

ORIGINAL CONTRIBUTION

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Comparison and HPLC quantification of antioxidant profiling of ginger rhizome, leaves and flower extracts

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Abstract

Background: In the present era, the attention of nutritionist diverted towards the bioactive entities present in natural sources owing to the presence of health boosting perspectives against lifestyle related disarrays.

Methods: In this context, different parts of ginger crop i.e. rhizome, leaves and flower of variety Suravi (ID no. 008) were used for the preparation of ginger extracts with 50% methanol, 50% ethanol and water via rotatory shaker for 45 min. After that, different phytochemical analysis and in vitro analyses were carried out to determine the antioxidant potential of these extracts. Lastly, the best selected extracts from each part was quantified through HPLC.

Results: The results of current investigated indicated that ethanol extract proved to have maximum quantity of phytoceutics as compared to methanol and water. The maximum TPC, flavonoids, flavonols, DPPH assay, antioxidant activity, FRAP assay, ABTS assay and metal chelating potential was observed in ginger leaves as 780.56 ± 32.78 GAE/100 g, 253.56 ± 10.65 mg/100 g, 49.54 ± 1.74 mg/100 g, $75.54 \pm 3.17\%$, $77.88 \pm 3.27\%$, 105.72 ± 4.44 μ mole TE/g, 118.43 ± 4.97 μ mole TE/g and $35.16 \pm 1.48\%$, respectively followed by ginger flowers and ginger rhizome. The lowest antioxidant activity was estimated in ginger rhizome. On the basis of phytochemical profiling and in vitro analyses, ethanol extracts of ginger flowers, leaves and rhizome were selected for the quantification through HPLC.

Conclusion: The findings proved that maximum 6-gingerol was present in ginger leaves (4.9 mg/g) tackled by ginger flowers (2.87 mg/g) and ginger rhizome (1.03 mg/g).

Keywords: Phytochemical screening, In vitro analysis, Quantification, HPLC, Gingerol

Background

Owing to the raising amount of remedies and their side effects on the health stratum of individuals forced the community to replace them with some phytodrugs. The food material that is mostly utilized as the source of nutraceuticals are fruits, vegetables along with a number of spices that are used on daily basis in normal lifestyle. These food commodities enclosed extraordinary number of bioactive moieties that can be illustrated by volatile and non-volatile assay. The phytochemicals extracted

from these foods have numerous medicinal properties [1]. In this context, herbs and spices are mostly used for the purpose of seasoning and to provide taste as well as flavor to the food products. These herbs and spices have already extensively used in almost all folk medicines and traditional food products that were used to improve the health of community. From previous decades, ginger rhizome is being broadly used to treat many lifestyle related disorders [2].

Along with ginger rhizome, ginger leaves and flowers can also be used to demonstrate health benefits of phytochemicals present in them that can compelled their extraction over and above to characterization in many food products to lead them at the rank of designer foods. A number of extraction

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techniques have been used to extract and quantify the bioactive entities of ginger rhizome, flowers and leaves classified as phenolic compounds. These bioactive or phenolic compounds have retained a strong position in inherit and chemical activities of ginger family [3]. In the phytochemistry of plants, phenolic moieties have been recommended as the most important bioactive compound that are liable to be used as health boosting ingredients and can be recommended as antioxidants. These phytochemicals are highly reactive in nature and can oxidize the free radical molecule such as superoxides owing to the scavenging property and can limit the process of lipid peroxidation [4].

For the prophylactic potential of ginger rhizome, flowers and leaves, it is necessary to get the bioactive entities in extracted form from the original source. For the extraction of phytoceutics from ginger parts, the extraction depends upon the particle size of bioactive moiety along with its chemical nature. The rhizome, flower and leaves of ginger based extracts have diverse classes of total phenolic entities that are easily soluble in all the polar solvents. For the purity of bioactive compounds, it is recommended that the ginger especially flower and leaves should be free from fat, waxes and chlorophyll [5].

