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Chemical constituents and biological activities of different extracts from ginger plant (*Zingiber officinale*)

Emad A. Shalaby[1], Sanaa M. M. Shanab[2], Rehab M. Hafez[2] and Abeer E. El-Ansary[1*]

Abstract

Background Ginger is widely used in traditional food and folk medicine. It was known to contain monoterpenoids, sesquiterpenoids, phenolic compounds and its derivatives as well as aldehydes, ketones, alcohols, and esters. The current work aims to evaluate the antioxidant and anticancer activities of different extracts from ginger peels and residues.

Methods Ginger samples were collected from local market and extracted using water and ethanol solvent. Four different antioxidant assays were used (DPPH, ABTS, potassium permanganate and methylene blue), the anticancer activity was determined using HepG2 cell line and the active ingredients for the promising extract were identified using HPLC/UV.

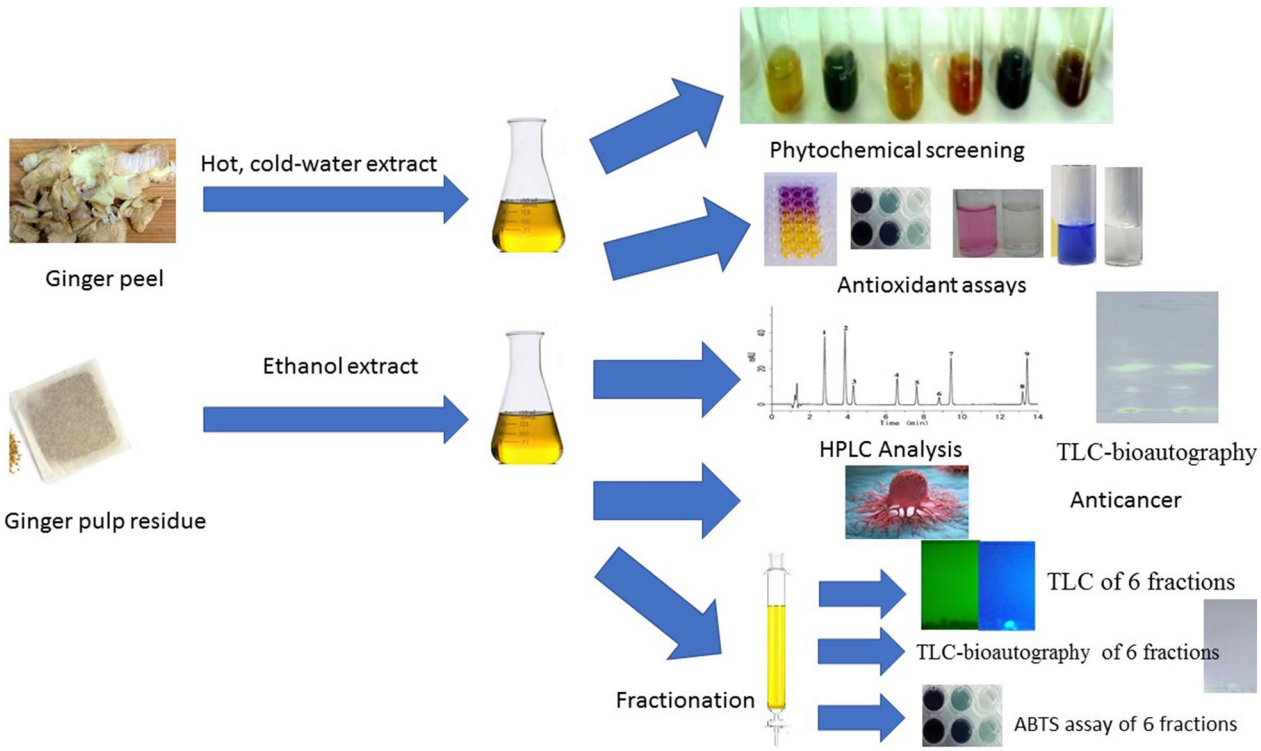
Results The obtained results recorded that hot water extract of ginger peels was the promising extract exhibiting promising antioxidant activity. Fractionation of this promising extract was achieved by silica gel column chromatography with petroleum ether/ethyl acetate as mobile phase. Six fractions were produced. Thin layer chromatography ($TLCF_{254}$) was used for separation of active compounds and bioautography confirmed their antioxidant efficiency. Higher antioxidant activity and cytotoxicity against HepG2 cell line was recorded by fraction No. 4. Cold water extract of ginger peels exhibited comparatively higher antioxidant efficiency while both aqueous peel extracts showed antibacterial efficiency against four Gram-positive and Gram-negative bacterial strains using well diffusion assay.

Conclusion Ginger peels hot water extract is the promising extract as antioxidant, antiradical, antibacterial and anticancer.

Keywords Ginger, Extracts, Antioxidant activities, Antibacterial activities, Anticancer activity, Phytochemical screening, HPLC

*Correspondence:
Abeer E. El-Ansary
abeeransary@agr.cu.edu.eg
Full list of author information is available at the end of the article

Graphical Abstract



Background

Ginger [*Zingiber officinale* (L.) Rosc.] is the rhizome of monocotyledonous perennial plant widely used to flavor food and in folk medicine in several countries [1]. It was known to show numerous biological activities as antipyretic, antidiabetic, analgesic, anti-helmintic, antiviral, antioxidant, anti-inflammatory and anticancer activities [2–6].

Different chromatographic analysis is used to detect and determine the volatile compounds of low molecular weight in ginger [as GC/MS, GC-FID, LC/MS, HPLC] as well as monoterpenoids and sesquiterpenoids (as β-bisabolene, α–zingiberene). Gingerols are the main compound in ginger to which attributed its acridity. Gingerols are converted by heat to shogaols that make the characteristic spicy-sweet fragrance of ginger. Polar compounds in ginger can be detected by liquid chromatography (LC) [7].

The antimicrobial effect of ginger essential oils extracts and oleoresins is principally depends on their chemical composition, the solvent and method used for extraction, the procedure to which ginger is submitted [8]. Phenolic compounds as eugenol, zingerone, shogaols, gingerols, etc., and their synergistic relations with compounds like zingiberene, α- and β-bisabolene α-farnesene, are mainly in charge for the antimicrobial efficiency of ginger [6].

Most of these compounds are insoluble in water, so aqueous extracts displayed lesser activity compared to essential oils, oleoresins and organic extracts [9]. The high concentration of essential oil eugenol displayed higher antimicrobial activity.

It has been described that ginger has antioxidant [10–12], antiviral [2] antimicrobial [13, 14], and anticancer [5, 15, 16] properties.

The present research aimed to study the antioxidant, antibacterial, and anticancer activities of different ginger extracts (aqueous extract of cold and hot water as well as ethanol extract) of both ginger pulp and peel and to determine the active constituents to which attributed these activities.

Materials and methods

Plant material

Ginger [*Zingiber officinale* (L.) Rosc.] was commercially collected from the local market. Peels were separated from it before use.

Experimental research and field studies on plants (either cultivated or wild), including the collection of plant material, comply with relevant institutional, national, and international guidelines and legislation.

Chemicals and solvents

Doxorubicin (DOX), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (ethylbenzthiazoline-6-sulfonic acid ($ABTS^{+}$), ascorbic acid, L-glutamine, gallic acid and quercetin were acquired from Sigma-Aldrich (St. Louis, MO, USA), while dimethyl sulfoxide (DMSO) was purchased from PubChem Co. (Darmstadt, Germany).

