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Phytochemical and Antioxidant Analysis of Ginger (*Zingiber officinale*) Ethanolic Extract

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Abstract

Background: Ginger (*Zingiber officinale*) is famous for its antioxidant activity, and its antioxidant activity can potentially counteract oxidative stress-related diseases. Ginger pungency and odor are a result of its bioactive compounds, including gingerols, shogaols, paradols, and zingerone. In the present work, a study of the phytochemical profile and antioxidant activity of ginger and its therapeutic potentials was performed.

Method: Method: Dried rhizomes of ginger were extracted with 7% ethanol for 24 hours, and 18.2% dark brown, paste-like extract was yielded. The extract then was analyzed for bioactives with the use of high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). Antioxidant activity was measured with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

Results: GC-MS analysis revealed that the extract contained high concentrations of fatty acids and derivatives, comprising 92.91% of its contents. Trace residues of aldehydes and alkenes (0.02%) and hydrocarbons (13.58%) were detected. Gallic acid (71.25 ppm), hydroxybenzoic acid (49.25 ppm), ferulic acid (51.11 ppm), ellagic acid (20.25 ppm), and caffeic acid (11.58 ppm) phenolic compounds were confirmed in HPLC analysis. Ginger extract exhibited strong antioxidant activity, as same as activity observed with vitamin C, with an IC50 value of 65.11 µg/mL in the DPPH assay.

Conclusion: The current study confirms that the ethanolic extract of ginger possesses a high level of antioxidants, with a potential for numerous therapeutic uses.

Introduction

Ginger (*Zingiber officinale*), a zingiberaceous species in the family Zingiberaceae, native to South Asia, is a long-standing traditional medical herb [1]. Gingerols, shogaols, paradols, and zingerone, responsible for their pungent odor and strong, pungent, warm, sweet, and aromatic flavor, have a range of biologically active compounds [2]. Compounds in *Zingiber officinale* exhibit anti-inflammatory, antimicrobial, anticancer, and antioxidant activity [3].

Ginger is of interest for its antioxidant activity, and its activity can potentially counteract oxidative disease-related oxidative stress. Oxidative stress occurs when an organism fails to detoxify ROS generated in its tissue. ROS at high concentrations can induce oxidative damage in cellular structures, and oxidative damage can contribute to cardiovascular, neurodegenerative, and cancer-related diseases.

Ginger contains antioxidant compounds, and its activity can reverse oxidative damage through ROS-scavenging in an organism. Understanding the biochemistries of bioanalytic analysis in plant extracts is critical in discovering bioactive compounds for therapeutic activity.

There have been documented groups of phytochemicals such as phenolic acids, polyphenols, and fatty acids, that have been proven to enhance antioxidant activity in plant extracts [2]. Antioxidant activity in natural compounds must be examined in a manner that will unveil its therapeutic application in oxidative-related disease [4].

A comprehensive evaluation of ginger's ethanolic extract is significant in view of its widespread use in traditional medicine, its rich diversity in terms of its phytochemicals, and its potential therapeutic values.

In an attempt to gain a deeper insight into ginger's phytochemical composition and evaluate its antioxidant activity, this work seeks to present scientific backing for its traditional and therapeutic values.

Methods

Plant Materials and Extraction

Zingiber officinale rhizomes used were obtained from local market in Baghdad Province, Iraq. The plant was identified and authenticated in by the Iraqi National Herbarium, Directorate of Seed Testing and Certification, the Iraqi Ministry of Agriculture (certification number 2860 on April 2022).

The rhizomes were initially chipped and then ground using an electrical blender into a fine powder and left to air-dry. After that, 50 g of the powder was soaked in 200 mL of 70% ethanol (Alpha Chemika, India) and allowed to sit at room temperature in a conical flask overnight. A fine muslin cloth was then used to filter the mixture in order to get rid of any solid particles. The resultant combination was then filtered through the Whatman filter paper (No. 1) after the filtering residue was removed twice more using the same method.

