

Figure 4. Total Phenol Content (TPC) of fresh ginger in maceration and Soxhlet method for different solvents using GAE as a mean.

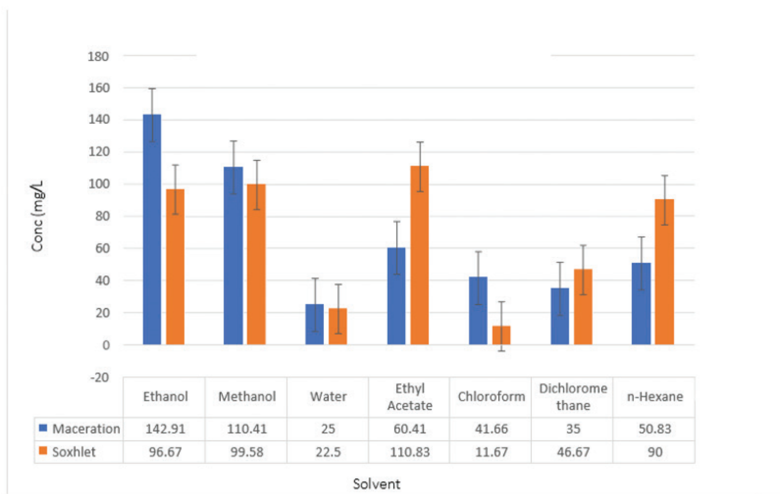

Figure 5. Total Phenol Content (TPC) of powder ginger in maceration and Soxhlet method for different solvents using GAE as a mean.

flask. 0.3 mL of 5% $NaNO_2$ solution was added for 5 minutes, followed by 10% $AlCl_3$ (w/w) for six-minute and 1.0 M NaOH (2 mL). Then, 2.4 mL of DW was added, and the absorbance of the reaction mixture was evaluated at 430 nm. By comparing the outcomes to the Quercetin standard curve, the results were given in mg quercetin/mL (Ghasemzadeh et al. 2010). The data were plotted as a chart scale for the two types of ginger fresh and powder in Figs 6, 7.

According to the research, TFC has shown various data trends compared to TPC. In fresh ginger, the results were from 530 to 26.67 mg/g Quercetin in the MAC method, while in SOX were by 406.67 to 10 mg/g, respectively. Dichloromethane and ethyl acetate had the highest TFC of *Zingiber officinale* extraction in the MAC technique for fresh ginger the results are explained in Table 3. As well as, in SOX the ethyl acetate and dichloromethane had the highest content. In this work, we indicate that the content could be affected by the polarity of the solvent used as discussed in previous literature data, as shown in Table 3.

When we used the MAC approach, the results of ginger powder ranged from 700 to 30 mg/g Quercetin, while those obtained from the SOX method ranged from 546.67 to 25.67 mg/g. Ethanol in MAC and ethyl acetate had the greatest levels of total flavonoid, respectively. On the other hand, ethyl acetate and dichloromethane had the greatest levels of flavonoid content in SOX the same result as fresh ginger. The advantages of employing the two extraction methods include learning the best method to utilize for extraction. In Table 3 the data are shown for the best solvents among other solvents for the TFC in powdered ginger.

Ethanol results from MAC and SOX were (700 and 513.3 mg/g), ethyl acetate (504.4 and 546.67 mg/g), and dichloromethane was (164.4 and 530 mg/g) so the MAC technique had manifested a greater result in ethanol, while for ethyl acetate the results were approximately close to the range. In addition, dichloromethane shows a crucial effect on the content of flavonoids in the SOX technique that offers a triple-fold increase in flavonoid content than

