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Investigation of Antioxidant Compounds in Ginger (Zingiber Officinale) for Cancer Treatment

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Abstract

Antioxidants, which eliminate reactive oxygen species (free radicals), are present in a wide range of food items and are generally known as scavengers. Antioxidants are crucial for maintaining human health as the body's natural defensive mechanisms are unable to function properly when exposed to excessive oxygen stress. Among herbs and spicy Ginger (Zingiber officinale Roscoe) extract and its components have anti-proliferative activity, anti-inflammatory, antioxidant and best as chemo-preventive agent. Prior research on in vitro examinations of ginger formulations and certain isolated components revealed that ginger exhibits anti-inflammatory properties, including the inhibition of Cox and the inhibition of nuclear factor KB. Additionally, it triggers apoptosis in endometrial cancer cells by activating P53. The aim of this study is to find components of ginger which lay path to overcome or kill Cancerian cells, avoiding chemo or radiation-based methods of curing. The Phenolic compounds were extracted utilizing methanol, Ethanol, Chloroform and ethyl acetate. High performance liquid chromatography (HPLC) is used to analyze the compound 2, 2-diphenyl-1, picrylhydrazyl (DPPH). Phenolic compounds were then analyzed utilizing HPLC and DPPH array. This result shows ginger extract have number of anti-oxidants, which can further be used for cancer treatment. The purpose of this paper is to study the anti-oxidant and anticancer compounds in the extract of Zingiber officinale for cancer research. Simultaneously, these results illustrate that ginger extract has vigorous anticancer activity against cancer.

Keywords: Antioxidants and anticancer compounds; Zingiber officinale; High performance liquid chromatography; 2, 2-diphenyl-1; picrylhydrazyl (DPPH).

Introduction

Antioxidants are acknowledged for their capacity to enhance wellness and reduce the likelihood of developing cancer, hypertension, and heart disease [1]. Antioxidants are substances that possess the ability to diminish the causes or consequences of oxidative stress. The identification of antioxidant activity in plants has been conducted by several researchers [2-4].

Oxidative stress may arise from several sources such as environmental factors, sickness, infection, inflammation, and the natural process of aging via the generation of reactive oxygen species (ROS). ROS contain free radicals and other oxygenated molecules that arise as a consequence of these processes. The human body naturally manufactures some endogenous antioxidants, but, the consumption of dietary antioxidants may provide an extra layer of protection. Flavonoids, polyphenolics, Vitamins C and E, and carotenoids are the predominant dietary antioxidants. The primary emphasis of the naturally occurring antioxidant is on food

plants, particularly herbs and spices [5]. Herbs and spices include phenolic chemicals, ascorbic acid, and carotenoids, which are known to have strong antioxidant characteristics [6].

Oxidative stress arises when the production of free radicals or ROS exceeds the antioxidant capacity of a biological system [7]. This phenomenon is implicated in heart diseases, neurodegenerative diseases, cancer, and the aging process [6]. There is increasing evidence suggesting that oxidative damage has a role in the development of chronic, age-related degenerative illnesses. However, dietary antioxidants have been shown to counteract this damage, hence reducing the risk of disease [8]. Antioxidants are compounds that, in small amounts relative to the material being oxidized, effectively slow down or block the oxidation process [9]. The word "antioxidant" has several meanings. An antioxidant is a chemical that counteracts oxidation or inhibits processes facilitated by oxygen or peroxides. In biological terms, antioxidants are compounds, whether they are artificially created or found in nature, that have the ability to hinder or postpone the decay of a product. Additionally, they may counteract the harmful consequences of oxidation in animal tissues. An agent that effectively mitigates the detrimental impacts of reactive species, such as ROS or RNS, on the normal physiological processes in humans. More than 75% of the global population continues to depend on plants and plant extracts for their healthcare needs [10].

Historical background

In 1879, Tresh [11] extracted a concentrated, oily, and pungent substance from ginger oleoresin, which he named gingerol. In 1917, researchers from England and Japan separately extracted two strong-smelling chemicals from ginger, known as gingerol and zingerone. The reference is from [12]. In 1927, a Japanese group released a detailed analysis of shogaol, a molecule found in ginger that has a strong taste and smell. The substance was named after the Japanese word for ginger, "shoga" [13, 14].

A multitude of analytical methods have been used to identify a minimum of 115 components in both dried and fresh ginger species. Gingerols are the primary components of fresh ginger and are significantly diminished in dried ginger. In contrast, shogaols, which are the primary results of gingerol dehydration, are more abundant in dried ginger compared to fresh ginger [15]. A total of 31 gingerol-related chemicals have been discovered in the methanolic crude extracts of freshly harvested ginger rhizome [16]. Ginger has been divided into a minimum of 14 bioactive compounds, namely (6)-gingerol, (4)-gingerol, (10)-gingerol, (8)-gingerol, (14)-shogaol, (6)-paradol, 1-dehydro-(10)-gingerdione, (6)-shogaol, tetrahydrocurcumin, (10)-gingerdione, methoxy-(10)-gingerol, hexahydrocurcumin, gingerenone A, and 1,7-bis-(4′ hydroxyl-3′ methoxyphenyl)-5-methoxyhepthan-3-one [17]. The composition of ginger in a given sample is influenced by factors such as the nation it comes from, the commercial processing it undergoes, and whether it is dried, fresh, or processed [18]. Among the bioactive compounds that give Jamaican ginger its pungent taste, such as (6)-, (8)-, and (10)-gingerols and (6)-gingerol, (6)-shogaol is found to be the most prevalent pungent bioactive ingredient in the majority of the oleoresin samples examined [19]. Phylogenetic study has shown that ginger samples from various geographic locations cannot be genetically differentiated. However, metabolic profiling has revealed specific quantitative variations in the levels of (6)-, (8)-, and (10)-gingerols [20]. A study conducted by Schwertner et al. [21] analyzed the levels of (6)-, (8)-, and (10)-gingerols and (6)-shogaol in 10 ginger-root dietary supplements obtained from various health food shops and pharmacies. The findings of the study were concerning.

A multitude of plants from the Zingiberaceae family, often known as the ginger family, have a long-standing record of being used for therapeutic purposes in traditional medicine systems. Ginger (Zingiber officinale) is the most well-known plant that has been extensively studied in terms of its pharmacological and therapeutic properties over the last thirty years [22, 23]. Ginger, scientifically known as Zingiber officinale Roscoe, is a plant that resembles a reed and is incapable of reproducing sexually. It has a strong and fragrant underground stem called

a rhizome, which it depends on for asexual reproduction. The exact origins of this species are uncertain; however, it is thought to have originated from either India or South-East Asia [10, 24]. Ginger has an extensive historical background, serving as both a culinary ingredient and a medicinal herb. It is referenced in ancient Sanskrit manuscripts, as well as in classical Arabic, Buddhist, Roman and Greek literature [14]. By the ninth century, it was extensively used in Europe [24]. In 1547, it was first shipped out of Jamaica, where it subsequently became a substantial agricultural product [10]. The cultivation of this plant has expanded to many subtropical and tropical locations such as Africa, India, the West Indies, China, and Australia. In 2000, the global output of this plant was predicted to be over 100,000 tons [25]. The rhizome of ginger, sometimes referred to as Rhizoma Zingiberis in the field of pharmacy, is utilized in numerous traditional medicinal practices such as Traditional Chinese therapy, Ayurveda, and Western herbal therapy [26]. The traditional applications of this substance include a wide variety of ailments, such as flatulence, dyspepsia, nausea, colic, colds, vomiting, migraine, flu, and rheumatic and muscular diseases [26].