The yield as well as the efficiency of bioactive entities are depended on the polarity of solvent along with the concentration of flavonoids present in the sample from which it is isolated. The solvent also have strong impact on the quantity as well as quality of total phenolic compounds (TPC) and total flavonoids. According to review, ethanol elucidated highest amount of TPC as compared to methanol, acetone, ethyl acetate and water. However, still more experimental trails are required to determine the effect of solvent on the phytochemical profiling of different parts of ginger crop [6].

After the process of extraction, the chromatographic method are used as technique to save the environment with more accuracy rate, precise and reproducible conclusions such as fast pressure column, gas chromatography and liquid chromatography. Each of these technique, is reliable on the nature of elucidated compound by keeping in view the sensitivity of the instrument. For this purpose, high performance liquid chromatography (HPLC) is a conclusive instrument for the quantification and characterization of bioactive components present in the extracts of different parts of ginger. Different parameters are used in HPLC depending upon the assorted difference in the structure of bioactive entities extracted in extracts such as column temperature, type of detector used, pressure difference as well as wavelength [7].

The quantification of bioactive entities of ginger extracts through HPLC depicted that the most abundant bioactive ingredient was gingerol as 3.436 mg/g however, the amount of gingerol decreased and converted to shogaol by increasing the temperature during the drying process of ginger. Among gingerol series, 6-gingerol was from 1.030–3.046 mg/g in fresh ginger followed by 8-gingerol 0.105–0.312 mg/g and 10-gingerol 0.078–0.425 mg/g [8].

Materials and methods

Procurement of materials

Ginger rhizome, leaves and flowers as raw material with special reference to variety Suravi (ID no. 008) was procured from South China and stored in Functional and Nutraceutical Research Section of NIFSAT, UAF. All the reagents HPLC graded as well as analytical graded along with their standards were ordered from Sigma Aldrich (Sigma Aldrich Tokyo, Japan) and Merck (Merck KGaA, Darmstadt, Germany).

Sample preparation

All the parts of ginger crop (rhizome, leaves and flowers) were cut into small parts up to the required size and then sun dried in maximum sun light timing and then processed to remove all the fatty material and chlorophyll. The resultant 50% dried ginger cuts were analyzed for further chemical properties.

Preparation of ginger extracts

The extracts were prepared by using minimally processed ginger rhizome, leaves and flowers by using 50% v/v methanol, 50% v/v ethanol and water for 45 min as discussed in the protocol of Arutselvi et al. [9] and mentioned in Table 1. Following that, the methanol and ethanol-based extracts were subjected to the rotary evaporator (Eyela, Japan) to eliminate water molecules from extracts and then extracts were stored for further analyses.

Table 1 Treatments for solvent extraction

Treatment	Solvent	Part
T ₁	Methanol	Rhizome
T ₂	Ethanol	Rhizome
T ₃	Water	Rhizome
T ₄	Methanol	Leaves
T ₅	Ethanol	Leaves
T ₆	Water	Leaves
T ₇	Methanol	Flower
T ₈	Ethanol	Flower
T ₉	Water	Flower

Methanol = (50% methanol + 50% water)

Ethanol = (50% ethanol + 50% water)

Phytochemical screening test

Total phenolic contents (TPC)

For the determination of total phenolic contents (TPC) of ginger rhizome, leaves and flowers were measured separately by using Folin-Ciocalteu method as prescribed by Chan et al. [10]. This method is based on the formation of phosphotungstic blue as reduced form of phosphotungstic acid and resulted in increased absorbance owing to the increased number of aromatic phenolic groups. For this purpose, 50 μ L of ginger extracts along with 250 μ L of Folin-Ciocalteu' reagent and 750 μ L of sodium carbonate (20% solution) were added in a washed and dried test tube then the total volume of test tube was raised to 5 mL by the addition of distal water. After that, the sample was stored for 2 h and the absorbance were measured at 765 nm by using spectrophotometer (CECIL CE7200) in contrast with control and blank samples. The results of absorbance were then compared with the standard of gallic acid equivalent (mg gallic acid per 100 g of extract).