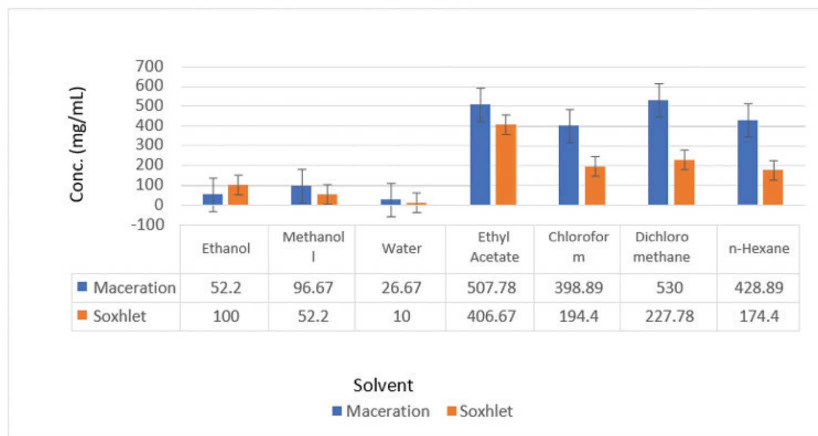

Figure 6. Total Flavonoid Content (TFC) of fresh ginger in maceration and Soxhlet method for different solvents using quercetin as a mean.

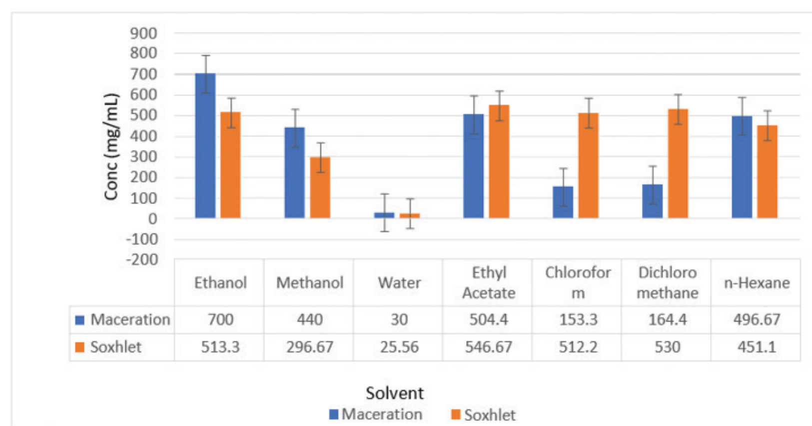

Figure 7. Total Flavonoid Content (TFC) of powder ginger in maceration and Soxhlet methods for different solvents using quercetin as a mean.

MAC (Figs 6, 7). Further experimental work may be needed, but in comparison with the method, both were good to find TFC in plants extracted using these two solvents which also suggests a good polarity.

HPLC analysis

Calibration curves

By comparing retention times with standards and UV absorbance ratios after co-injection of samples and standards, it was possible to identify the five compounds present in the extracts. Using the standard calibration curves, the amounts of gallic acid, caffeic acid, naringin, rutin, and quercetin were determined. The calibration curves of gallic acid, caffeic acid, naringin, rutin, and quercetin were constructed (x-axis represents concentration while the y-axis represents peak area) with the correlation coefficients (R^2) 0.9997, 0.9998, 0.9998, 0.9988 and 0.9978 for gallic acid, caffeic acid, naringin, rutin, and quercetin, respectively and regression equations; $y = 12256x - 18765$, $y = 15381x - 14938$, $y = 7750.7x - 7473.5$, $y = 2058.2x - 1842.3$, and $y = 5055x - 46671$ for gallic acid, caffeic acid, naringin, rutin, and quercetin, respectively.

The Linearity range, equations, correlation coefficients (R^2), slope, intercept, limit of detection (LOD), and limit of quantification (LOQ) for each were also recorded (Table 4) which indicated that the proposed method exhibits a good sensitivity for the simultaneous quantification of the above compounds. The method of validation was carried out following the ICH's guidelines for linearity range, LOD, and LOQ (ICH 2005). Based on the standard deviation of the response (SD) and the slope (S) of the calibration curves, the determination of LOD and LOQ was performed using the following formulas: LOD=3.3(SD/S) and LOQ=10(SD/S), respectively.