2 All About Ginger

2.1 Ginger chemistry

The rhizome of ginger contains secondary metabolites that may be categorized into two main groups: volatile chemicals, which can be extracted using steam distillation, and nonvolatile phenolic compounds. The primary focus is on the principal phenolic compounds, which are known for their pungent qualities. The pharmacological effect of ginger rhizome is mostly attributed to molecules from the non-volatile pungent phenolic family [27].

2.2 Non-volatile compounds

The pungency of ginger is attributed to phenolic chemicals. The primary component found in the fresh rhizome is a group of similar phenolic alkanones called gingerols, along with related compounds like ginger diols. The primary component in these substances is (6)-gingerol, whereas 8- and 10-gingerol are present in smaller amounts [13, 28]. Upon exposure to heat or alkali treatment, gingerols undergo conversion into a sequence of homologous shogaols by dehydration, and into the compound zingerone [13]. The shogaols exhibit a higher level of pungency compared to the gingerols [28]. Gingerols, which are phenolic ketones, may be transformed into shogaols, zingerone, and paradol [29]. These chemicals are accountable for the sense of heat in the mouth [30].

2.3 Gingerols in fresh ginger

6-gingerol is the most pungent chemical found in fresh ginger roots. Ginger also contains other gingerols of different chain lengths (n6 to n10), but 6-gingerol is the most prevalent among them. Shogaols, which are the dried version of gingerols, are present in limited amounts in fresh ginger root and are mostly found in dried and heat-treated roots. Among them, 6-shogaol is the most prevalent [15].

The precise mechanisms by which ginger exerts chemo preventive effects are not fully comprehended, but it is believed to involve the stimulation of carcinogen-detoxifying enzymes [31], as well as the display of antioxidant [32] and anti-inflammatory properties [15]. Ginger has the ability to prevent the activation of nuclear factor-κB caused by different substances. This has been demonstrated in a study developed by Aktan et al. [33] in 2006. Additionally, ginger has been found to decrease the expression of genes regulated by nuclear factor-κB that are participated in cell growth and the formation of new blood vessels, such as interleukin 8 and vascular endothelial growth factor.

2.4 Anti-oxidant activity

6-shogaol has been identified as a very effective chemical found in ginger, with strong anti-inflammatory and antioxidant properties. The analysis of 6-shogaol and 6-gingerol in ginger extract has been studied using many techniques, as stated by Melianita et al. [34]. However, high-performance liquid chromatography (HPLC) is the most often used approach. Ginger

extracts have shown antioxidant properties in several in vitro test methods, including the scavenging of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals.

3. Methodology

The experimental work was done in Laboratory of biotechnology at PCSIR LAHORE.

3.1 Plant material and extraction procedure

Two kilograms of fresh ginger were acquired at a local market in the city of Sargodha. The botany department validated the taxonomic classification of this plant.

The rhizomes were cleansed, cut, and sun-dried for a duration of three days, after which they were pulverized using a grinder.

3.2 Preparation of extracts

A sequential extraction was conducted utilizing solvents with varying degrees of polarity. A total of 25 grams of dry ginger powder was extracted in a sequential manner using chloroform, followed by ethyl acetate, ethanol, methanol, and a mixture of 50% ethanol and 50% water. The extraction process was carried out at room temperature (25±2oC) and normal atmospheric pressure. The samples were shaken at a speed of 100 revolutions per minute for one hour and then left overnight using a Wrist Action Shaker (Figure 3.1). The model being referred to is the Burrell Model 75.

The extract was filtered using Whatman paper, and the operation was repeated. The final filtrate was poured onto trays, placed in an open area, and left to dry for a period of three days (as shown in Figure 3.2). The scratched dried material from the tray is gathered and placed into separate sample bottles. Currently, each sample is quantified at a concentration of 0.05 milligrams, which is dissolved in 10 milliliters of the same solution used to create the extract. Next, vigorously agitate the sample to uniformly dissolve it in the solution. The extract is prepared for further action.

Figure 3.1; Wrist Action Shaker. (Burrell Model 75)

Ginger extracts were subjected to agitation with appropriate solvents in a shaker for about one hour to facilitate extraction. The resulting mixture was then further agitated to get a purer residue.

Figure 3.2-2
Ginger extract prepared with methanol solvent placed in trays for drying.

3.3. DPPH free radical scavenging activity

Antioxidant capacity

The antioxidant activity of natural substances was assessed utilizing the DPPH radical, a stable free radical [35]. The presence of polyphenol components in plant extracts contributes to their antioxidant activity by enabling them to serve as hydrogen or electron donors and to scavenge free radicals [22]. Therefore, the purple hue of 2,2-diphenyl-1-picryl hydrazyl (DPPH) will be transformed into α, α-diphenyl-β-picrylhydrazine, resulting in a yellow coloration [36]. According to Suhaj [37], the scavenging of the stable radical DPPH is regarded a reliable and simple method for assessing the antioxidant scavenging activity.

The inherent existence of antioxidants in plants, as well as their combination with other antioxidants, can result in both an additive effect (where the cumulative effect is expected to be the sum of the individual effects) and a synergistic effect (where the cumulative effect is greater than the sum of the individual effects) [38]. Graversen et al. [39] and Roberts and Gordon [40] discovered that plant polyphenols have a synergistic impact when combined with other antioxidants contained in plant material. Furthermore, further research has been established to examine the combined impact of antioxidants, as shown by studies conducted by Liu et al. [41], Altunkaya et al. [42], and Romano et al. [43].

The test sample, consisting of 25 units out of a total of 5 samples, was combined with a solution containing 0.004% DPPH and methanol. Place 3 milliliters of DPPH into the test tubes. Next, introduce concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 µl into separate test tubes. The mixture was agitated vigorously and allowed to rest for 30 minutes.

Absorbance of the resulting solution was measured at 517 nm in a UV-visible spectrophotometer (Figure 3.4).

Figure 3.3-1 Spectrophotometer (Thermo-Nicolet Evolution 300)
Samples are ready to get their spectrum with the help of Spectrophotometer.