Cell lines

Human hepatocellular cancer cell line (HepG-2) was obtained and propagated in Vacsera (Giza, Egypt).

Bacterial strains

Gram-negative bacteria [*Escherichia coli* (ATCC 25922) and *Salmonella* sp (ATCC 14028)] and Gram-positive bacteria [*Bacillus cereus* (ATCC 33018) and *Staphylococcus aureus* (ATCC 25923)] were used as tested strains for antibacterial activities.

Sample extractions

1. *Cold water* extraction: 150 g of air-dried ginger peels were ground and extracted three times with distilled water at room temperature.
2. *Hot water extraction*: 150 g of air-dried ginger peels were ground and extracted three times with hot water at 90 °C for 30 min.
3. *Pulp* extraction: Twenty-five grams of air-dried grind ginger pulp was extracted three times with 75 ml of ethanol (70%) at room temperature.

All extracts were filtered using filter paper and each was kept apart in brown bottles until use.

Phytochemical screening of ginger peels

Detection of alkaloids

Wagner's and iodine tests were managed using the method of Shaikh and Patil [17]. Wagner's test was accomplished by adding 2 drops of Wagner's reagent to 3 ml of the extract solution along the sides of the test tube. The positive result showed brown/reddish precipitate.

Iodine test was carried on by adding 5 drops of iodine solution to 3 mL of extract solution in a test tube. Positive result revealed blue color, which disappears on boiling and reappears on cooling.

Detection of phenolic compounds

Ferric chloride test was attained according to the protocol of Shaikh and Patil [17]. Water extract solution was added to a few drops of 5% ferric chloride solution and positive result showed as a dark green/bluish-black color.

Detection of flavonoids

Four tests were conducted to detect the existence of Flavonoids according to Shaikh and Patil [17].

Alkaline reagent test was carried out by adding 1 mL extract to 2 mL of 2% NaOH solution with a few drops diluted HCl. The positive result looked like an intense yellow color, which turns colorless with the addition of diluted acid.

Lead acetate test was made by mixing 1 mL extract with a few drops of 10% lead acetate solution. Yellow precipitate will be a positive result.

Ginger peels extract was added to a mixture of 5 mL diluted ammonia solution and concentrated H_2SO_4 in the test tube for ammonia test. Yellow color appeared as a positive result concentrated H_2SO_4 test was conducted by adding concentrated H_2SO_4 to the plant extract. The positive result appeared as an orange color.

Detection of carbohydrates

Test for starch Three milliliters of water extract were added to 5 mL (5%) of KOH solution in the test tube. Positive result displayed canary coloration [17].

Detection of reducing sugars

Benedict's test was attained by adding equal amount of Benedict's reagent to ginger peels extract in the test tube and then boiled for 2 min. Yellow, red or green colors will exhibit as positive result [17].

Detection of glycosides

Modified Borntrager's and concentrated sulfuric acid tests were accomplished according to the method of Shaikh and Patil [17]. Borntrager's test was completed by mixing ginger peels extract with ferric chloride solution in the test tube and boiled for 5 min, cooled and then an equal volume of benzene was added. The benzene layer is separated and finally, ammonia solution was added. The positive result showed rose pink to blood-red colored solution.

Concentrated H_2SO_4 test was carried out by adding five ml of plant extract to a test tube filled with two mL glacial acetic acid, drop of 5% $FeCl_3$, and concentrated H_2SO_4. The positive result displayed the formation of brown ring.

Detection of tannins

NaOH test was used to detect tannins by the method of Shaikh and Patil [17]. 4 mL of plant extract was added to 40 mL 10% NaOH and then shaken well. Emulsion is formed indicating positive result.

Detection of proteins and amino acids

Biuret and ninhydrin tests were done according to Shaikh & Patil [17].

Biuret test was achieved by adding 2 mL filtrate to a mixture containing one drop of 2% copper sulfate solution, 1 mL of 95% ethanol, and KOH pellets in the test tube. The positive result is a pink-colored solution in the ethanolic layer.

Ninhydrin test was carried out by mixing 2 mL filtrate with 2 drops of ninhydrin solution (10 mg ninhydrin dissolved in 200 mL acetone) in the test tube and then heated. Positive result was a purple-colored solution.

Phenolic compounds in extracts were identified using high-performance liquid chromatography/UV (HPLC)

Identification and determination of plant extract phenolics were accomplished by Agilent 1260 Infinity HPLC Series, equipped with Quaternary pump, the column used: a Kinetex 5 µm EVO C18 100 × 4.6 mm, operated at 30 °C. The best separation was obtained with the following gradient: at 0 min, 5% B; at 20 min, 10% B; at 50 min, 30% B; at 55 min, 50% B; at 60 min, 100% B; at 100 min, 50% B and 50% C; at 110 min, 100% C until 120 min. The solvent flow rate was 1 ml/min and separation was performed at 35 °C. The injected volume was 20 µl. Detection: VWD detector set at 284 nm.

Thin layer chromatography (TLC)

This method was achieved according to El-fayoumy et al. [18] The active compounds were separated from the hot aqueous extract of ginger peels using precoated silica gel plates (TLC F_{254}) and benzene:acetone (9:1 v/v) as mobile phase.

Antioxidant activity using bioautography

The 2,2diphenyl-1-picryl hydrazyl radical (DPPH) was used as a spray reagent in a rapid TLC screening method for antioxidant activity. TLC done on both ginger peel extracts (hot water and cold water). The sheet was left to dry, and antioxidant activity was determined by spraying TLC plates with 0.2% of DPPH in methanol. The incidence of an antioxidant compound was qualitatively demonstrated by the appearance of yellow or white spots on a purple background according to the method of Gaber et al. [19].

Fractionation of plant extract by column chromatography

This strategy was performed for purifying chemicals based on their hydrophobicity or polarity and then determining each fraction's activity. The chromatographic column (40 cm length, 2.5 cm diameter) was packed with 150 g silica gel (60–120 mesh for column chromatography) using petroleum ether as solvent. Five grams of crude hot water extract of ginger peels were ground very well with silica gel powder and then placed on the top of the packed column. The column was then sequentially eluted with 100% petroleum ether and increased the polarity with ethyl acetate solvent, the polarity increased by 15% between each mobile phase mixture (Table 1).

Determination of total phenolic compounds

Phenolic compounds were determined using the technique described by Singleton and Rossi [21]. 100 µl of the extract was mixed with 750 µl of Folin–Ciocalteu reagent (10%). After 3 min, 750 µl of saturated sodium carbonate solution (6%) was added to the mixture. The reaction mixture was kept in the dark for 90 min. The absorbance was measured at 725 nm using a spectrophotometer. Phenolic compounds were calculated based on the standard curve of gallic acid.

Determination of total flavonoids

Flavonoids were determined using the method defined by Zhishen et al. [22]. 125 µl of the extract was mixed with 75 µl of $NaNO_2$ (5%) and was incubated for 6 min. Then 150 µl $AlCl_3$ (10%, w/v) was added to the mixture. After 5 min, 750 µl NaOH (1 M) was added to the mixture and then incubated in the dark for 15 min. The solution was mixed well, and the absorbance was measured spectrophotometrically against a blank at 510 nm. Quercetin

Table 1 Fractions of the promising ginger peels hot water extract by column chromatography, using petroleum ether and ethyl acetate as a mobile phase mixture

Solvent/sample no.	Fractions number					
	1	2	3	4	5	6
Petroleum ether	100	80	60	40	20	0
Ethyl acetate	0	20	40	60	80	100

served as the standard compound for the preparation of the calibration curve.