HPLC analysis of selected flavonoids and phenolic compounds

For separation, the Reversed-Phase High-Performance Liquid Chromatography (RP- HPLC) method was employed. The separation temperature, mobile phase composition, and analytical column were the main focuses of the optimization process. The C18 analytical column produced positive results and showed good stability over a broad pH range. Consequently, we decided to use NUCLEODUR 100-5 C18 ec, 5 µm, 250 mm × 4.6 mm (MACHEREY-NAGEL, Germany). It was not feasible to

Table 4. 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 (μg/mL)	0.05–400	0.05–400	0.05–400	1.0–100	0.05–400
Regression equation	y = 12256x - 18765	y = 15381x - 14938	y = 7750.7x - 7473.5	y = 2058.2x - 1842.3	y = 5055x - 46671
Correlation coefficient (R^2)	0.9997	0.9998	0.9998	0.9988	0.9978
Slope	12256	15381	7750.7	2058.2	5055
Intercept	18765	14938	7473.5	1842.3	46671
LOD (μg/mL)	0.006	0.005	0.009	0.034	0.014
LOQ (μg/mL)	0.018	0.014	0.028	0.10	0.043

separate mixed standards that eluted close to each other (close retention time); however, upon using longer columns (250 mm), the analysis time was adequate. The standards for phenolic and flavonoids produced retention times (RTs) that were consistent with the polarity of the compounds for phenolic and flavonoids (Mradu et al. 2012). During the analytical process, a combination of methanol and 0.2% formic acid solution was utilized as the mobile phase in gradient mode.

The stationary phase of the chromatographic column stationary phase would be harmed if the formic acid concentration was too high. As a result, the gradient elution method outlined in the work of Xue et al. was utilized to separate the target compounds using 0.2% formic acid and methanol as the mobile. The temperature was fixed at 40 °C, and the flow rate was kept constant at 1 mL/min with an injection volume of 4 μL. All the compounds were separated under these conditions in 14.5 minutes with good resolution between the peaks of the matrix and the analyte.

The temperature of the chromatographic column was varied between 25 and 45 °C as part of a temperature optimization to better understand how temperature affects the separation. As column temperature varied, it was seen that there was a difference in retention time and signal-to- noise ratio. As anticipated, retention time somewhat decreased as the temperature of the chromatographic column rose. As can be seen from the standard mixture injection in Fig. 8B, the best results were obtained at a temperature of 40 °C where the peaks were baseline separated and the signal-to-noise ratio was lower.

Different sample preparation techniques may be necessary depending on the plant matrix, which comes at a price in terms of money and time spent on the sample. Majors found that the majority (around 61%) of the analysis was spent on sample preparation. The straightforward sample preparation techniques used in this investigation may be significantly related to the results thus obtained.

RP-HPLC analysis of selected phenolic acid and flavonoids is present in Tables 5–8. A representative chromatogram of blank injection is shown in Fig. 8A. On the other hand, a representative injection from the sample extract is also shown in Fig. 8C and thus it can be concluded that no interferences are observed indicating the selectivity of the current method. Based on the data obtained, the concentration of the common flavonoid's quercetin was the highest in all extracts used whether using maceration or the Soxhlet method (Tables 5–8).