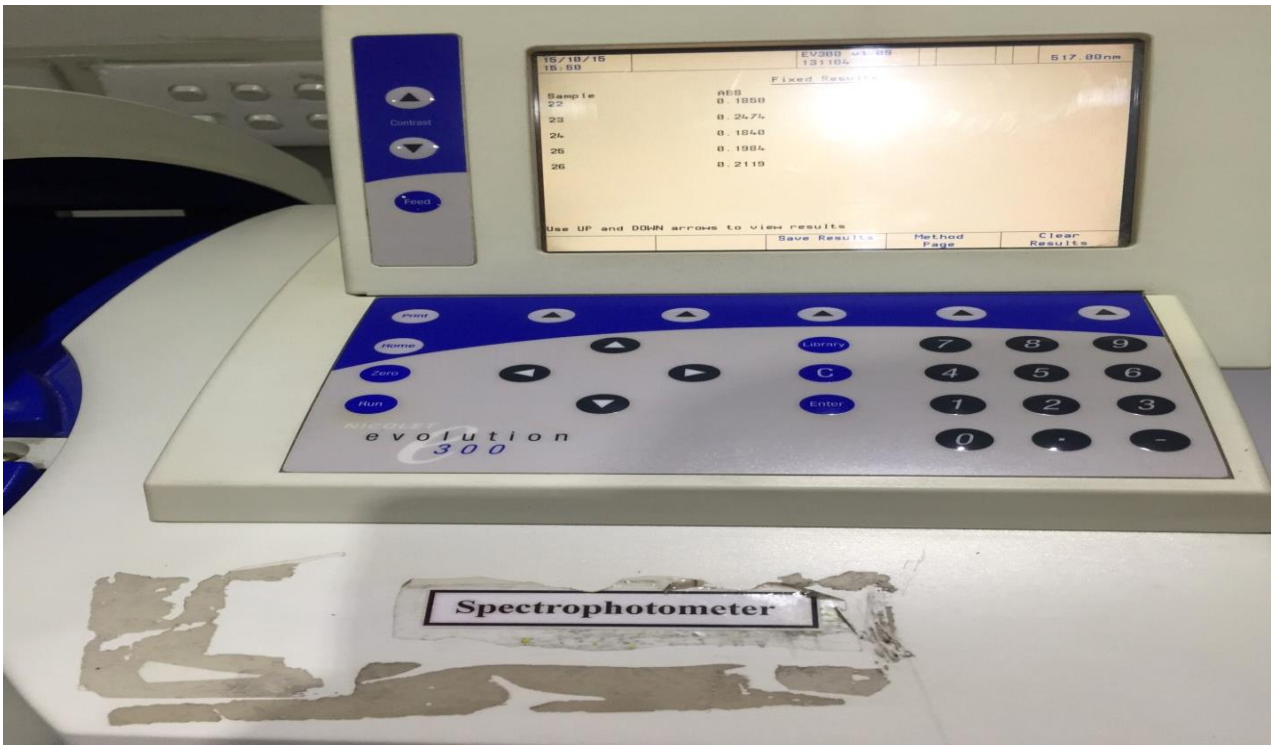

Figure 3.3-2 Monitor of Spectrophotometer.

3.4 HPLC (High performance liquid chromatography).

HPLC is a widely employed method in analytical chemistry and biochemistry for the identification, quantification, and separation of substances.

HPLC equipment consist of a mobile phase reservoir (Figure 3.4-1), a separation column (Figure 3.4-3), a detector, an injector (Figure 3.4-4), and a pump (Figure 3.4-2) for the separation of substances. Compounds are formed by introducing a sample combination onto the column. Various fractions were separated as they traveled through the column due to their distinct partitioning activities between the mobile and stationary phase. Degas the mobile phase to eliminate the production of air bubbles.

The samples for examination were transported in small quantities into the mobile phase stream. The mobility of the analytes inside the column is hindered by particular physical or chemical interactions with the stationary phase. The retardation might vary based on the analyte, as well as the nature of the mobile and stationary phases.

The retention time refers to the moment when a certain analyte is released, and it serves as the unique characteristic used to identify that analyte under specific circumstances.

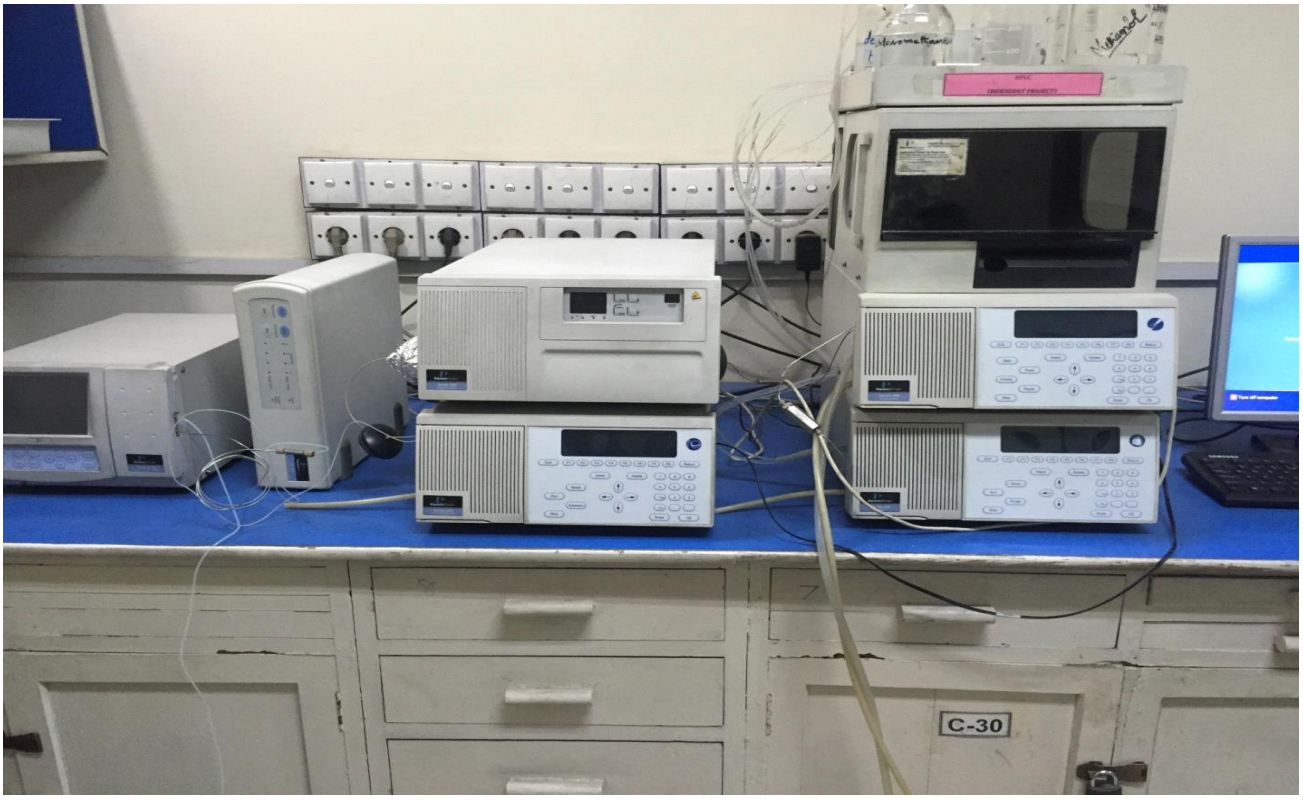

Figure 3.4-1

HPLC (Perkin elmer Series 200) complete system of HPLC with PC (Ws), Link, Pump, Detector, Series 200 Autosampler.