Antioxidant activity methods

DPPH, ABTS, methylene blue, and $KMnO_4$ methods were used to assess the free radical scavenging ability of various antioxidant agents.

DPPH radical assay

The antioxidant activity of plant extract was determined according to Burits and Bucar [23]. One milliliter of the extract was mixed with 1 mL of a DPPH solution (0.03% w/v in methanol). After an incubation of 30 min of reaction at room temperature in a dark place, the absorbance of the solution was measured at 517 nm (using a spectrophotometer). Control was prepared by the same technique without extract. Ascorbic acid (100 ppm) was used as a natural antioxidant standard. Radical scavenging activity (%) was calculated from the following equation:

$$\text{Scavenging activity (\%)} = [(\text{A control} - \text{A sample}) / \text{A control}] \times 100.$$

ABTS radical assay

The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) [ABTS] assay was done as described by Re et al. [20]. The radical prepared by mixing equal volume (1/1, v/v) from ABTS (7 mM) and potassium persulfate and the mixture was kept in the dark at room temperature from 4 to 16 h until the reaction was accomplished and the absorption was stable. After incubation, The ABTS solution was diluted with distilled water to an absorbance of 0.700 ± 0.05 at 734 nm. Assessment has been made by mixing 0.9 ml of ABTS solution with 0.1 ml of extract or column fractions and mixing for 45 s. The absorbance was assessed after incubation for 1 min. Calculate the decrease of absorption by the equation mentioned by Gaber et al. [19]:

Activity (%) $= [(Ac - At)/Ac] \times 100$ in comparison with ascorbic acid as natural antioxidant standard (100 ppm), where Ac is the absorption of ABTS and At is the absorption of extract.

$KMnO_4$ as non-radical assay

The scavenging effects of crude extract were investigated following the method of Gaber et al. [19], where 1.0 mL of 0.02 M $KMnO_4$ solution (in methanol) was added to a test tube containing 1.0 ml of extract. The mixture was vortexed for 1 min and reserved at room temperature for 30 min in the dark. The absorbance of all the sample solutions and ascorbic acid as natural antioxidant standard was measured at 514 nm. The percentage of scavenging activity (%) was calculated as the following:

$$\text{Antioxidantactivity (\%)} = (\text{control} - \text{sample}) \times 100/\text{control}.$$

The antioxidant activities of the samples were in comparison with ascorbic acid as a natural antioxidant standard (100 ppm) and $KMnO_4$ solution as control (0.02 M).

Methylene blue as non-radical assay

The scavenging effects of crude extract were evaluated according to the method described by Gaber et al. [19].

One ml of 0.002 M methylene blue solution (in water) was added to a test tube containing 1.0 ml of sample. The mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark. The absorbance of all the samples and ascorbic acid as natural standard were measured at 663 nm.

The percentage of scavenging activity (%) was calculated as the following: antioxidant activity (%) = (control − sample) × 100 /control, where methylene blue solution (0.002 M) is considered as control.

Effect of extract pH on antioxidant activity

One ml from the promising extract was exposed to different pH degrees, then, the antioxidant activity was determined before and after exposure.

Antibacterial activity by well diffusion assay

The antibacterial activities of different extracts were evaluated by the well diffusion method against *Bacillus cereus* (ATCC 33,018), *Escherichia coli* (ATCC 25922), *Salmonella* sp (ATCC 14028), and *Staphylococcus aureus* (ATCC 25923). The melted agar medium (1.5% agar) was inoculated with 10% (v/v) of the bacterial culture broth. Wells were made in the agar plate and 100 µL of the extract or standard drug was added into the respective wells. After incubation for 24 h at 37 °C, zones of inhibition were measured. Growth inhibition was recorded positive in the presence of a detectable clear zone (mm) around the wells [24].

Anticancer activity

Cell culture

A human hepatocellular cancer cell line (HepG-2) was acquired from Vacsera (Giza, Egypt) in 1, 200, 000 cells in quantity, and it was ethically approved to work with it. Cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 100 µg/mL streptomycin, 100 units/mL penicillin, and 10% heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO_2 atmosphere at 37 °C [25].

Cytotoxicity assays

The cytotoxicity of crude extracts tested against HepG-2 cells by SRB-assay as described by Skehan et al. [26]. Exponentially growing cells were collected using 0.25% Trypsin–EDTA and plated in 96-well plates at 1000–2000 cells/well. Cells were exposed to each test extract for 72 h and subsequently fixed with TCA (10%) for 1 h at 4 °C. After many times of washing, cells were exposed to 0.4% SRB {sulforhodamine B (SRB), 2-(3-diethylamino-6-diethylazaniumylidene-xanthen-9-yl)-5-sulfobenzene-sulfonate} solution for 10 min in a dark place and then washed with 1% glacial acetic acid. After drying overnight, Tris–HCl was used to dissolve the SRB-stained cells and color intensity was measured at 540 nm [25].

Antiradical activity

The reduction of ABTS can thus be monitored by measuring the decrease in its absorbance at 734 nm during the reaction according to the method described by Brand-Williams et al. [27]. The antiradical activity (AU515) was calculated according to the equation: AU734 = (A0–A1) – (A0K–A1K), where AU734 is the antiradical activity of the extract, A0 the absorbance of the sample at the beginning of the reaction (0 min), A1 the absorbance of the sample after incubation times (20–320 s) of the reaction, A0K the absorbance of the control sample at the beginning of the reaction, and A1K the absorbance of the control sample after incubation times (20–320 s) of the reaction.

Statistical analysis

Data were exposed to an analysis of variance and the means were compared using the least significant difference (LSD) test at 0.05 and 0.01 levels using SPSS version 22.0 computer program [28].

Results and discussion

The current study aims to measure and demonstrate multiple biological activities of the ginger pulp and peels. Hence, several tests and assays were used to measure different phytochemical compounds then evaluation of the antioxidant activity using several methods for the pulp and peels extracts using DPPH, ABTS, methylene blue, and $KMnO_4$, assays. Moreover, HPLC was used for separation and identification of the phenolic compounds and flavonoids in hot water extract of ginger peels and then column chromatography technique was applied on promising extract (hot water extract) to purify chemicals based on their polarity and then determining each fraction's activity using TLC and bioautography. Additionally, total contents of phenolic and flavonoid compounds were determined. TLC was used for both extracts of ginger peels and then antioxidant activity by bioautography was performed. The effect of pH was investigated on the antioxidant activity of the promising ginger extract. Antibacterial, anticancer and antioxidant activities were measured for ginger peels extracts. In addition, antiradical activity for ginger peels and pulp extracts was performed. Based on the previous published articles it was known that ginger is always used in medications, herbs and spices, and its peels are usually discarded. Hence, the present study aimed to determine the phytochemical compounds such as phenols and flavonoids content and evaluate the biological activities of different ginger peels and pulp extracts such as antioxidant; anticancer, antiradical, and antibacterial activity. Also, column chromatography was used for separation of six fractions from the promising ginger peel extract.

Phytochemical screening of ginger peels extracts

The preliminary qualitative screening for phytochemicals of ginger peels (cold and hot) extracts revealed the occurrence of secondary metabolites such as flavonoids, phenols, tannins, carbohydrates, reducing sugars, and glycosides in both extracts (Table 2).