Flavonoids

Total flavonoid content of ginger rhizome, leaves and flowers (individually) were determined by using spectrophotometer method in which the flavonoid-aluminum complex was formed as mentioned by Bushra et al. [11]. For the determination of total flavonoid, quercetin was used as standard. For this purpose, 1 mL of ginger extract was added with 5 mL of distal water and 0.3 mL of sodium nitrite (5% v/v solution) in a volumetric flask of 10 mL. After the rest of 5 min, 2 mL of NaOH (1 M solution) and 0.6 mL of AlCl₃ (10% w/v solution) was added. After that. The absorbance was measured at 510 nm by using spectrophotometer. The data was expressed as quercetin equivalent in mg/100 g of extract).

Flavonols

The flavonols were estimated by following the guidelines of Kumaran and Karunakaran, [12]. The standard used was quercetin because it is almost present in all herbs and spices and can be easily extracted in pure form. According to this method, 1 mL of ginger extract of ginger rhizome, flower and leaves was mixed with 3 mL of sodium acetate (5% solution) and 1 mL of aluminum trichloride (2% solution). After the resting time of 150 min, the absorption was measured at 440 nm by utilizing spectrophotometer. The standard used for flavonols was quercetin curve that was estimated by using 1 mg of quercetin in 0.150 to 0.05 mg in 1 mL methanol solution. The same chemicals were used for control and blank samples except any ginger extract. The value for total flavonols was expressed as mg quercetin per 100 g of extract.

In vitro studies

Free radical scavenging activity (DPPH assay)

DPPH as 1, 1-diphenyl-1-picrylhydrazyl is a non-reactive, highly colored and stable oxidizing radical that ended by the formation of DPPH-H (a yellow colored hydrazine) linked up with the abstraction of hydrogen atom in free form from the phenolic compound or/and antioxidant. To depict the DPPH assay of ginger extracts (rhizome, leaves and flowers), the method of Gupta and Prakash, [13], sample solution was prepared by mixing 0.025 mL of ginger extract (separately) in 10 mL of respective solvent in which extract was prepared. Following this, 3 mL of freshly prepared DPPH solution (1% in methanol) was dissolved in extract solution. The rest timing of this reaction was 15 min at ambient location and then the value was measured by spectrophotometer at 517 nm. Likewise, the absorbance of control and blank samples were also determined by UV/Visible spectrophotometer at same wavelength. The results of DPPH assay were expressed in percentage.

Antioxidant activity (AA)

The antioxidant activity of ginger rhizome, leaves and flowers extracts (individually) was depended on the combined oxidation of linolenic acid with β -carotene. The antioxidant activity of ginger leaves was elucidated by following the protocol of Abd El-Baky and El-Baroty, [14]. According to their protocol, 2 mg of β -carotene was dissolved in a test tube by adding 20 mL of chloroform then aliquot (3 mL) of this mixture was added separately with 40 mg of linolenic acid in a flask along with 400 mg of tween 20 as emulsifier. After adding all the reagents, the mixture was then subjected to the evaporation by using rotary evaporator for 10 min at 40 °C to remove chloroform from the mixture. After evaporation, the mixture was mixed with 100 mL of distal water by placing the sample at vortex mixer that helps to prepare emulsion. In the last 2 mL of this emulsion was mixed with thoroughly with 0.12 mL extract of ginger and incubated in water bath at 50 °C for half hour. Then the absorbance of mixture was measured by spectrophotometer using 470 nm wavelength. The antioxidant activity of ginger extracted were depicted as inhibition percentage against control value by following the equation:

$$AA (\%) = \frac{\text{Degradation rate of control} - \text{Degradation rate of sample}}{\text{Degradation rate of control}} \times 100$$

Ferric reducing antioxidant power (FRAP) assay

The reducing potential of ginger rhizome, leaves and flowers were determined by calculating the ability of

extracts to reduce ferric tripyridyltriazine into blue colored ferrous ions that can be measured at 593 nm wavelength by using UV/Visible Spectrophotometer as mentioned by Chan et al. [10]. For this purpose, FRAP reagent was prepared by dissolving 2.5 mL 20 mM of ferric chloride and 2.5 mL of 10 mM TPTZ with 25 mL 0.1 M of acetate buffer (pH 3.6). After that, the mixture was incubated for 10 min at 30 °C. To estimate the reducing potential of ginger extracts, 100 µL of ginger extract was mixed with 1.5 mL of FRAP reagent along with 100 µL of distilled water then the absorbance was measured by using spectrophotometer at 593 nm. The curve for calibration was drawn by using 0–500 µmol/mL of trolox and expressed as µmol trolox equivalent/g of sample.