Figure 3.4-2 HPLC.
Perkin elmer series 200 Autosampler and pump.

Figure 3.4-3 HPLC.
Perkin elmer series 200 column oven and Uv/Vs Detector.

Figure 3.4-4 HPLC**.** Detector system.

HPLC analysis

The analysis of plant extracts was conducted utilizing a Perkin Elmer series 200 HPLC instrument equipped with a UV/VIS detector. The detector utilizes a visible tungsten lamp with a wavelength range of 360–700 nm, as well as a deuterium lamp for UV detection with a wavelength range of 190–360 nm. The detector operates by illuminating one bulb at a time. The approach used a wavelength of 228nm. The mobile phase was transferred at a flow rate of 1 mL/min. The volume of the injection was 10 µL. Data was gathered using a UV/visible diode array detector. Retention time is a method used to distinguish various fractions of phenolic chemicals found in ginger. The UV spectra were obtained using pure synthesized standards provided by the Department of Pharmacy at PCSIR. For the creation of standards, we used absolute ethanol (99%) of analytical grade. The quantification of the mentioned substances was performed using standard curves generated using unmodified standards.

Sample preparation

The Fresh rhizomes were washed, then the epidermis was eliminated and they were sliced into roughly 1.5 × 1.5 × 1.5 mm cubes. The sample, weighing 5 grams, was placed in a centrifuge tube. Then, an amount of 99% ethanol that was twice the weight of the sample was added. The preparation was subjected to sonication for almost 20 minutes, followed by centrifugation at a speed of 4000 revolutions per minute for five minutes. The liquid portion was transferred to a transfer pipette and then delivered to a brown glass vial. It was stored at a temperature of 4°C and filtered using a Millipore Automation Certified Filter before being injected onto the HPLC column.

Method

An examination of the extracted samples was performed using reversed-phase HPLC on an Agilent Perkin Elmer series 200 HPLC system equipped with a column. The mobile phase A used in the experiment was HPLC-grade water received from the laboratory. The mobile phase

B, on the other hand, comprised of HPLC-grade acetonitrile and 0.05% trifluoroacetic acid, which were present in both phases. The mobile phase used for gradient elution consisted of solvent A and solvent B, with a volume ratio of 70:30 (v/v) at the beginning and 10:90 (v/v) after twenty minutes. This was followed by a ten-minute period with a constant volume ratio of 10:90 (v/v) between solvent A and solvent B. The mobile phase was transferred at a flow rate of 1.00 mL/min. The injection volume was 10.0 µL, and the column was maintained at a temperature of 40 ºC. The absorption spectra were obtained utilizing a UV/visible diode array detector, covering the wavelength range of 200 to 400 nm, with quantification at 228 nm.

Standards

The synthetic standards of (6)-gingerol, (8)-gingerol, (6)-shogaol, and (8)-shogaol with a purity of 99% were acquired from the Department of Pharmacy, PCSIR (LAHORE). The synthetic standard of [10]-gingerol with a purity of 98% was also obtained from the same source. Every standard solution with a predetermined concentration was dissolved in 99% ethanol and prepared in a 10 mL volumetric flask. A composite standard solution was prepared by combining 100 µL of each separate standard solution. A ten-fold dilution of the mixed standard was used in the investigation. The standard compounds had concentrations ranging from 15 to 25 µg/mL. The identification and recognition of shogaols and gingerols within the sample were determined by comparing their retention duration and UV-spectra with the standards.

The quantification was established using standard curves constructed using pure standards.

Gingerols quantification in test samples

Rhizome ginger extracts were analyzed. The 228nm peak areas, mean concentrations of (6)-, (8)- and (10)-gingerol were calculated. A classic HPLC trace is shown in (Figure.3.4-5).

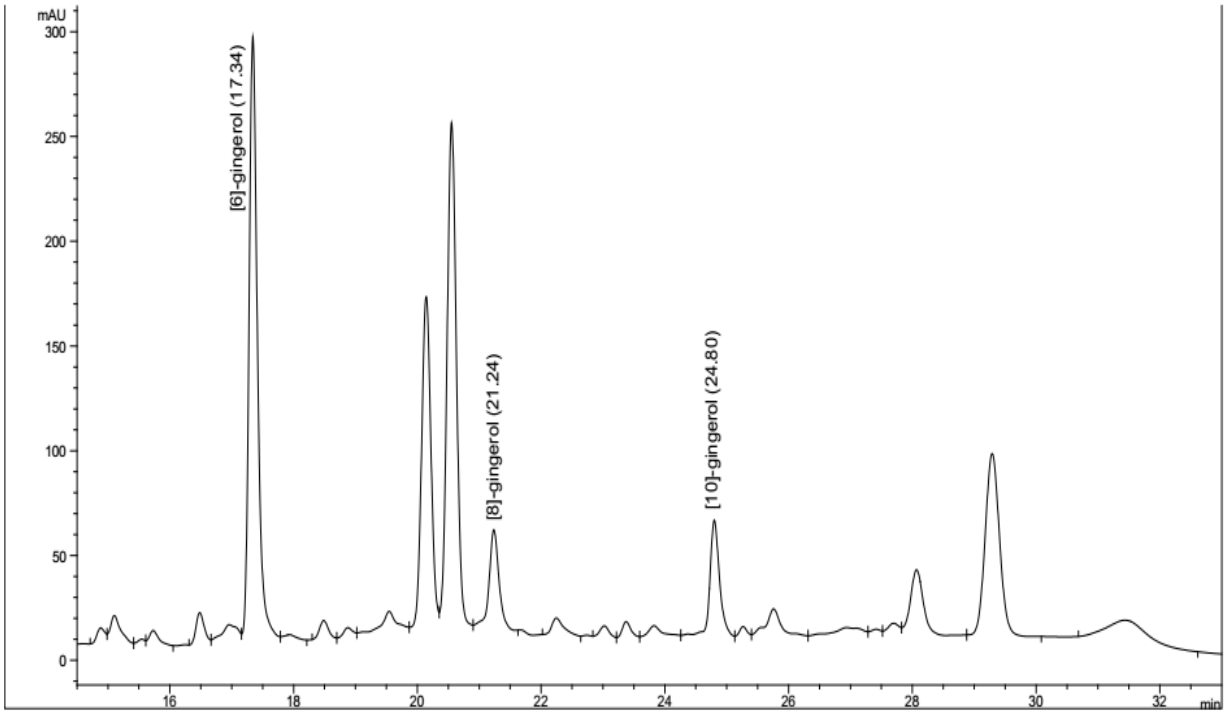

Figure 3.4-5. Ethanol extract of fresh ginger in HPLC Chromatogram

Gingerols stability

The ethanolic extracts were assayed. The (8)- and (10)-gingerol mean concentrations did not modify and thus seems to be constant in ethanolic solution.