Table 2 Phytochemical screening of both extracts from ginger peels

Test name	Ginger peels	
	Cold water extract	Hot water extract
Alkaloids		
Wagner's	ND	+
Iodine	ND	+
Phenolics and flavonoids		
Alkaline reagent	+	+
Lead acetate	+	+
Ammonia	+	+
Conc. H_2SO_4	+	+
Carbohydrates		
Starch	+	+
Reducing sugar		
Benedict	+	+
Glycosides		
Conc. H_2SO_4	+	+
Modified Borntrager test	+	+
Tannins		
10% NaOH	+	+
Proteins and amino acids		
Biuret	ND	ND
Ninhydrin	ND	ND

+ present, *ND* not detected

Phytochemical screening was measured and determined for the active compounds which have pivotal activities in aqueous extracts of ginger peels using some qualitative tests for detecting the occurrence of flavonoids, phenols, tannins, carbohydrates, reducing sugars, glycosides, and alkaloids. These results were in agreement with previous data obtained by Osabor et al. [29] and Arawande et al. [30]. They described the presence of different natural products in ginger such as plant acids, tannins, alkaloids, phenolic, amino acids derivatives, reducing sugars, and glycosides. These active compounds are proved to display antifungal, antibacterial, antitumor, and antioxidant agents as recorded by Shalaby [31], Shalaby et al. [32], Rampogu et al. [33].

Phytochemicals have disease defensive characteristics and function as antioxidants, enzyme stimulating substance, anticancer and antibacterial agents as well as having hormonal properties. Plants with a high concentration of these phytochemicals are referred to as therapeutic plants [29]. Phytochemical screening also exhibited positive results in phenols and flavonoids.

Many investigations have shown that the various ginger extracts, the oil and some of its phytochemicals own free radical scavenging, antioxidant and antiperoxidative properties.

The extract was observed to scavenge, hydroxyl, superoxide, nitric oxide and $ABTS^{*+}$ radicals in a dose-dependent manner in vitro [34-36]. Cell free assays also reveal that the ginger extract prevents enzymatic lipid peroxidation, iron/ascorbate-induced oxidation of the membrane lipids and cumene hydroperoxide [37-39]. The antioxidant activity of ginger extract was unaffected by thermal denaturation when boiling for 30 min at 100 °C, suggesting that the spice components in addition to imparting flavor to the food [37], it possess potential health benefits by preventing the lipid peroxidation [40].

Red ginger water extracts obstruct angiotensin-I converting enzyme in rat hearts [38]. Its oleoresins are toxic, and its volatile oils have antimicrobial activity against *Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa and Salmonella typhimurium* [39]. In addition, ginger oil is described to reduce the H_2O_2-induced oxidative damage [40].

6-Gingerol, an important component in ginger, have good antioxidant properties [40], scavenger of peroxyl radicals [41], and cause a dose-dependent inhibition of nitric oxide production [40]. In addition, it can reduce peroxidation of phospholipid liposomes in the presence of iron(III) and ascorbate [41, 42].

Hot water extract of ginger peels revealed the highest result extracts almost in all methods. So as promising extract, HPLC and column chromatography were used to determined active compounds in hot water extract which were found to be 9 components.

Phenolic compounds identified using high-performance liquid chromatography /UV (HPLC).

Qualitative screening of phytochemicals in ginger peel aqueous extracts revealed the presence of numerous active compounds as recorded in Table 2. Determination of these active ingredients was performed using HPLC and petroleum ether/ethyl acetate as mobile system. HPLC chromatogram recorded 9 active compounds in the promising hot water extract of ginger peels.

Thin layer chromatography (TLC) of hot and cold water extracts

The TLC of hot water extract of ginger peels and cold water extract of ginger peels revealed that they have strong antioxidant activity as represented in Fig. 1.

Antioxidant activity of hot water extract by bioautography

The occurrence of an antioxidant compound was demonstrated by the appearance of yellow or white spots on a purple background (DPPH) as represented in Fig. 1.

Thin layer chromatography (TLC) of the six fractions

Fractionation of the promising hot water peel extract was performed using silica gel column chromatography with petroleum ether/ethyl acetate as mobile phase system. Six fractions were produced. TLC was performed on the 6 fractions to determine the antioxidant activity of each fraction.

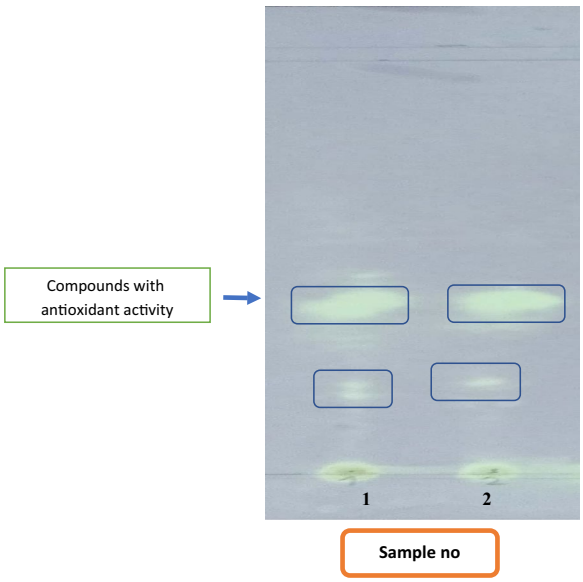


Fig. 1 TLC-bioautography of hot water extract of ginger peels (1) and cold water extract (2) of ginger peels sprayed with DPPH reagent

Hot and cold ginger peel extracts were tested for their antioxidant efficiency using TLC F_{254} and benzene/acetone (9:1 v/v) as mobile phase system as shown in Fig. 1, spraying with the DPPH recorded the higher antioxidant activity of peel extracts where the spots turned yellow to white on the purple background (Fig. 1). Application of the same TLC procedure on the six fractions of the promising hot water extract revealed their antioxidant activity with DPPH.

TLC of hot water and cold water extracts from ginger peel samples showed spots under UV light at 365 nm and 245 nm. This indicated the presence of antioxidant activity. Then antioxidant activity by bioautography was performed after spraying with DPPH for both samples of ginger peels as illustrated in exhibited antioxidant activity as yellow spots on TLC sheets. Similar results were exhibited with ABTS, methylene blue, and $KMnO_4$ confirming this result.

Antioxidant activity by bioautography

All of the six fractions exhibited antioxidant activity (qualitative test) but the fourth fraction has the highest antioxidant activity against DPPH spraying.

Antioxidant activity (%) of fractions from hot water extract

The antioxidant activity of hot water extract of ginger peels against ABTS was performed for the six fractions (quantitative test) as shown in Table 3. The results showed that all the 6 fractions exhibited variable antioxidant activities, but fraction number 4 represents the most efficient antioxidant activity against ABTS radical (59.51 ± 0.51%) followed in descending orders by fraction number 5 (58.04 ± 0.48%) and the lowest antioxidant activity was recorded by fraction number 3 (37.14 ± 0.11%).

Column chromatography was used for separation of active compounds in the promising hot water peel extract. Six fractions with different concentrations were produced using petroleum ether and ethyl acetate with hot water extract of ginger peels to determine the active compounds in the extract. Table 3 reveals that fraction number 4 recorded the highest biological activities as an antioxidant, when sprayed with DPPH and when measured against ABTS (59.51 ± 0.51%) followed by fraction number 5, 1, 6, 2 (58.04 ± 0.48, 50.53 ± 0.28,49.79 ± 0.23, 48.89 ± 0.15%, respectively). Last fraction having antioxidant activity was fraction number 3 (37.14 ± 0.11%). These results may be due to the high content of antioxidant compounds in hot water extract of ginger peels as shown in Table 3.