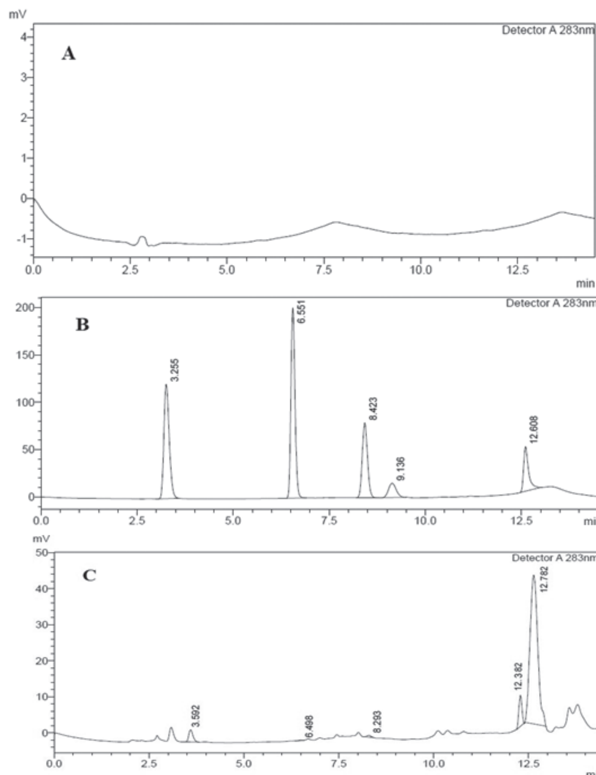

Figure 8. Chromatograms of (**A**): blank injection, (**B**): standard phenolic and flavonoid compounds mixture (100 μg/mL), and (**C**): sample injection of fresh ginger extract using ethyl acetate solvent by SOX method using HPLC separation. Gallic acid, caffeic acid, naringin, rutin, and quercetin using HPLC. Peak identification based on the standard injection: Gallic acid (RT: 3.255 min); caffeic acid; (RT: 6.551 min); naringin (RT: 8.423 min); rutin (RT: 9.136 min); and quercetin (RT: 12.608 min).

Additionally, the lowest concentration among the selected compounds examined was naringin using the maceration extraction method and, in most cases, it was not detected. The lowest concentration among the selected compounds examined was rutin using Soxhlet extraction method, and in most cases, it was not detected. In brief, the HPLC analysis of the flavonoid components in fresh or powdered ginger revealed that quercetin had the highest concentration of all the extracts, whether they were made using the Soxhlet or Maceration, regardless of the type of solvent used. Quercetin was then followed by gallic acid, caffeic acid, rutin, and finally naringin. Quercetin was the highest in fresh and powder ginger extracts using the Soxhlet method compared with the other compounds.

Table 5. HPLC analysis of phenolic and flavonoid compounds extracted from fresh ginger by different solvents using the maceration method. Results are expressed in μg/mL.

Components	Methanol	Ethanol	Ethyl acetate	Water	Dichloromethane	Chloroform	*n*-hexane
Gallic acid	< LOD	2.36±0.005	1.73±0.076	1.78±0.042	ND	ND	ND
Caffeic acid	< LOD	0.99±0.026	1.16±0.043	1.08±0.012	ND	ND	ND
Naringin	ND	1.08±0.014	ND	ND	ND	ND	ND
Rutin	< LOD	1.16±0.091	4.18±0.078	14.52±0.032	ND	ND	ND
Quercetin	71.15±1.22	62.92±1.22	9.72±0.33	12.73±0.027	9.84±0.003	10.66±0.043	ND

All analyses are the mean value of triplicate measurements ± standard deviation. ND: Not detected.

Table 6. HPLC analysis of phenolic and flavonoid compounds extracted from powder ginger by different solvents using the maceration method. Results are expressed in μg/mL.

Components	Methanol	Ethanol	Ethyl acetate	Water	Dichloromethane	Chloroform	*n*-hexane
Gallic acid	1.64±0.76	1.76±0.055	1.29±0.087	1.61±0.019	ND	1.79±0.154	ND
Caffeic acid	1.15±0.221	1.03±0.093	ND	1.05±0.33	1.01±0.042	0.99±0.032	1.00±0.87
Naringin	ND	ND	ND	ND	ND	ND	1.98±0.027
Rutin	1.95±0.056	1.82±0.004	1.06±0.076	1.49±0.75	ND	1.27±0.076	ND
Quercetin	9.66±0.054	9.97±0.003	11.06±0.065	11.96±0.10	12.16±0.009	11.25±0.43	ND

All analyses are the mean value of triplicate measurements ± standard deviation. ND: Not detected.

Table 7. HPLC analysis of phenolic and flavonoid compounds extracted from fresh ginger by different solvents using the Soxhlet method. Results are expressed in μg/mL.

Components	Methanol	Ethanol	Ethyl acetate	Water	Dichloromethane	Chloroform	*n*-hexane
Gallic acid	2.02±0.087	1.64±0.93	3.64±0.096	1.62±0.008	ND	1.53±0.87	ND
Caffeic acid	1.00±0.098	0.99±0.65	1.13±0.066	0.92±0.054	ND	0.99±0.043	ND
Naringin	1.06±0.088	1.05±0.013	1.47±0.032	1.06±0.11	1.16 ± 0.96	1.10±0.86	ND
Rutin	ND	ND	ND	1.21±0.001	ND	ND	ND
Quercetin	18.86±0.054	9.59±0.051	15.62±0.007	10.20±0.053	9.43 ± 0.05	10.58±0.34	11.53±0.65

All analyses are the mean value of triplicate measurements ± standard deviation. ND: Not detected.