Results

25 g of ginger was sequentially extracted using 100 ml of each solvent: chloroform, ethyl acetate, ethanol, methanol, and a mixture of 50% ethanol and 50% water. This process resulted in the extraction of compounds from the ginger.

The selection of an appropriate extraction technique is crucial for the efficient extraction of a diverse spectrum of bioactive molecules, including both polar and non-polar compounds, from plant materials. Each extract was tested for antioxidant properties.

4.1 Solvent V/s active components

The extraction method varies according to the specific bioactive component being extracted. Phenolic acids and glycosides of various flavonoids are typically extracted using water, alcohol, or a combination of water and alcohols. Methanol has been identified as the optimal solvent for extracting flavones, saponins, lactones, polyphenols, and some terpenoids.

4.2 Antioxidant activity of ginger extract

To understand nature and function of anti-oxidant activity of the extracts various techniques like HPLC, DPPH assay was tested. BHA was utilized as a standard for anti-oxidant assay.

4.3 DPPH assay

We own a total of five ginger extracts. Below is a tabular description for each extract, including the percentage of inhibition, while the measurements were collected at 517 nm. The calculation of percent inhibition is determined using a specific formula.

(A blank – A sample/ A blank)* 100

Tabular description at respective concentrations 0.2, 0.4, 0.6, 0.8, 1.0.

For % inhibition (DPPH) of chloroform, ethanol, methanol, ethyl acetate, 50% ethanol + 50% water for ginger extract. Keeping blank as standard with concentration of 1.3639.

Sr #	Sample code	Concentration	Absorbance	% inhibition
1	C1	0.2	0.8513	37%
2	C2	0.4	0.5742	57%
3	C3	0.6	0.4305	68%
4	C4	0.8	0.3160	76%
5	C5	1.0	0.2850	79%

Table 4 .1: % inhibition (DPPH) of chloroform extract of ginger at respective concentrations 0.2, 0.4, 0.6, 0.8, 1.0. Keeping blank as standard with concentration of 1.3639.

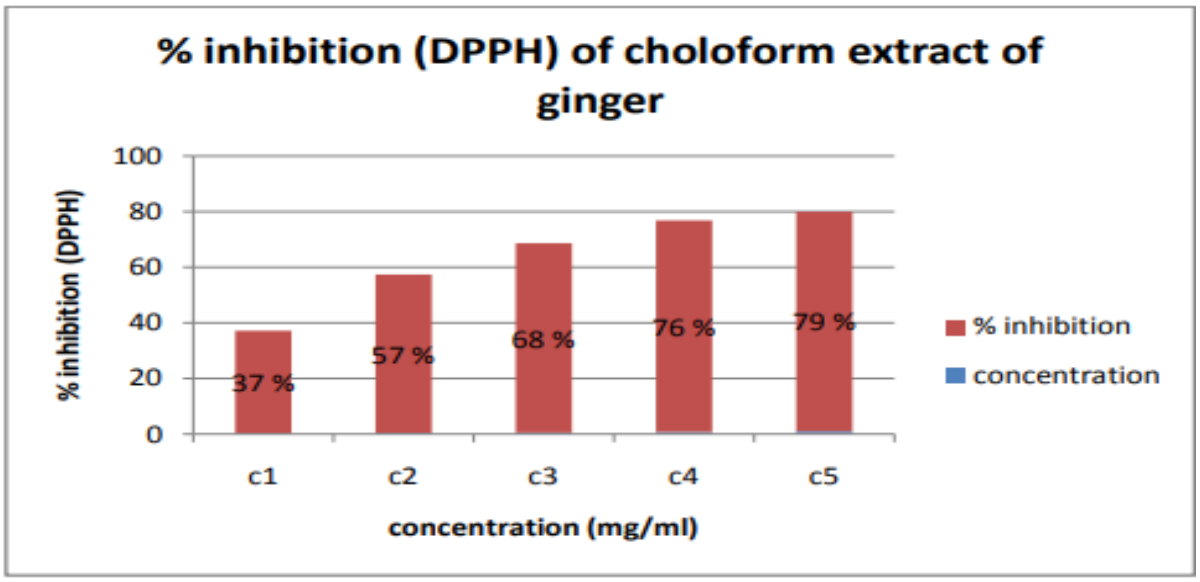

Figure 4.1 % inhibition (DPPH) of ginger extract increases as concentration of chloroform increases.

Sr #	Sample code	Concentration	Absorbance	% inhibition
1	EA1	0.2	0.7811	40%
2	EA2	0.4	0.5736	57%
3	EA3	0.6	0.4318	68%
4	EA4	0.8	0.2653	80%
5	EA5	1.0	0.2474	81%

Table 4.2 % Inhibition (DPPH) of ethyal acetate extract of ginger at respective concentrations 0.2, 0.4, 0.6, 0.8, 1.0. Keeping blank as standard with concentration of 1.3639.

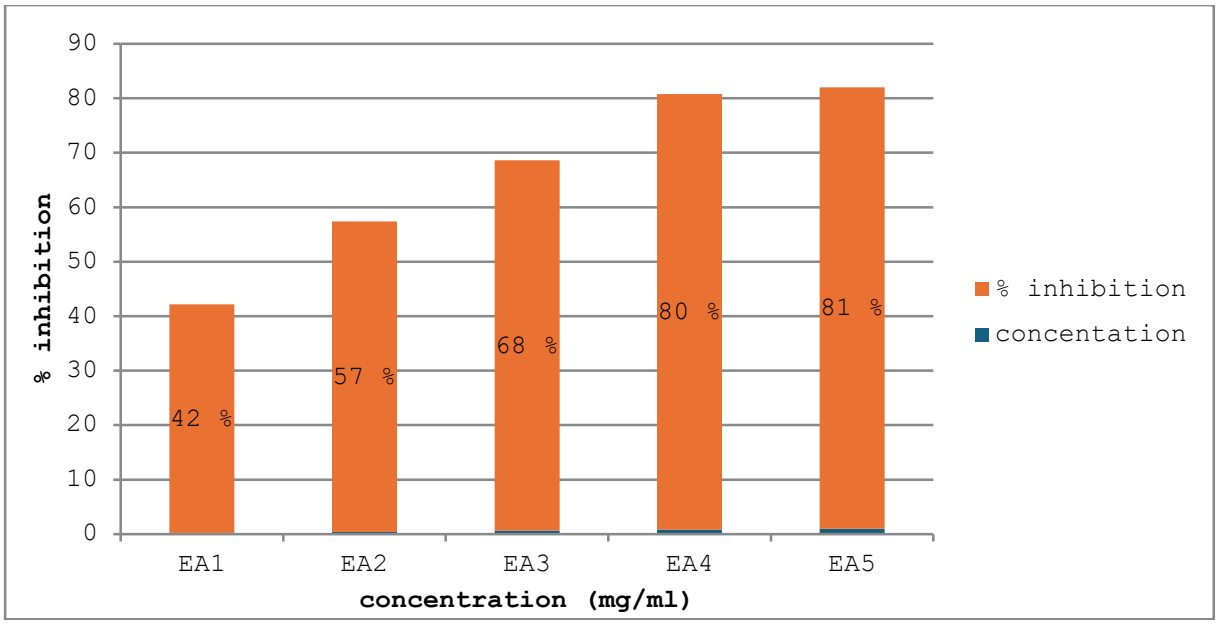

Figure 4.2 % Inhibition (DPPH) of ethyl acetate of ginger extract.
% inhibition (DPPH) of ginger extract increases as concentration of ethyl acetate increases.