To indicate the antioxidant substances in the promising fraction to which attributed this activity, the water-soluble antioxidant substances (in the hot water extract): phenolic and flavonoids must be estimated.

Determination of total phenolic compounds

The obtained data in Table 4 reveal that hot water extract of ginger peels contain more phenolic compounds (0.333 ± 0.04%) compared with cold water extract (0.323 ± 0.01%).

From the data presented in Table 4 for the determination of total phenolic compounds, it was clearly obvious that phenolic content was high in hot water extract than cold water extract. High levels of hot water extract were reported (0.333 ± 0.04 g/100 g fresh weight) compared to cold water extract (0.0323 ± 0.01 g/100 g fresh weight). Hot water extraction helps in releasing high molecular weight compounds and this may be the reason behind

Table 3 Antioxidant activity (%) of different fractions of hot water extract against ABTS radical

Fraction number	Antioxidant activity (%)
1	50.53 ± 0.28^{c}
2	48.89 ± 0.15^{cd}
3	37.14 ± 0.11^{e}
4	59.51 ± 0.51^{a}
5	58.04 ± 0.48^{b}
6	49.79 ± 0.23^{c}

Values are means of three replicates ± SE

The values in the column followed by the same letter are not significantly different at $P = 0.01$

Table 4 Total phenolic compound content (as g/100 g fresh weight) of aqueous extract from ginger peels

Ginger	Total phenol (g/100 g fresh weight)
Cold water extract	0.323 ± 0.01^{a}
Hot water extract	0.333 ± 0.04^{a}

Values are means of three replicates ± SE

The values in the column followed by the same letter are not significantly different at P = 0.01

Table 5 Total flavonoid content (as g/100 g fresh weight) of aqueous extract from ginger peels

Ginger	Total flavonoid (g/100 g fresh weight)
Cold water extract	0.0637 ± 0.006^{b}
Hot water extract	0.0968 ± 0.005^{a}

Values are means of three replicates ± SE

The values in the column followed by the same letter are not significantly different at $P = 0.01$

this result; phenolics act as antioxidants by scavenging free radicals, metal chelation so it's to prevent a wide spectrum of chronic diseases and improve health and these results agreed with Marrelli et al. [43] and Ali et al. [44].

Determination of total flavonoids

The data presented in Table 5 showed that hot water extract of ginger peels exhibited higher flavonoids content (0.0968 ± 0.005 g/100 g) than cold water extract (0.0637 ± 0.006 g/100 g).

Flavonoid determination shown in Table 5 revealed that hot water extract exhibited higher antioxidant activity (0.0968 ± 0.005 g/100 g fresh weight) than cold water extract (0.0637 ± 0.006 g/100 g fresh weight) and this confirmed the results shown in Table 1 in phytochemical screening in (alkaline reagent, lead acetate, ammonia, and Conc. H_2SO_4 tests). High levels of flavonoids and total phenolic (TP) contents in hot water extract of ginger peels in comparison with cold water extract of ginger peels of medicinal plants have been reported in previous studies as Ali et al. [44] who reported high content of flavonoids in ginger; which used as reducing agents, free radical scavengers, and assists the body to suppress the inflammatory response that may be caused by allergy or bacteria as reported by Watson [45].

According to Pokorny et al. [46], the inhibition mechanism of oxidation by antioxidants was performed in two ways, the binding/ capture of free radicals (primary antioxidants) and other mechanisms that do not directly capture free radicals (secondary antioxidants), such as binding or chelating metals, oxygen capture, singlet, the conversion of hydrogen peroxide becomes non-radical.

Correlation between different antioxidant activity assays with flavonoids and phenols of hot water extract and cold water extract of ginger peels was performed (Table 6). The table revealed that there are great correlation between ABTS with DPPH, methylene blue, phenolics and flavonoids. However, no correlation observed with KMnO4, while DPPH results were correlated with methylene blue, phenolics and flavonoids obtained data.

DPPH radical scavenging activity and ABTS methods were used for their sensitivity and stable radicals. α- tocopherol and ascorbic acid were used as standards in many research because both are widely used in the human body: α- tocopherol, a lipid phase chain-breaking antioxidant, played an important role in lipid peroxidation, while ascorbic acid acts as water phase chain-breaking antioxidants that scavenge radicals found in the water compartment [40].

Evidence in the literature profusely exhibits that ginger has strong in vivo and in vitro antioxidant properties. Aqueous and ethanol ginger extract has cascade of natural antioxidants, which had significant scavenging ability to superoxide radicals and lipid peroxidation [47].

pH measurement

Data in Table 7 reveal that alkaline sample with hot water extract exhibited higher antioxidant activity against ABTS (97.46 ± 0.53%) followed by acidic sample (79.42 ± 0.76%) when both were compared to the control (93.71 ± 0.41%).

The obtained data recorded in Table 7 of pH measurement of hot water extract of ginger peels revealed that hot water extract of ginger peels have higher antioxidant activity in the alkaline medium (97.46 ± 0.53) than acidic medium (79.42 ± 0.76) and this result may be due to ginger peels being more effective in alkaline pH. This result is very quintessential because use of the peels in food as a medication or cure may be advised without decreasing its effectiveness.

Table 7 Antioxidant activity of hot water extract of ginger peels at different pH degree measurement against ABTS radical assay

pH degree	Antioxidant activity (%)
Acidic	79.42 ± 0.76^{c}
Alkaline	97.46 ± 0.53^{a}
Control (hot water extract)	93.71 ± 0.41^{b}

Values are means of three replicates ± SE

The values in the column followed by the same letter are not significantly different at $P = 0.01$

Table 6 Correlation between different antioxidant activity assays with flavonoids and phenols of hot water extract and cold water extract of ginger peels

Parameters	ABTS	DPPH	$KMnO_4$	Phenolics	Flavonoid	Methylene blue
ABTS	–	0.74	− 1	0.65	0.74	0.32
DPPH	–	–	− 1	0.71	0.91	0.85
KMnO4	–	–	–	− 1	− 1	− 1
Methylene blue	–	–	0.21	0.31	0.48	–
Phenolic compound	–	–	–	–	0.85	–

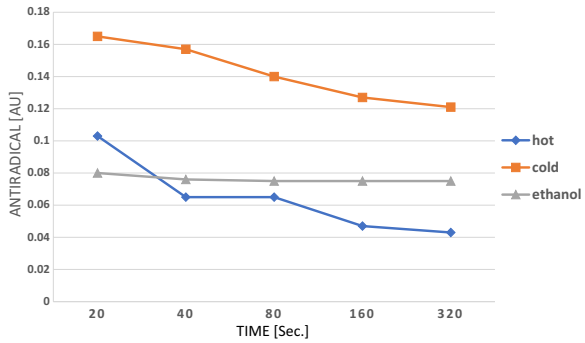


Fig. 2 Antiradical activity (as AU) of different extracts of ginger residues

Antiradical activity

The obtained results illustrated in Fig. 2 demonstrated that the antiradical activity was shown to be incubation time dependent. Hot water extract showed the highest antiradical activity represented as AU or antiradical unit (0.043) against ABTS, followed by cold water extract (0.121) and finally, ethanol pulp extract (0.075) at 320 s of incubation.