A vacuum rotary evaporator (Heidolph, Germany) was used to concentrate the mixture at 40°C. Ultimately, the concentrated extract was heated to 45 °C in an oven until the solvent evaporated. Before being employed for phytochemical investigations, the resultant extract was kept in a sterile container [5]. The yield was computed using the following formula:

$$\text{Yield (\%)} = \frac{\text{Weight of ginger extract}}{\text{Weight of dried ginger powder}} \times 100$$

Analysis of Phytochemicals

Gas Chromatography-mass Spectrometry (GC-MS)

A Trace GC Ultra coupled to a Data System Quadrupole II (DSQ II) mass spectrometer (Thermo Scientific, Bremen, Germany) was used for the GC-MS analysis. For the separation, a Solid Phase Microextraction (SPME) fiber was used, and a 10-minute desorption process was carried out in the GC injection port to achieve the desired results.

The column used was a Stabilwax-DA-Crossband-Carbowax-polyethylene-glycol (Restek, Bellefonte, PA) measuring 30 mm long, 0.32 mm internal diameter, and 1 µm film thickness. The oven temperature program was as follows: initial temperature (40 °C/3 minutes), ramped to 220 °C (at 15 °C/minute), and held (1 minute). Helium was used as the carrier gas, with a constant flow rate of 1.5 mL/minute.

The MS ion source temperature was held at 225 °C, and the electron impact (EI) mode was set with an energy of 70 electron Volts (eV). The mass range was set from 33 to 250 mass/charge (m/z) ratio. The method was adjusted in light of the research conducted by [6].

High Performance Liquid Chromatography (HPLC)

In addition to GC-MS phytochemical analysis, ginger ethanolic extract was examined further by HPLC (Sykamn, Germany). The system was equipped with a C18-Octadecylsilane (ODS) column measuring 250 × 4.6 mm with a particle size of 5 µm. A 10 µL sample was introduced into the system for analysis.

The mobile phase consisted of two solvents: solvent A (95% acetonitrile + 0.01% trifluoroacetic acid) and solvent B (5% acetonitrile + 0.01% trifluoroacetic acid), flowing at a rate of 1 mL/minute. The gradient program started with 10% solvent A for the first 5 minutes, increased to 25% solvent A between 5-7 minutes, and then to 40% solvent A between 7-15 minutes before returning to the initial conditions.

Phenolic compounds were detected using a UV-visible detector set at a wavelength of 278 nm [7].

Antioxidant Activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH assay was carried out based on the method of [8] with some modifications. DPPH (ALPHA AESAR, Thermo Fisher, USA) and vitamin C (ascorbic acid) (Avonchem Ltd, UK) were used as the free radical and the standard antioxidant, respectively.

The ethanol was of analytical grade and obtained from a local supplier. The absorbance of the samples was recorded using a digital UV-spectrophotometer (ALS Lab, Germany).

A stock solution of vitamin C (1 mg/mL) and a stock solution of DPPH (0.1 mM) were made in ethanol. Different concentrations of vitamin C (10 to 100 µg/mL) and ginger ethanolic extract (10 to 100 µg/mL) were prepared by diluting the stock solutions with ethanol. Then, 100 µL of each sample or standard was added into the DPPH (100 µL) in a 96-well microplate and for 30 minutes was kept in dark at room temperature.

The absorbance was measured at 517 nm against a blank of methanol using a microplate reader. The DPPH radical scavenging activity was calculated as percentage using the following formula:

$$\text{Scavenging \% (DPPH)} = \frac{\text{A control - A sample}}{\text{A sample}} \times 100$$

Where A control is the absorbance of the control (DPPH solution + ethanol), and A sample is the absorbance of the sample or vitamin C (standard) + DPPH. The IC_{50} value, a concentration of a sample or a standard that reduces fifty percentage of DPPH radicals, was obtained from the linear regression equation of the percentage of scavenging activity versus the concentration.

The activity of antioxidant of the samples was evaluated based on their IC_{50} values according to [9] as follows : very strong: <50 µg/mL, strong: 50-100 µg/mL, medium: 100-250 µg/mL, weak: 250-500 µg/mL, and not active: >500 µg/mL.

Results

The ethanolic extraction procedure of fresh dried ginger rhizomes used in current study yielded 18.2% of a dark brown paste-like thick sauce with a pronounced bitter flavor.