Sr #	Sample code	Concentration	Absorbance	% inhibition
1	E1	0.2	0.8017	41%
2	E2	0.4	0.4626	66%
3	E3	0.6	0.3332	75%
4	E4	0.8	0.2434	82%
5	E5	1.0	0.1840	86%

Table 4.3 % inhibition (DPPH) of ethanol extract of ginger at respective concentrations 0.2, 0.4, 0.6, 0.8, 1.0. Keeping blank as standard with concentration of 1.3639.

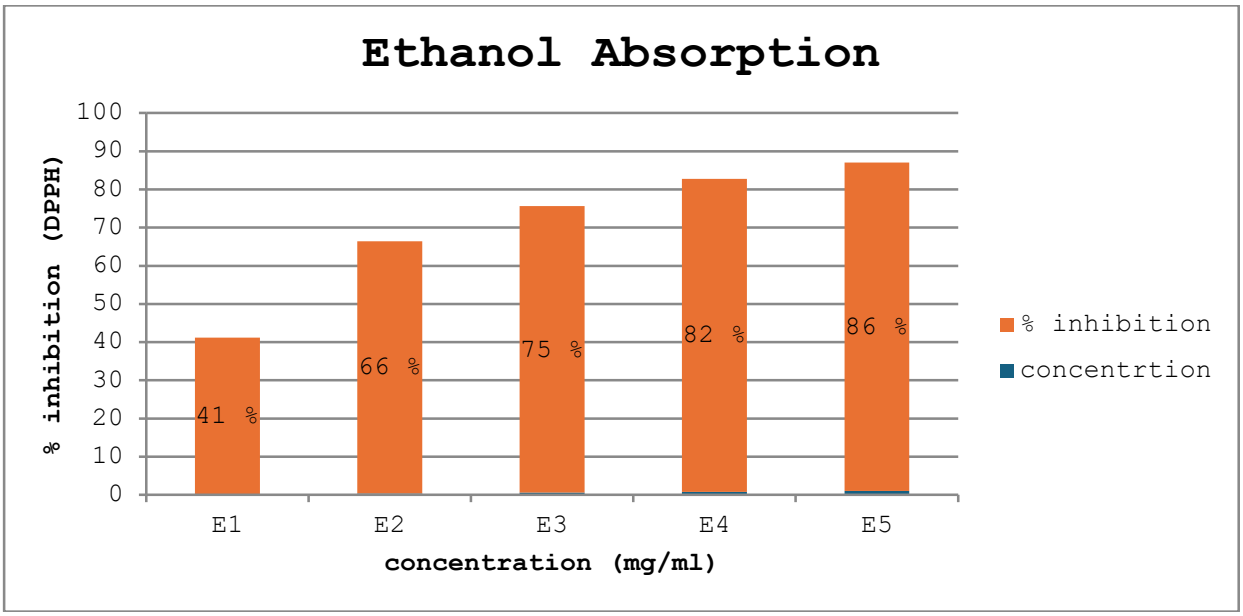

Figure 4.3 % Inhibition (DPPH) of ethanol of ginger extract
% inhibition (DPPH) of ginger extract increases as concentration of ethanol increases.

Sr #	Sample code	Concentration	Absorbance	% inhibition
1	M1	0.2	0.7882	42%
2	M2	0.4	0.4348	68%
3	M3	0.6	0.2829	79%
4	M4	0.8	0.2267	83%

5	**M5**	**1.0**	**0.1684**	**87%**

Table 4.4 % inhibition (DPPH) of methanol extract of ginger at respective concentrations 0.2, 0.4, 0.6, 0.8, 1.0. Keeping blank as standard with absorbance of 1.3639.

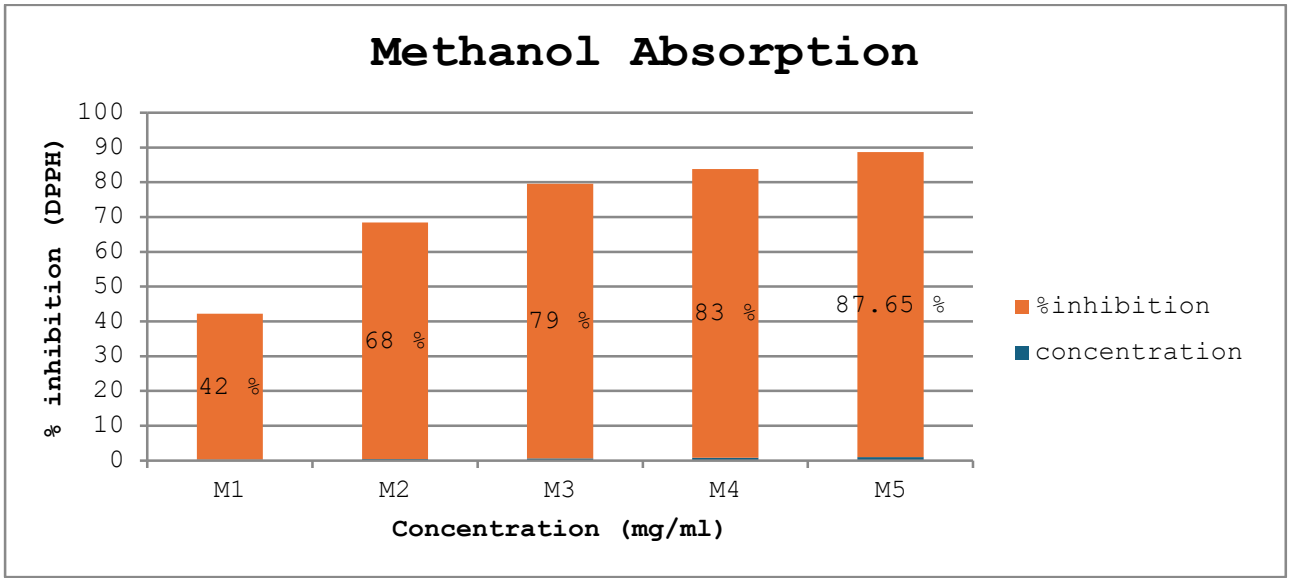

Figure 4.4 % inhibition (DPPH) of methanol of ginger extract.
% inhibition (DPPH) of ginger extract increases as concentration of methanol increase.