The obtained results in Fig. 2 that illustrated antiradical activity of different extracts of ginger showed that hot water extract of ginger peels exhibited higher antiradical activity (0.103: 0.043) than cold water extract of ginger peels (0.165: 0.121) and the lowest antiradical activity was recorded by ethanol pulp extraction (0.08: 0.075) from (20:320 secs) of incubation; and this result may be due to higher phenolic compounds recorded in Table 4.

Antioxidant activity methods

Antioxidant activity of the ginger extracts was tested using radical assays (DPPH & ABTS) and non-radical (methylene blue & $KMnO_4$), as illustrated in Table 8 and Fig. 3.

Table 8 Antioxidant activity (%) of aqueous and ethanolic extracts from ginger peels and residues against different antioxidant assays

Samples	Antioxidant assay			
	DPPH	ABTS	KMnO4	Methylene blue
Cold water extract	80.40 ± 0.64^{b}	14.67 ± 0.54^{c}	94.88 ± 0.48^{b}	27.51 ± 0.56^{c}
Hot water extract	84.15 ± 0.60^{a}	74.71 ± 0.36^{b}	91.63 ± 0.55^{c}	34.66 ± 0.17^{b}
Ethanolic extract	24.7 ± 1.34^{d}	92 ± 0.74^{a}	42.45 ± 0.38^{d}	27.41 ± 0.58^{c}
Ascorbic acid	76.42 ± 0.31^{c}	73.93 ± 0.21^{b}	99.09 ± 0.06^{a}	59.69 ± 0.26^{a}

Values are means of three replicates $\pm$ SE

The values in the column followed by the same letter are not significantly different at $P = 0.01$

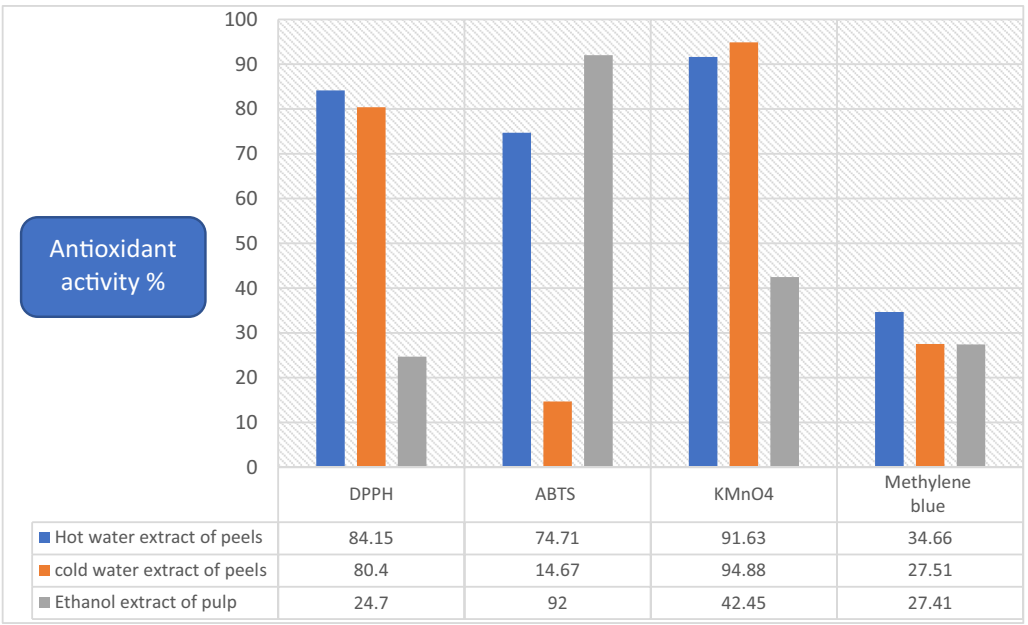
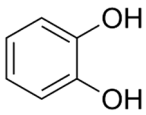
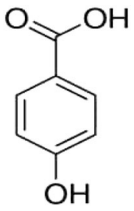
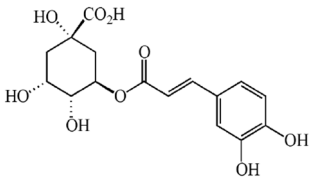
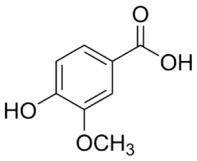
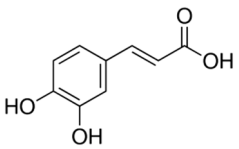
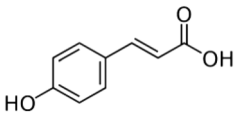
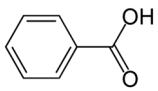
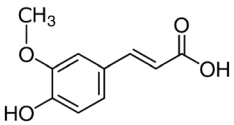
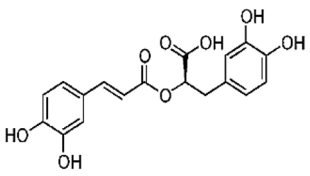


Fig. 3 Antioxidant activity (%) of different ginger extracts of peels and pulp against four different antioxidant assays

Table 9 Phenolic and flavonoid compounds in ginger peel extract analyzed by HPLC

No.	R.T (min)	Compounds name	Conc mg/Kg	Chemical Structure	Biological activities	References
1	5.464	Catechol	1.92		Antimicrobial activity	[50]
2	7.510	p- Hydroxy benzoic acid	21.94		Antimicrobial, antiestrogenic, anti-inflammatory, antiplatelet antiviral, and antioxidant	[51]
3	9.117	Chlorogenic acid	1.95		Antioxidant, antidiabetic, antimicrobial, anticarcinogenic, anti-inflammatory, and antiobesity strategies	[52]
4	9.717	Vanillic acid	0.388		Antioxidant, anti-inflammatory, and neuroprotective effects	[53]
5	10.245	Caffeic acid	0.913		Antioxidant, anti-inflammatory, and anticarcinogenic activity	[54]
6	13.205	p-Coumaric acid	1.17		Antioxidant, anticancer, antimicrobial, anti-inflammatory, antiplatelet aggregation	[55]
7	14.330	Benzoic acid	31.41		Antimicrobial activity	[56]
8	15.182	Ferulic acid	0.393		Antioxidant, anti-inflammatory, antiviral, antiallergic, antimicrobial, antithrombotic, anticarcinogenic, and hepatoprotective actions	[57]
9	22.030	Rosemarinic acid	47.80		Antioxidant and anti-inflammatory and antidiabetic	[58]

DPPH assay

The obtained data in Table 8 revealed that hot water extract exhibited the highest antioxidant activity against DPPH by 84.15 ± 0.60% followed by cold water extract (80.40 ± 0.64%) when compared with the natural standard which is ascorbic acid (76.42 ± 0.31%).

ABTS assay

Data as represented in Table 9 exhibited significant antioxidant activity of hot water extract of ginger peels (74.71 ± 0.36), which was significantly like the standard (73.93 ± 0.21), then followed by cold water extract with the ABTS (14.67 ± 0.54).

$KMnO_4$ assay.

The obtained results in Table 10 revealed that cold water extract represented the highest antioxidant activity against $KMnO_4$ (94.88 ± 0.48) followed by hot water extract (91.63 ± 0.55) in comparison with ascorbic acid (99.09 ± 0.06).

Methylene blue assay

The obtained data in Table 11 exhibited that hot water extract represented the highest antioxidant activity against methylene blue (34.66 ± 0.17%) followed by cold water extract (27.51 ± 0.56%) compared to ascorbic acid (59.69 ± 0.73%).