Phytochemical Analysis

GC-MS

The composition of bioactive compounds in *Zingiber officinale* Roscoe ethanolic extract was examined by GC-MS. The identified compounds, groups they belong to, along with their retention time, area percentage, molecular formulas, and molecular weights are shown in Table 1.

The analysis conducted using GC-MS indicated that ginger extract predominantly consists of fatty acids and their derivatives, accounting for 92.91% of the total composition. Additionally, minor amounts of aldehydes (0.01%), alkenes (0.01%), and hydrocarbons (13.58%) were also detected.

The composition of fatty acids and their derivatives comprises saturated fatty acids (3.9%), monounsaturated fatty acids (22.33%), polyunsaturated fatty acids (32.84%), and fatty acid esters (33.84%). The chemical compounds known as aldehydes and alkenes encompass cinnamaldehyde and hexadecadiene. The hydrocarbon compounds encompass indane and hexadecane.

These compounds exhibit a range of biological activities and offer potential health advantages, including but not limited to anti-inflammatory, anti-cancer, anti-diabetic, anti-obesity, anti-atherogenic, antimicrobial, antifungal, antiviral, insecticidal, and antioxidant properties. The compound that exhibited the highest abundance was 9-Octadecenoic acid(Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester, commonly known as the ester of hydroxy oleic acid. This particular compound constituted approximately 27.36% of the total area.

The aforementioned compound is classified as a fatty acid ester that originates from oleic acid, a monounsaturated omega-9 fatty acid. The compound that was found to be the second most abundant was oleic acid, constituting 22.33% of the total area.

In addition to oleic acid, two other isomers were present. The compounds under consideration are 9-octadecenoic acid, commonly known as (E)-Elaidic acid, and 6-Octadecenoic acid, also known as Petroselinum acid. These compounds are classified as the trans and cis isomers of oleic acid, respectively. The compound that ranked third in terms of abundance was 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (also known as methylated linoleate), comprising 17.62% of the total area.

The substance in question is a methyl ester derived from linoleic acid, a polyunsaturated omega-6 fatty acid. Linoleic acid is classified as an essential fatty acid, which is a type of fatty acid that humans are unable to synthesize and therefore must acquire through dietary means.

The compound identified as 1H-Indene, 2-butyl-5-hexyloctahydro- (Indane) was found to be the fourth most prevalent compound, comprising 13.58% of the total area.

This particular compound is classified as a hydrocarbon and is a member of the indane family. Indanes, an aromatic compounds class, are characterized by the presence of a ring of five-members fused to a benzene ring.

The compound that ranked fifth in terms of abundance was 9,12-Octadecadienoic acid (Z,Z)-, linoleic acid as commonly known. This particular compound constituted approximately 15.22% of the total area. The compound in question bears resemblance to the aforementioned linoleic acid, albeit lacking the presence of a methyl ester group.

The compound identified as n-Hexadecanoic acid (palmitic acid) was found to be the sixth most abundant compound, representing 3.9% of the total area.

This compound is a saturated fatty acid that is extensively distributed in the natural environment. In addition to the six prominent peaks, there were three additional minor peaks observed, which corresponded to the compounds 9,17-Octadecadienal, (Z)-(cinnamaldehyde), (+)-(Z)-1,9-Hexadecadiene, and (+)-(Z)-1-Hexadecene.

The above-mentioned compounds exhibit characteristics of aldehydes and alkenes, displaying a range of aromatic and unsaturated properties. Cinnamaldehyde is a widely recognized compound utilized as a flavoring agent, possessing notable properties such as anti-inflammatory, antimicrobial, antidiabetic, and anticancer effects. Hexadecadiene and hexadecanes are hydrocarbons with unsaturated bonds, which have been documented to possess insecticidal and antifungal characteristics.

During the GC-MS analysis, it was observed that certain compounds exhibited co-elution with another compound, resulting in the inability to determine their individual area percentages.

Consequently, it is assumed that the area percentages of certain compounds are equivalent to the compound that was previously reported under that peak. As an illustration, it was observed that 9-Octadecenoic acid, also known as (E)-Elaidic acid, and 6-Octadecenoic acid (Petroselinum acid) exhibited co-elution with Oleic acid within peak 5.