Sr #	Sample code	Concentration	Absorbance	% inhibition
1	**E1+H2O**	**0.2**	**0.9869**	**27%**
2	**E2+H2O**	**0.4**	**0.7928**	**41%**
3	**E3+H2O**	**0.6**	**0.6211**	**54%**
4	**E4+H2O**	**0.8**	**0.5567**	**59%**
5	**E5+H2O**	**1.0**	**0.4516**	**66%**

Table 4.5 % inhibition (DPPH) of 50% ethanol and 50% water extract of ginger at respective concentrations 0.2, 0.4, 0.6, 0.8, 1.0. Keeping blank as standard with concentration of 1.3639.

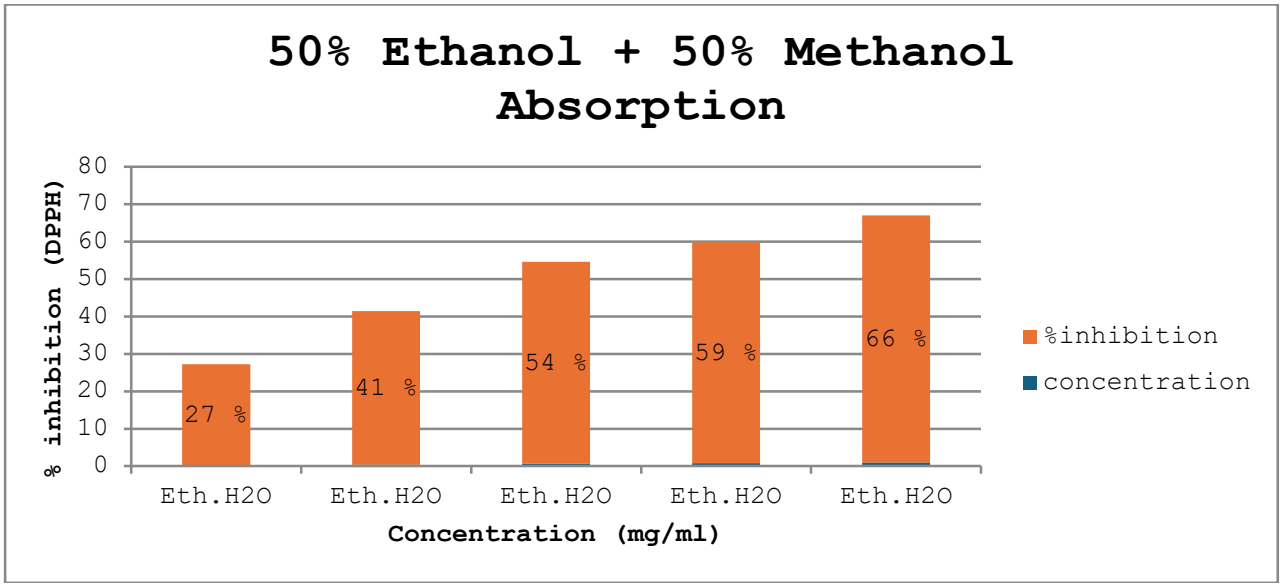

Figure 4.5 % inhibition (DPPH) of 50% ethanol + 50% water of ginger extract. % inhibition (DPPH) of ginger extract increases as concentration of 50% ethanol + 50% water increases.

Sr #	Sample code	Concentration	Absorbance	% inhibition
1	C1	0.2	0.8513	37%
2	EA1	0.2	0.7811	40%
3	E1	0.2	0.8017	41%
4	M1	0.2	0.7882	42%
5	E1+H2O	0.2	0.9869	27%

Table 4.6 % Inhibition (DPPH) of chloroform, ethylene acetate, ethanol, methanol, 50% ethylene + 50% water extract of ginger at respective concentrations 0.2. Keeping blank as standard with concentration of 1.3639.

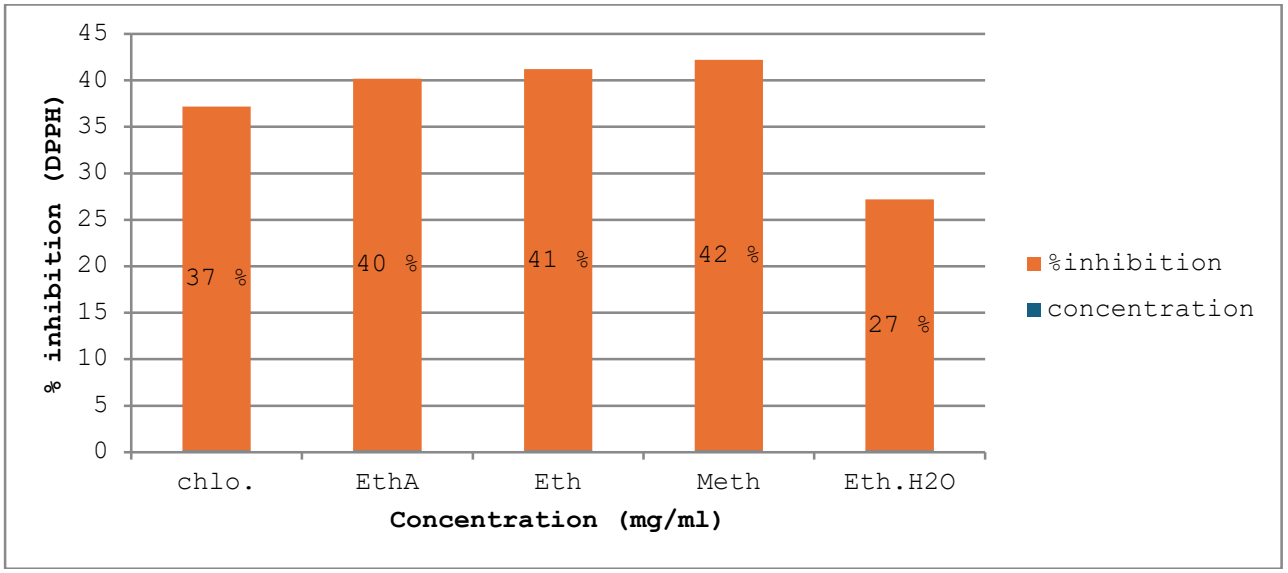

Figure 4.6
% inhibition (DPPH) of chloroform, ethyl acetate, ethanol, methanol, 50% ethanol + 50% water of ginger extract. % inhibition (DPPH) of ginger extract shows relative increase with use of chloroform, ethyl acetate, ethanol, methanol, but decrease abruptly for ethanol and water solvent.

Discussion

Cancer has emerged as the predominant global illness and is now classified as the second greatest cause of death globally, behind cardiovascular disease. Prostate cancer is the most frequent cancer in men, impacting 111 nations, whereas breast cancer is the most prevalent in females, involving 145 countries globally [26]. Approximately 80% of the global population lives in underdeveloped nations [44]. Consequently, 56% of new cancer diagnoses and 64% of cancer-related fatalities occur in these countries [26]. Consequently, cancer is regarded as a significant health peril in Asian nations [45]. Cancer has emerged as the primary cause of mortality in Asian Pacific nations [46], resulting in 3 million new cases and 2 million fatalities across Asia [47].