Table 8. HPLC analysis of phenolic and flavonoid compounds extracted from powder ginger by different solvents using the Soxhlet method. Results are expressed in μg/mL.

Components	Methanol	Ethanol	Ethyl acetate	Water	Dichloromethane	Chloroform	*n*-hexane
Gallic acid	1.56 ±0.006	1.57 ±0.054	1.65 ± 0.76	2.45 ± 0.028	2.58 ± 0.006	2.54 ± 0.032	ND
Caffeic acid	1.11 ± 0.006	1.05 ± 0.22	0.92 ± 0.005	1.10 ± 0.11	1.01 ± 0.003	1.04 ± 0.007	ND
Naringin	1.22 ±0.054	1.33 ± 0.11	1.34 ±0.096	1.20 ± 0.72	1.00 ± 0.077	ND	0.11 ± 0.10
Rutin	ND	1.21 ± 0.12	ND	ND	ND	1.13 ± 0.15	1.12 ± 0.043
Quercetin	9.30 ± 0.028	10.06 ± 0.076	9.67 ± 0.054	11.82 ± 0.66	9.61 ± 0.009	9.45 ± 0.053	15.83 ± 0.032

All analyses are the mean value of triplicate measurements ± standard deviation. ND: Not detected.

Tables 5 and 6 showed that in all cases quercetin was the highest among all the compounds analyzed. It has been reported that depending on the subunit location and chain length, quercetin derivatives' anticancer effectiveness varies from quercetin. Quercetin and its different derivatives had different anticancer effects than quercetin. Inserting a phenolic hydroxyl group, such as by etherification (*O*-alkylation), can substantially decrease cancer cell proliferation (Lotfi et al. 2023). In addition, several nanoparticles, including chemotherapeutic drugs like mitoxantrone (MTX) and adriamycin (ADR), were added to the surface of the quercetin. The synergistic anticancer effects of this modified quercetin may be increased while chemotherapeutic drug adverse effects and drug resistance may be decreased (Saha et al. 2016).

Naringin is a flavonoid that is thought to have a beneficial impact on human health as an anti-inflammatory, free radical scavenger, antioxidant, and activator of glucose metabolism. It was found to have an inhibitory impact on carcinogens and is the main flavanone in ginger. Naringin's concentration in ginger was low, ranging from 0.11 to 1.47 μg/mL, even though little information has been acquired about it. Naringin concentration in ginger was affected whether it is powder or fresh or using maceration or Soxhlet method of extraction. Generally, fresh ginger had a higher concentration than powdered ginger.

Some of the most crucial functional elements in ginger include phenolic acids and flavonoids. As a result, we comprehensively assessed the composition and concentration of a few selected phenolic acids and flavonoids in fresh and

powdered ginger different extracts. By choosing 5 standards based on their availability in our laboratory, we did an extensive examination of the potential phenolic acids and flavonoids in ginger. The extract samples also had unknown peaks, but due to their small peak areas, they were not included in the analysis of the principal phenolic acids and flavonoids. Mass spectrometry should be used in the future to ascertain the nature of this unidentified chemical.

MTT cell proliferation assay antiproliferative

The MTT assay is a method used to assess cellular metabolic activity which in terms to indicates cell viability, proliferation, and cytotoxicity (Fig. 9). The concentrations vs. Survival% values were plotted, and the IC_{50} value for the 28 ginger extracts was measured. Results were summarized (Table 9).

All 7 powdered ginger extracts extracted by maceration showed high cytotoxicity activity (IC_{50}<50 µg/mL) against HT-29 colorectal adenocarcinoma. Similarly, all powdered Ginger extracted by Soxhlet showed high cytotoxicity activity, except those extracted using water, which showed mild cytotoxicity activity (IC_{50} 100–200 µg/mL).