Consequently, it is presumed that their respective area percentages are also 22.33. In a comparable manner, it was observed that 9-Octadecenoic acid (Z)-2,3-dihydroxypropyl ester (specifically, the ester of hydroxy oleic acid) co-eluted with 9-Octadecenoic acid (Z)-2-hydroxy-1-(hydroxymethyl)ethyl ester (also an ester of hydroxy oleic acid) under peak 6. Consequently, the area percentage of the former compound is presumed to be 27.36, mirroring that of the latter compound.

HPLC

The ginger ethanolic extract was subjected to HPLC analysis, revealing the existence of diverse phenolic compounds. The identified compounds, retention times, and amounts are summed up in Table 2.

These phenolic compounds are known to play a significant role in the antioxidant properties shown by ginger. Gallic acid, with a retention time of 10.22 minutes and an area percentage of 20.65, was identified as the predominant compound, comprising 71.25 parts per million (ppm) of the total quantity.

The compound hydroxybenzoic acid, with a retention time of 13.26 minutes and an area percentage of 21.44, exhibited the second highest abundance among the compounds analyzed at a concentration of 49.25 ppm. Ellagic acid emerged as the third most prevalent compound, with a retention time of 4.88 minutes and an area percentage of 21.25%. On the other hand, ferulic acid, comprising 15.49% of the total area and 50.11 ppm in quantity, occupied the fourth position in terms of abundance.

Group	Subgroup	Compound	RT	Area %	Qual
Fatty acids and their derivatives	Saturated fatty acids	n-Hexadecanoic acid (Palmitic acid)	56.056	3.9	99
	Monounsaturated fatty acids	Oleic acid	61.635	22.33	99
		9-Octadecenoic acid,(E)-(Elaidic acid)	-	-	98
		6-Octadecenoic acid (Petroselinum acid)	-	-	95
	Polyunsaturated fatty acids	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (methylated linoleate)	57.022	17.62	91
		9,12-Octadecadienoic acid (Z,Z)- (Linoleic acid)	61.440	15.22	99
	Fatty acid esters	9-Octadecenoic acid(Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester (Ester of hydroxy oleic acid)	62.189	27.36	93
Aldehydes and alkenes	Aldehydes	9,17-Octadecadienal,(Z)-(cinnamaldehyde)	57.022	0.01	90
	Alkenes	(+)-(Z)-1,9-Hexadecadiene	57.022	0.01	89
Hydrocarbons	Indanes	1H-Indene,2-butyl-5-hexyloctahydro-(Indane)	61.212	13.58	60
Total area%:	-	-	-	100	-

Table 1: Compounds and groups they belong to, along with their retention time (RT), area percentage (Area%), quality index (Qual) as detected in the ginger (*Zingiber officinale* Roscoe) ethanolic extract by GC-MS.

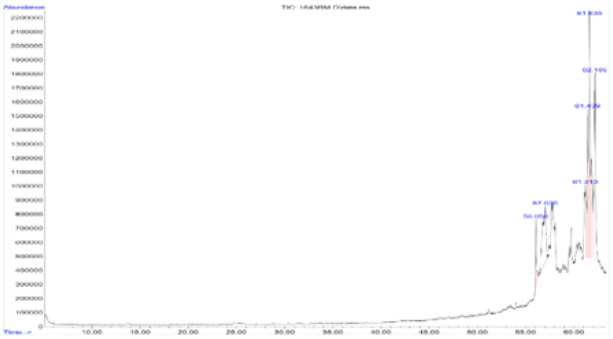

Figure 1: Total ion chromatogram (TIC) of ginger ethanolic extract analyzed by GC-MS. Peaks correspond to various compounds identified in the extract, categorized into their respective chemical groups. The compounds are categorized into fatty acids, aldehydes, alkenes, and hydrocarbons.

Antioxidant Activity

The activity of antioxidant of the ethanolic ginger extract was assessed by DPPH free radical-scavenging assay. The results, presented in Figure 3, show activity

of DPPH radical scavenging and IC50 values for both the ginger ethanolic extract and vitamin C.