The antioxidant activity of both extracts of ginger peels and ginger pulp was evaluated using DPPH and ABTS, radical scavenging methods and, $KMnO_4$ and methylene blue as non-radical scavenging methods. The obtained results recorded in Fig. 3, revealed that ABTS, and methylene blue methods go parallel while $KMnO_4$ showed different results. These results revealed that the highest antioxidant activity of ethanol extract of the ginger pulp was against ABTS which was considered higher than ascorbic acid as natural standard (92 ± 0.74%). Then when tested against $KMnO_4$, the antioxidant activity was highly decreased (42.45 ± 0.38) as compared to ascorbic acid as natural standard (99.09 ± 0.06). The lowest antioxidant activity was measured against methylene blue (27.41 ± 0.58) as compared to ascorbic acid as natural standard (59.69 ± 0.26).

Table 11 The cytotoxicity assay with different water extracts for ginger peels at different concentrations

Ginger	Cytotoxicity (%)		
	250 ppm	500 ppm	1000 ppm
Cold water extract	12.3 ± 1.0^{b}	21.7 ± 1.8^{b}	34.33 ± 2.15^{b}
Hot water extract	32.5 ± 1.70^{a}	57.9 ± 1.57^{a}	86.8 ± 3.57^{a}
Doxorubicin	The anticancer standard that gave 95% at 10 ppm		

Values are means of three replicates ± SE

The values in the column followed by the same letter are not significantly different at $P = 0.01$

Antioxidant activity had been done with several assays such as DPPH, ABTS, methylene blue, and $KMnO_4$. In DPPH assay Table 8, hot water extract of ginger peels has recorded the highest antioxidant activity followed by cold water extract of ginger peels which may be due to its content of active ingredients with antioxidant activity as p-hydroxy benzoic acid, chlorogenic, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, and rosemarinic as displayed in Table 9 and the highest amount of phenolic and flavonoid compounds as shown in Tables 4 and 5. These results are in agreements with the results obtained by Aboul-Enein et al. [48]; Shalaby et al. [49] and El-fayoumy et al. [18] they reported that there were strong correlations among the antioxidant activity (determined by DPPH, and methylene blue), and phenolic compounds concentration in plant species.

The obtained results against ABTS in Table 8 and Fig. 3 recorded that ethanol pulp extract showed significantly highest antioxidant activity against ABTS followed by hot water extract of ginger peels and finally cold water extract of ginger peels when compared to ascorbic acid as natural standard; these results are consistent with Elizabeth et al. [59] who reported that ginger peels have higher antioxidants than ginger pulp, which may be due to less concentration used in this project or different conditions.

Table 10 Antimicrobial activity of the aqueous extracts from ginger peels

Ginger	Antimicrobial activity (mm)			
	Escherichia coli	*Salmonella* sp	*Staphylococcus aureus*	*Bacillus cereus*
Cold water extract	5.5 ± 0.33^{c}	6.0 ± 0.30^{b}	8.4 ± 0.71^{b}	4.3 ± 0.18^{c}
Hot water extract	8.9 ± 0.62^{b}	5.0 ± 0.24^{c}	9.0 ± 0.79^{a}	5.9 ± 0.34^{b}
Novobiocin	–	–	8.5 ± 0.57^{b}	18.5 ± 0.57^{a}
Polymyxin	18.66 ± 0.57^{a}	21.33 ± 0.57^{a}	–	–

Values are means of three replicates ± SE

The values in the column followed by the same letter are not significantly different at $P = 0.05$

$KMnO_4$ analysis revealed that [in Table 8 and Fig. 3] cold water extract of ginger peels was recorded as a highest antioxidant agent than other extracts; followed by hot water extract and the lowest antioxidant activity against $KMnO_4$ were ethanol pulp extraction. These results may be due to released compounds when grinding the extract and this agreed with Elizabeth et al. [59] who said that ginger peels have higher antioxidant activity than ginger pulp and unpeeled ginger with acetone extract.

According to the data shown in methylene blue, Table 8 and Fig. 3, hot water extract of ginger peels has the strongest antioxidant among different extracts, which may be due to its composition of active ingredients with antioxidant activity; almost all of these active ingredients have antioxidant activity as seen in Table 9 and the highest amount of phenolic compounds are shown in Table 4. These findings are consistent with those reported by Aboul-Enein et al. [48] and El-fayoumy et al. [18], Faten and Emad [60], Ahmeda etal. [61] who found a high correlation between antioxidant activity (as measured by methylene blue), and total phenolic concentration in different plant species. So, hot water extract of ginger peels followed in descending order by cold water extract and finally ethanol pulp extract.

The results of DPPH, ABTS, $KMnO_4$, and Methylene blue were examined (Table 8 and Fig. 3) and it was clearly shown that antioxidant activity improved in a dose-dependent manner [62, 63]. The antioxidant activity of different extracts of ginger was evaluated using DPPH, ABTS, as radical scavenging methods and $KMnO_4$, and methylene blue non-radical scavenging methods (Fig. 4).

According to the results, we can conclude that hot water extract had the maximum antioxidant activity against DPPH and methylene blue because they both has a strong correlation as shown in Table 6 with each other followed by cold water extract and ethanol pulp extract while $KMnO_4$ revealed that the cold extract has the highest antioxidant activity followed by a hot extract of ginger peels then ethanol pulp extract. As shown with ABTS, ethanol pulp extract revealed the highest antioxidant activity followed by hot water extract of ginger peels and then cold water extract in comparison to ascorbic acid as a natural standard.

Phenolic compounds were identified using High-Performance Liquid Chromatography /UV (HPLC). The developed HPLC method was reliable with high accuracy and precision. The clear and separated peaks at retention times of 5.464, 7.510, 9.117, 9.717, 10.245, 13.205, 14.330, 15.182, and 22.03 min were obtained for catechol, p- Hydroxy benzoic acid, chlorogenic, vanillic acid, caffeic acid, p-coumaric acid, benzoic acid, ferulic acid, and rosemarinic, respectively (Table 9).

Well diffusion assay

Ginger has strong antibacterial properties. In vitro studies have shown that active components of ginger inhibit the growth of colon bacteria, *Escherichia coli,* Proteus *sp.,* Staphylococci and Salmonella [62, 63].

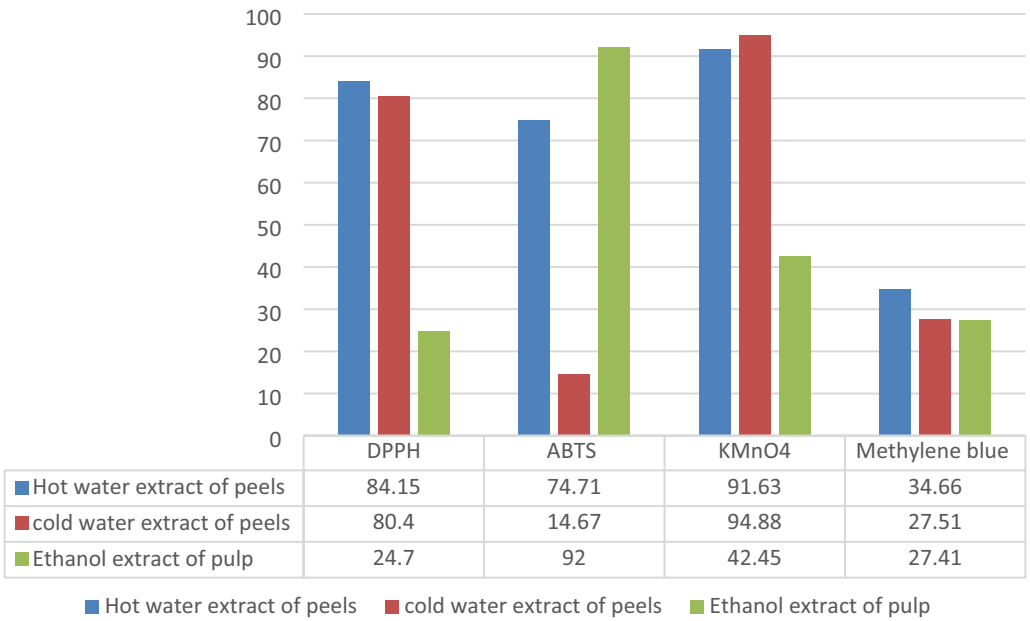


Fig. 4 Antioxidant of different ginger extracts of peels and pulp against four antioxidant determination methods