In contrast, fresh ginger extracted by maceration, using methanol or *n*-hexane showed a lack of cytotoxicity activity. Only fresh extract using dichloromethane showed high activity. While extracts of ethanol, water, ethyl acetate, and chloroform showed mild cytotoxic activity.

Furthermore, fresh ginger extracted by Soxhlet using ethanol, methanol, and *n*-hexane solvents showed no cytotoxic activity against HT-29 colorectal adenocarcinoma (IC_{50}>200 µg/mL). On the other hand, ethyl acetate extracts of fresh ginger showed strong cytotoxicity activity (IC_{50}<50 µg/mL). While fresh extracted by water, chloroform, and dichloromethane showed mild cytotoxic activity.

These results are consistent with the literature, where Shailah et al. showed that HT 29 CRC cells were more vulnerable to the anticancer activity of dried ginger extract with an IC_{50} (455 µg/mL) much less than HCT 116 cells (496 µg/mL). The cytotoxic activity is probably due to the presence of gingerol which is known to inhibit the growth of HCT 116 CRC cells and liver HepG2 cancer cells. Similarly, Al-Tamimi et al. found that ginger exhibited the lowest IC_{50} among other studied plants, with 40 µL/mL on mucus-secreting HT-29 and 60 µL/mL on non- mucus-secreting HT-29 cells. In this work, the anticancer efficacy of *Zingiber officinale* extract shows a clinically significant, according to the outcomes at a safe dose that is clinically promising to the patients with minimal side effects.

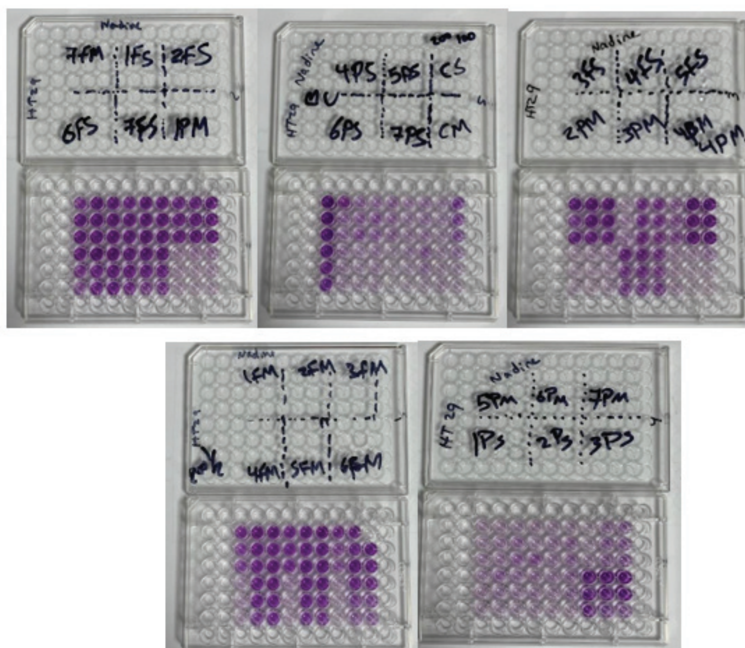

Figure 9. MTT cell proliferation assay Antiproliferative.1FM: Ethanol in fresh ginger MAC,2FM: Methanol in fresh ginger MAC, 3FM: Water in fresh ginger MAC, 4FM: Ethyl acetate in fresh ginger MAC, 5FM: Chloroform in fresh ginger MAC, 6FM: Dichloromethane in fresh ginger MAC, 7FM: *n*-hexane in fresh ginger MAC. 1PM: Ethanol in powder ginger MAC, 2PM: Methanol in powder ginger MAC, 3PM: Water in powder ginger MAC, 4PM: Ethyl acetate in powder ginger MAC, 5PM: Chloroform in powder ginger MAC, 6PM: Dichloromethane in powder ginger MAC, 7PM: *n*- hexane in powder ginger MAC. 1FS: Ethanol in fresh ginger SOX, 2FS: Methanol in fresh ginger SOX, 3FS: Water in fresh ginger SOX, 4FS: Ethyl acetate in fresh ginger SOX, 5FS: Chloroform in fresh ginger SOX, 6FS: Dichloromethane in fresh ginger SOX, 7FS: *n*- hexane in fresh ginger SOX. 1PS: Ethanol in powder ginger SOX, 2PS: Methanol in powder ginger SOX, 3PS: Water in powder ginger SOX, 4PS: Ethyl acetate in powder ginger SOX, 5PS: Chloroform in powder ginger SOX, 6PS: Dichloromethane in powder ginger SOX, 7PS: *n*- hexane in powder ginger SOX.