Compound	RT	Area	Height	Area%	Height%	W05	Quantity	Qual
Gallic acid	10.22	3841.22	714.56	20.65	20.14	0.33	71.25 ppm	99
Hydrobenzoic acid	13.26	2521.69	412.66	21.44	21.44	0.25	49.25 ppm	99
Ellagic acid	4.88	2854.11	458.99	21.25	21.55	0.35	20.25 ppm	99
Ferulic acid	7.86	1632.00	248.99	15.49	15.23	0.14	50.11 ppm	99
Caffeic acid	3.10	1526.48	325.68	19.15	19.20	0.24	11.58 ppm	99

Table 2: Identified compounds in the ginger ethanolic extract, with their retention times (RT), peak areas and heights, relative percentages, peak width (W 05), quantified concentrations (ppm), and identification quality (%), as determined by HPLC.

The ginger extract displayed a dose-dependent increase in DPPH radical scavenging activity, highlighting its antioxidant potential. The IC50 values for the ginger ethanolic extract and vitamin C were 65.1 µg/mL and 52.54 µg/mL, respectively.

These findings indicate that vitamin C has a slightly higher antioxidant capacity than the ginger extract, which served as the positive control.

According to the classification of antioxidant activity based on IC50 values proposed by Jun et al. (2003), both ginger extract and vitamin C demonstrated a strong antioxidant activity.

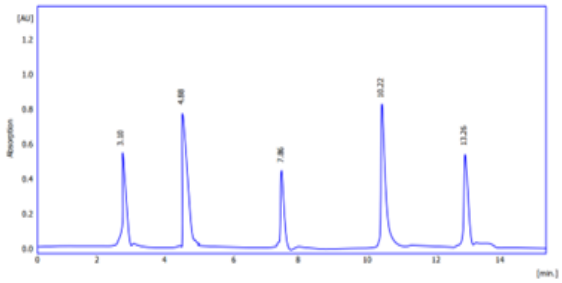

Figure 2: High-performance liquid chromatography (HPLC) chromatogram of ginger ethanolic extract recorded at 280 nm. Peaks are labeled with their respective retention times and identified compounds: caffeic acid (3.10 min), ellagic acid (4.88 min), ferulic acid (7.86 min), gallic acid (10.22 min), and hydrobenzoic acid (13.26 min), representing the phenolic compounds present in the extract.

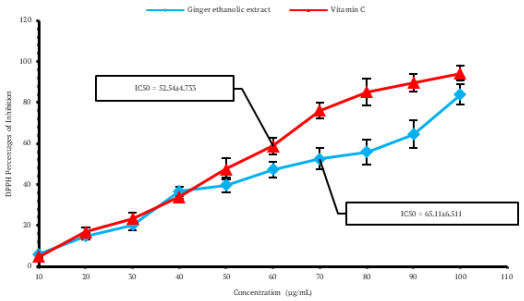

Figure 3: DPPH radical scavenging activity and IC_{50} values (Mean±SEM, n=3) of ginger ethanolic extract and vitamin C. Antioxidant activity of ginger ethanolic extract and vitamin C against DPPH free radical.

Discussion

Yield

The yield of ginger extract is an important parameter that reflects the efficiency of the extraction process. The extraction process in current study of ginger root yielded 18.2% of a thick paste dark-brown paste-like thick sauce texture, with a pungent bitter taste Similar result was reported by [10], who reported a black thick extract with 19.405% yield mango ginger extracted by 70% ethanol in a maceration method. In a study conducted by [11] the acetone ginger extracts obtained from two different geographically sourced ginger, Iraqi ginger and Jamaican ginger, were in the form of a yellowish-brown concentrate, with a lower yield for Iraqi ginger (0.841%) compared to Jamaican ginger (1.069%). The authors suggested this difference could be related to the differences in cultivation conditions [12]. Factors such as the type of solvent used, drying method, extraction time, temperature, and particle size of grinded ginger can all affect the yield [13-16].