The exact death rate and number of cancer cases in Pakistan remain uncertain [47]. Considering the absence of a cancer registry database The Karachi Cancer Registry (KCR) was established to ascertain the incidence rates of several forms of cancer in Pakistan from 2000 to 2008 [48]. Ginger has been utilized as a spice as well as medicinal in China and India since ancient times. It was also recognized in Europe starting in the 9th century and in England starting in the 10th century for its therapeutic qualities [49]. Ginger is furthermore utilized to alleviate a range of gastrointestinal ailments, including colic, morning sickness, gas, upset stomach, heartburn, bloating, flatulence, diarrhea, lack of appetite, and dyspepsia (discomfort after eating). Ginger is advised in the Indian Ayurvedic medical system to improve food digestion [50].

In addition to these, ginger has been documented as an analgesic for conditions such as arthritis, muscular soreness, low back pain, chest pain, stomach discomfort, and menstruation pain. It is effective in the treatment of upper respiratory tract infections, bronchitis and cough. According to Shukla and Singh [51], it is advisable to use this substance as an anti-inflammatory treatment for joint issues.

Ginger has antiviral properties that make it effective in treating cold and flu because of its warming impact [52]. Ginger is utilized as a taste enhancer in food and drinks, as well as a scent in soaps and cosmetics [53].

In light of the aforementioned research, attempts have been undertaken to isolate antioxidants from ginger and subsequently use them for cancer management [15].

Research conducted on animal models has demonstrated that ginger and its phenolic components, namely 6-gingerol, effectively inhibit the development of cancer in the skin, gastrointestinal tract, colon, and breast. Ginger extracts have undergone testing to evaluate their antitumor promoting and apoptotic capabilities in various in vitro cell lines, such as gastric, leukemia, ovarian, prostate, and lung cancer. The precise mechanisms by which ginger exerts chemo preventive effects are not fully comprehended, but it is believed to involve the activation of carcinogen-detoxifying enzymes [31], as well as the presence of antioxidant [32] and anti-inflammatory properties [15].

In order to achieve this objective, fresh ginger was procured from the local market at PCSIR Lahore. The ginger was then dried and an extract was made using a series of solvents, including ethyl acetate, chloroform, ethanol, methanol, and a mixture of 50% ethanol and 50% water. The extraction process was carried out at room temperature (25±2oC) at normal atmospheric pressure. A wrist shaker was used, oscillating at a speed of 100 revolutions per minute for a duration of one hour, after which it was left undisturbed overnight. The extract was filtered using Whatman paper and the operation was repeated.

The ultimate filtrate was poured onto trays and there after exposed to the open air for a period of three days to facilitate drying. The dried material from the tray was scraped and then collected in separate sample bottles. Each sample was tested at a concentration of 0.05 mg, diluted in 10 ml of the same solution used to make the extract. Then were shook to dissolve sample in solution homogeneously. The extract was prepared for further action. The presence of antioxidants in ginger was investigated using DPPH test and HPLC activity.

The indigenous entity of antioxidants in plants, as well as their alliance with other antioxidants, can upshot in both a totaling outcome (where the amalgam effect is expected to be the sum of the sole effects) and a synergistic effect (where the commingle effect is greater than the whole of the distinct effects) [38, 39] and Roberts and Gordon [40] search out that plant polyphenols have a synergistic influence when unite with other antioxidants contained in plant material. Furthermore, further exploration has been developed to pursue the combined impact of antioxidants, as shown by studies run by Liu et al. [41], Altunkaya et al. [42], and Romano et al. [43].

A total of 25 test samples, selected from a pool of 5 samples, were combined with a solution containing 0.004% DPPH and methanol. 3 milliliters of DPPH solution were added to the test tubes. Subsequently, concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 µl were applied to each test tubes. The solution was agitated vigorously and allowed to rest for a duration of 30 minutes. The absorbance of the solution obtained was quantified at a wavelength of 517 nm using a UV-visible spectrophotometer. The radical scavenging activity was quantified by assessing the reduction in DPPH absorbance. Higher levels of free radical scavenging activity were indicated by a drop in absorbance. Graphs were generated utilizing the recorded results.

Aims and Objectives

This research encompasses the study of the photochemical properties and biological effects of Ginger. Numerous new compounds have been identified, analyzed, and tested for their biological activity. The objective of this study is to find and isolate active phenolic components in Ginger and assess their antioxidant properties for cancer research using various methodologies. The phenolic compounds that found were subsequently used in vitro experiments involving several cancer cell lines.

Statistical Analysis: Data were statistically elaborated by analysis of variance using the multiple comparison test used in SPSS Version 18. The significance differences were considered at $p<0.05$.

Conclusion

The current treatment approach relies on synthetic medications, such as anti-diabetic, anti-inflammatory, radiation treatments, and chemotherapy, which are effective but also have significant unfavorable side effects. An affordable, efficient, and reliable solution is required to regulate the progression of illnesses by modulating genetic, metabolic, antioxidant, and other related activities. Ginger has significant efficacy in inhibiting NFkB, COX2, and LOX, promoting apoptosis, activating tumor suppressor genes, and regulating several biological processes. The components of ginger contribute to a sense of hope over the unique treatment technique. Further investigation should prioritize clinical studies to examine its efficacy and elucidate its precise function in modulating biological pathways.

Direction of future research

The Zingiberaceae family, consisting of over 1100 species over more than 50 genera, serves as a significant reservoir of secondary metabolites. There is currently little or no information available on the photochemistry or pharmacological characteristics of most of these species. Therefore, there is a significant opportunity for future studies into the family as a whole. Regarding future research stemming directly from the current study, there is much opportunity for exploration. The current treatment approach relies on synthetic medications, such as anti-diabetic, anti-inflammatory, radiation treatments, and chemotherapy, which are effective but also have significant unfavorable side effects. An affordable, efficient, and safe solution is required to regulate the progression of illnesses by modulating genetic, metabolic, antioxidant, and other related activities. Ginger has significant efficacy in inhibiting NFkB, COX2, and LOX, promoting apoptosis, activating tumor suppressor genes, and regulating several biological processes. The components of ginger generate hope over the unique treatment approach. Further investigation should prioritize clinical studies to examine its efficacy and precise involvement in the modification of molecular pathways.

Author's Contributions:

Aisha Iqbal: Methodology, Visualization, Writing–original draft, Writing–review & editing.
Arif Muhammad Khan: Supervision, Methodology, Visualization, Writing–original draft, Writing–review & editing. All authors have read and agreed to publish the manuscript.

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Data Availability

All data generated or analyzed during this study are included in this manuscript.

Competing interests

The authors declare that they have no conflicts of interest to report regarding the present study.

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