Table 9. MTT cell proliferation assay results, % cells survival and IC_{50} of different ginger extracts.

Solvent	% Survival in powdered Ginger/ MAC different concentrations			IC_{50} (µg/mL)	% Survival in powdered Ginger/ SOX different concentrations			IC_{50} (µg/mL)
	200 (µg/mL)	100 (µg/mL)	50 (µg/mL)		200 (µg/mL)	100 (µg/mL)	50 (µg/mL)	
Ethanol	11.97	12.42	15.48	< 50	11.83	14.19	13.08	< 50
Methanol	11.2	11.23	12.14	< 50	12.56	11.06	13.01	< 50
Water	34.33	38.43	34.68	< 50	45.91	51.34	64.59	124.68
Ethyl acetate	12.07	11.01	10.9	< 50	17.43	22.85	21.85	< 50
Chloroform	11.51	12.24	15.58	< 50	17.39	13.25	14.89	< 50
Dichloromethane	10.7	12.07	18	< 50	12.52	10.82	11.93	< 50
n-hexane	8.87	18.52	20.52	< 50	7.10	10.37	19.25	< 50
Solvent	**% Survival in fresh Ginger/ MAC different concentrations**			**IC_{50} (µg/mL)**	**% Survival in fresh Ginger/ SOX different concentrations**			**IC_{50} (µg/mL)**
	200 (µg/mL)	**100 (µg/mL)**	**50 (µg/mL)**		**200 (µg/mL)**	**100 (µg/mL)**	**50 (µg/mL)**	
Ethanol	42.50	70.16	77.11	172.89	62.75	72.21	67.13	> 200
Methanol	59.62	78.50	76.85	> 200	52.63	55.90	65.88	> 200
Water	37.46	64.52	74.46	153.66	49.46	62.75	66.75	195.94
Ethyl acetate	12.90	52.90	61.95	107.25	12.42	42.09	38.43	< 50
Chloroform	10.89	58.54	57.01	117.42	24.63	68.07	78.40	141.60
Dichloromethane	9.60	37.88	36.70	< 50	44.49	62.92	69.25	170.10
n-hexane	56.19	70.17	73.15	> 200	56.80	53.46	56.90	> 200

Conclusions and future recommendations

With a run time of less than 14.5 minutes, the HPLC analysis method was able to successfully separate and quantify the five phenolic and flavonoid compounds standards (gallic acid, caffeic acid, rutin, naringin, and quercetin) and their extract. This study showed that the phenolic and flavonoid contents of ginger can vary depending on and different extracts from the ginger plant (fresh or powder). Significant differences between fresh and powdered ginger were found in the HPLC results, as well as in differences based on the method of extraction utilized. Finally, the suggested method/procedure can be used to identify specific phenols quickly and accurately in other plant extracts without the need for additional sample preparation, providing crucial knowledge about these significant chemicals with potential health advantages.

Future recommendation is to determine the components of phenolics and flavonoids that might regulate plant growth, more investigation is necessary. Future investigations should focus on this by examining the effects of various polyphenols alone or in combination on particular plant processes. Additionally, the determination of common compounds present in ginger such as gingerols, paradols, and shogaols could be conducted.

With a focus on CRC, the current review highlights the cytotoxicity activity of ginger. In this work, we evaluated the anticancer effect against CRC lines in different extracts from ginger plants (fresh or powder), and they were effective against HT-29. But crude powder extracts showed greater cytotoxic activity against HT-29 with (IC_{50}<50 µg/mL), therefore, it might be considered a promising anticancer agent for use in CRC treatment. Further cytotoxicity studies are needed to determine their effect and to confirm the precise safe dose for patient compliance.

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