Phytochemicals

GC-MS

The GC-MS analysis identified various phytochemicals in the ginger ethanolic extract, including fatty acids, alkenes, aldehydes, and indene derivatives, all falling into five distinct groups. Most significantly, fatty acids emerge as the preeminent category, constituting 45.45% of the identified compounds. These findings align harmoniously with prior research highlighting ginger's rich repository of bioactive components, notably fatty acids, and hydrocarbons [17,18&19] zingiberene, curcumene, α-Bergamotene, gingerol, zingerone, Caryophyllene and elemene [20-22].

Among the identified fatty acids are n-Hexadecanoic acid (palmitic acid), 9,12-octadecadienoic acid (Z,Z)-(linoleic acid), oleic acid, and 9-octadecenoic acid (E)-(elaidic acid). According to [17], ginger oil analyzed by GC-MS exhibited the presence of linoleic acid (79.3%), 9,12-Octadecadienoic acid (Z,Z) (15.22%), palmitic acid (12.3%), and 12-hydroxy-oleic acid (2.8%). Such fatty acids have been documented for their various bioactivities, involving antioxidative, anti-inflammatory, and antimicrobial attributes [22, 23]. Notably, oleic acid, characterized as a monounsaturated fatty acid, has garnered recognition for its potential cardiovascular benefits and facilitation of improved insulin sensitivity [24]. With these fatty acids, ginger ethanolic extract may have beneficial health effects and effective therapeutic applications.

Additionally, alkenes and aldehydes, both accounting for 9.09% of the ginger ethanolic extract, appear as prominent constituents within the ginger ethanolic extract. The established anti-inflammatory and antioxidant attributes of (+/-)-(Z)-1,9-Hexadecadiene

and 9,17-Octadecadienal (Z-) [25] add a layer of significance to these findings. Hence, the ginger ethanolic extract holds promise as a potential source of antioxidants to alleviate oxidative stress and inflammation, aligning with previous in vivo studies [26-31].

One of the noteworthy components of the ginger ethanolic extract, as found by GC-MS analysis, is 1H-indene,2-butyl-5-hexyloctahydro-. This indene derivative accounted for 9.09% of the compounds that were identified. Indenes have been found to possess several biological properties, like anti-cancer, anti-inflammatory, and antibacterial actions [32]. The existence of this indene derivative in ginger extract provides support for the putative medicinal properties of the spice.

Further constituents such as zingiberene, curcumene, α-Bergamotene, gingerol, zingerone, Caryophyllene, and elements have also been previously reported [20,21&2] However, in the current study, such compounds were not detected. There are several possible reasons for this discrepancy, as abundant published works suggest. The composition of phytochemicals in ginger can vary depending on various factors, such as the ginger variety, conditions of growing, cultivation, storage, and processing methods [1].

Collectively, and most importantly, it has been reported that the composition of ginger rhizomes fluctuates in metabolite, elemental, pharmacological, and nutritional value composition depend on the original geographical area [22, 17]. It was suggested that ginger grown on ecological plantations exhibits enhanced nutritional and pharmacological properties, accompanied by reduced concentrations of toxic elements, in comparison to ginger from conventional cultivars [17]. The study further demonstrated that ginger cultivars from various regions differ markedly in both their elemental and metabolite profiles. Ginger cultivated on ecological plantations in Japan was found to contain significantly higher levels of macro- and trace elements essential for human nutrition, while also exhibiting lower concentrations of heavy metals—specifically cadmium, lead, and nickel—when contrasted with samples from Nigeria, Australia, and China.

Additionally, the yield of ginger extract is an important parameter that reflects the efficiency of the extraction process. The extraction process in the current study of ginger root yielded 18.2% of a thick, dark-brown paste-like sauce with a pungent bitter taste. A similar result was reported by [10], who reported a black thick extract with a 19.405% yield of mango ginger extracted by 70% ethanol in a maceration method. In a study conducted by [10], the acetone ginger extracts obtained from two different geographically sourced ginger, Iraqi ginger, and Jamaican ginger, were in the form of a yellowish-brown concentrate, with a lower yield for Iraqi ginger (0.841%) compared to Jamaican ginger (1.069%). The authors suggested that this difference could be related to the differences in cultivation conditions [12].