The present work revealed that the well diffusion assay of both aqueous ginger peel extracts against Gram-positive and Gram-negative bacteria had very faint antibacterial activity (>0.1 mm inhibition zone) compared to the standard synthetic novobiocin and polymyxin (Table 10).

The results reported in Table 10 of well diffusion assay revealed the antibacterial activity of both extracts of ginger peels (hot and cold water extract) with different strains as *Escherichia coli*, *Salmonella* sp (Gram-negative bacteria) and *Staphylococcus aureus* and *Bacillus cereus* (Gram-positive bacteria) with the positive control that supposed to have 18.66 ± 0.57, 21.33 ± 0.57 mm inhibition zones with polymyxin, as well as 8.5 ± 0.57 and 18.5 ± 0.57 mm inhibition zones with novobiocin, respectively as standard drugs. Which in contrast with Elizabeth et al. [59], the ginger peels were effective against *Bacillus cereus* strain and consistent with Elizabeth et al. [59] when they said that ginger peels are ineffective against *Escherichia coli*, *Salmonella* sp and *Staphylococcus aureus* which may be due to less concentration used in this investigation (essential oils with antibacterial activity are insoluble in water).

Anticancer activity

All hot water extract concentrations expressed high cytotoxicity over HepG-2 cell line than cold water extract concentrations of ginger peels (Table 11).

Various biological activities including antimicrobial activity such as ferulic acid, catechol, p- hydroxy benzoic acid, p- coumaric acid, and benzoic acid; Also, it was found that most of the compounds have antioxidant activity like chlorogenic, p- hydroxy benzoic acid, vanillic acid, caffeic acid, p- coumaric acid, ferulic acid, and rosemarinic. Moreover, anticancer activity exhibited by ferulic acid, caffeic acid, and p- coumaric acid; for the anti-inflammatory, rosemarinic, ferulic acid, p- coumaric acid, vanillic acid, caffeic acid, Chlorogenic, and p- Hydroxy benzoic acid were found to be anti-inflammatory agents. Additionally, there are other biological activities in hot water extract of ginger peels as antidiabetic, antiobesity, antiestrogenic, antiplatelet, antiviral, antiallergic, antithrombotic, and hepatoprotective actions (Table 9).

It was observed in anticancer activity recorded in Table 11 that hot water extract has higher anticancer activity at different concentrations 250 ppm, 500 ppm, and 1000 ppm (7.5 ± 0.70, 9.6 ± 0.57, and 35.66 ± 0.57%) than cold water extract of ginger peels (zero, 5 ± 1.0 and 14.33 ± 1.15%), respectively to the concentrations used. All these results are compared to Doxorubicin as a standard at 10 ppm. These results may be due to the higher molecular weight compounds of released molecules during boiling of hot water extract and to the compounds identified by HPLC with anticancer activity. These results were in an agreement with Nguyen et al. [64] and it's may be used in a form of tablets in the treatment of a wide range of other cancers, including lung, bladder, cervical, various GL cancers (gastric, pancreatic, liver, colorectal) and ovarian cancers which may be also in an agreement with Marrelli et al. [43] as well as Prasad and Tyagi [65] due to its higher antiradical activity and it may be used in the form of medication to capture free radicals once it enters the body. It was shown that ginger exhibited an important role in cancer prevention by activating and inactivating various molecular pathways as reported by Rahmani et al. [66].

So, based on the obtained results we may use ginger especially its peels in medicine in the form of tablets or maybe in food in the form of soap to prevent the damage caused by free radicals to the body's cells, heart disease, and also may be used as a serum on the skin to prevent wrinkles and fine lines that caused by free radicals and environmental factors due to its antioxidant activity (exhibited by several methods).

Herbs are very important in the medical field. The expansion was required in this field to treat a wide spectrum of diseases with natural herbs. So, we recommend that ginger peels should not be discarded anymore due to their proved assiduous importance in the medical field as antioxidant, anticancer, and antiradical. Also, they contain very high content of phenolics and flavonoids which in turn have a huge importance in multiple fields specifically in the medical field. These findings were agreed with Ali et al. [67]. who reported that ginger cytotoxicity to HT29, HT116 and MCF-7 cancer cells was mainly associated with the presence of 6-shogaol and 6-gingerol in extracts, which appeared as strong cytotoxic effect in rhizome compared to non-cytotoxic effect was evaluated in the callus extracts.

Conclusion

Ginger rhizome has been used for very long time as a spice in food. It contains polyphenol compounds (6-gingerol and its derivatives) which exhibited great antioxidant activity. Ginger peels have long been thrown away, but this investigation showed that its aqueous extracts (cold and hot water) exhibited great antioxidant and anticancer activities. Ginger pulp ethanol extract was found to undergo higher antioxidant activities. Volatile oils, oleoresins, phenolic and flavonoids compounds are responsible for the pronounced biological activities of ginger.

Abbreviations

GC/MS	Gas chromatography–mass spectrometry
GC-FID	Gas chromatograph-flame ionization detector
LC/MS	Liquid chromatography–mass spectrometry
HPLC	High performance liquid chromatography
DOX	Doxorubicin
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ABTS+	2,2′-Azino-bis (ethylbenzthiazoline-6-sulfonic acid)
DMSO	Dimethyl sulfoxide
HepG-2	Human hepatocellular cancer cell line
TLC F_{254}	Thin layer chromatography plate, silica gel coated with fluorescent indicator F254
SRB-assay	Sulforhodamine B assay
Trypsin–EDTA	Trypsin–ethylenediamine tetra acetic acid
TCA	Trichloroacetic acid
LSD	Least significant difference
TP content	Total phenolic content

Author contributions

Conceptualization: EAS, SMS, RMH and AEE. Data curation; EAS, SMS, RMH and AEE. Funding acquisition; EAS, SMS, RMH and AEE. Investigation; EAS, SMS, RMH and AEE. Methodology; EAS, SMS, RMH and AEE. Resources EAS, SMS, RMH and AEE. Software; EAS, SMS, RMH and AEE. Validation; EAS, SMS, RMH and AEE. Writing—original draft; EAS, SMS, RMH and AEE. Writing—review and editing EAS, SMS, RMH and AEE. Please add at the end: All authors read and approved the final manuscript.

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Availability of data and materials

The data used and analyzed in this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Biochemistry, Faculty of Agriculture, Cairo University, Giza 12613, Egypt. [2]Department of Botany and Microbiology, Faculty of Science, Cairo University, Giza 12613, Egypt.

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