Factors such as the extraction method (type of solvent used, form of ginger [fresh, drying method], extraction time, temperature, and particle size of ground ginger), part of the plant used (root, leaves, stem), and its age can all affect the yield [13,14,15,16; 33&34]

Overall, the wide variety of chemical substances found in ginger, including fatty acids, alkenes, aldehydes, and indene derivatives, indicates the broad potential of ginger for various biological activities. The aforementioned findings provide additional support for the recognized significance of ginger as a rich botanical resource in both traditional and contemporary therapeutic applications.

HPLC

The phytochemical results in the current study of ginger ethanolic extract showed a different range of bioactive compounds. The presence of phenolic compounds in the ginger extract was measured in HPLC. These compounds (caffeic, ellagic, ferulic, gallic, and hydroxybenzoic acids) are known for their antioxidant, anti-inflammatory, and antidiabetic properties [35,36]. Caffeic and ferulic acids (a hydroxycinnamic derivative), and ellagic (a polyphenolic compound) have demonstrated antioxidant, anti-inflammatory, and antidiabetic activities [35,37,38]. The most abundant compound, gallic acid (71.25 ppm), a phenolic acid, is widely documented for its antioxidant and antidiabetic effects [36]. The hydroxybenzoic acid (49.25 ppm), an aromatic acid, is primarily noted for its antioxidant properties [37].

Identification phenolic components in ginger rhizomes have been reported in different studies. For example, benzoic acid (33.31 mg/100 g), ferulic acid (11.41 mg/100 g), and caffeic acid (6.71 mg/100g) as key phenolic constituents were reported by [38]. Tohma et al. (36) detected gallic acid at 39.6 mg/kg, caffeic acid at 91.2 mg/kg, ferulic acid at 224.7 mg/kg, and p-hydroxybenzoic acid (29.4 g/kg), while ellagic acid was not found in their analysis. These findings highlight the diverse phytochemical profile of ginger and its potential therapeutic properties [38]. Neither study detected ellagic acid, however, other phenols were detected in both studies were not detected in the current one, for example vanillin (89.4 g/kg, Tohma et al., 92020) vs 11.83 mg/100 g). These variations

highlight the potential influence of factors such as sample preparation, geographical origin, and analytical methods on the measured phenolic content in ginger rhizomes. According to [39], it was observed that normal and back plants, despite belonging to the same family and sharing similar morphological characteristics, exhibit a discernible biosynthetic pathway for the synthesis of their primary chemical constituents. Therefore, gingerol-associated phenolics were absolutely recognized in regular ginger, whereas methoxyflavones were obtained in black ginger.

Antioxidant

The DPPH assay is a well-fixed method for assessing the antioxidant activity of different substances, involving plant extracts. The method acts by measuring the reduction of the steady DPPH radical into its corresponding hydrazine form when antioxidants donate hydrogen or electrons, subsequent in a decrease in absorbance at 517 nm. This reduction means causes the DPPH radical, a stable nitrogen-centered free radical, to alter the color from violet to yellow. Antioxidants, or radical scavengers, are compounds that assist this reaction [8].

The effectiveness of antioxidants is determined by the percentage of inhibition at different concentrations, where higher inhibition values indicate stronger antioxidant activity. The antioxidant capacity is further evaluated using the IC50, a concentration required at which 50% of DPPH radicals are inhibited. A lower IC50 value reflects a stronger antioxidant potential.

The results of this study showed antioxidant activity of ginger extract and vitamin C increased with concentration, where the IC50 for the extract and vitamin C were 65.11 µg/mL and 52.54 µg/mL, respectively, indicating that vitamin C exhibits slightly stronger antioxidant activity compared to the ginger extract. This suggests that, under the experimental conditions, vitamin C, as it has a higher amount of hydroxyl groups [40], has a stronger antioxidant activity than the ginger extract [This indicates the ginger extract has antioxidant activity similar to vitamin C]. Comparing these results with the literature, [41] reported a maximum DPPH assay result of 65.30% for the ethanolic extract of ginger, which is close to the IC50 value reported here for the ginger extract. This suggests a similar antioxidant capacity in these two studies. Additionally, [21] reported that the DPPH assay of ginger in ethanol extract ranged up to 79%, which is close to the highest percentage of inhibition (83.904%) observed in the current study. [39] conducted antioxidant activity by DPPH assay of methanolic and aqueous extracts of 5 medicinal plants lemon balm, Asian pigeonwings, lemongrass, turmeric, and ginger. The authors reported that ginger aqueous extract had the highest antioxidant activity (88.05±0.31%). Although the authors in their study did not mention at which concentration this reduction was, neither using vitamin C as a positive control, the results were in line to ones reported in this study. In contrast, the work of [42&43] reported an extremely high DPPH assay result of 90.1% for ginger, although the concentration used (9 mg/mL) is considerably higher than the ranges tested in the current study (10-100 µg/mL). [36] reported that the ethanol and water extract of ginger scavenged 43.8% and 16.2% of DPPH free radicals at 30 µg/mL concentration, respectively. Using the same concentration, in this study, 20.21% DPPH free radicals were scavenged. In a study conducted by [44], scavenging activities against free radical in DPPH assay of ginger methanolic extract were found to be 39.6% at 0.25 mg, 64.7% at 0.5 mg, 77.6% at 0.75 mg, and 84.4% at 1 mg. Such results were within the range of our results (5.993% - 83.904%). [45] reported that essential oil of ginger in fresh form significantly inhibited 83.03% DPPH radicals, reaching up to at 240 µg/mL, which is higher than that reported in our study. Similarly, [46] found that a ginger oil extract inhibited 50% of DPPH radicals at 200 µg/mL concentration, a much higher concentration than the IC50 for the ginger extract in the present study. This suggests that the ginger oil may have a lower antioxidant activity than the ginger extract tested here, which was from dried ginger. On the lower end of the scale, [47] reported a DPPH assay result of just 7.8% for ginger rhizome, suggesting a considerably lower antioxidant activity than observed in our results. [48] used DPPH radical scavenging assay to evaluate ginger antioxidant activity (as ethanolic extract) and reported 12.43±3.84% DPPH free radical scavenging at 100 µg/mL increased to 58.52±0.21% at 1000 µg/mL. Similarly, lower antioxidant activity compared to current results was reported in a study conducted by [34], in which the ethanolic, methanolic, acetone, and ethyl acetate extracts of ginger exhibited 50% DPPH free scavenging of radicals activity at 0.499, 0.481, 0.654, and 0.501 mg/mL, respectively. These values were compared to the IC50 value of vitamin C, which was found to be 0.239 mg/mL.

While there is some variability in the reported DPPH assay results for ginger extracts in the literature, the study results of were generally in line with those of other studies, particularly when considering studies that used ethanolic extracts. Some variations in the results can be attributed to differences in extraction methods, plant parts used, and concentrations tested in different studies. The information confirms the antioxidant potential of ginger root ethanolic extracts, with them proving effective in inhibiting DPPH

radicals. Antioxidant activity could be present in phenolic compounds [36].

Recommendations

The current investigation examined the phytochemicals present in *Zingiber officinale* Roscoe's rhizomes and their antioxidant activity. Application of GC-MS and HPLC techniques encompassed a thorough analysis of bioactive compounds, such as unsaturated fatty acids, phenolic compounds, and indene derivatives. Application of the DPPH assay showed free radical-scavenging activity of the extract, proving its therapeutic value. The antioxidant and health-promoting activity of the detected compounds' potential therapeutic value necessitates future investigation according to these observations. The inclusion of ginger extract in functional foods and drugs could be a feasible direction for investigation. In vivo studies will be important for confirming the antioxidant and health-promoting activity of ginger extract in a physiologic environment.

Author Contributions

Conceptualization: Rasema M Hameed and Alaa K Mahmood; methodology: Rasema M Hameed; investigation: Rasema M Hameed and Alaa K Mahmood; data analyses: Rasema M Hameed; writing—original draft preparation: Rasema M Hameed; writing—review and editing: Rasema M Hameed and Alaa K Mahmood; supervision: Alaa K Mahmood. Both of the authors read and agreed to the published version of this manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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