ABTS (2, 2-azino-bis, 3-ethylbenzothiazoline-6-sulphonic acid) assay

ABTS assay is a method in which decolorization was estimated as mentioned by Kang et al. [15]. According to their protocol, ABTS reagent solution was freshly prepared by adding 5 mL of 14 mM ABTS solution with 5 mL of 4.9 mM potassium persulfate and then the mixture was stored for 16 h in dark place at ambient temperature. Then the solution was further diluted with respective solvent to produce absorbance of 0.7 ± 0.02 at wavelength of 734 nm. After preparation of standard absorbance solution, 1 mL of final solution was prepared by having 50 µL of ginger extract (either rhizome, leaves or flower) and 950 µL of ABTS solution. After 5 min of resting time at room temperature, the absorbance was recorded at 734 nm by using spectrophotometer and the outcome were compared with the blank and control ABTS solution. The ABTS assay was expressed as TEAC/g sample.

- TEAC: µmol trolox equivalent antioxidant capacity

Metal chelating potential

For the estimation of metal chelating potential of ginger rhizome, leaves and flower, ferrous ion chelation was performed as guided by Xie et al. [16]. In this procedure, 0.1 mL of ginger extract (separately) mixed with 0.05 mL of 2 mM FeCl₂. The mixture was mixed thoroughly for 10 min at ambient temperature then the absorbance of solution was observed by spectrophotometer at 562 nm. The following equation as used to express metal chelating potential of ginger extracts:

$$MC (\%) = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$

- A: Absorbance

Selection of best treatment for HPLC analysis

From the nine treatments aforementioned in Table 1, three best treatments each from rhizome, leaves and flower were nominated for HPLC analysis depending upon the antioxidant profiling tests and in vitro perspectives.

Characterization of active ingredients

The best selected treatment from ginger rhizome, leaves and flower were characterized for the concentration of bioactive compound i.e. gingerol by following the method mentioned by Salmon et al. [17] through HPLC (PerkinElmer, Series 200, USA) having shim packed C₁₈ column (CLC-ODS) having diameter properties of 15 cm × 4.6 mm and 5.0 µm range for particle size along with auto sampler. For the separation of bioactive compound isocratic HPLC grades acetonitrile and water (55:45 v/v) was used as mobile phase with 100 mL/min flow rate. Then 10 µL of sample was injected in the column at 40 °C temperature that was maintained for the complete process. At the end, the eluted material was assessed by using UV detector at 280 nm to calculate the amount of gingerol present in eluent. The amount was quantified by comparing the retention time of peaks in sample in contrast to gingerol standard peaks.

Statistical analysis

The obtained data for each parameter was subjected to statistical analysis to get accurate, precise and comprehensive conclusions. For the purpose, 2-way factorial design was applied by using Statistical Package (Statistix 8.1) to determine the level of significance as mentioned by Montgomery, [18]. Significant ranges were further compared by post-hoc Tukey's HSD test.

Results and discussion

Phytochemical screening test

Total phenolic contents

Phenolics over and above to the antioxidant perspectives owing to the valuable health boosting ability have been recognized as an essential ingredient in daily diet. It is depicted from mean values that total phenolic compounds have significantly affected by solvent and plant part however, the effect was observed as non-significant for part and solvent interaction. According to Table 2, the overall values of total phenolic compound (TPC) for methanol, ethanol and water extracts were 533.86 ± 21.60 , 687.16 ± 28.86 and 348.30 ± 12.19 mg gallic acid equivalent (GAE)/100 g, accordingly, however, the values regarding rhizome, leaves and flower part were 459.68 ± 18.39 , 615.78 ± 24.63 and 513.86 ± 20.55 mg GAE/100 g for rhizome, leaves and flower, accordingly. Maximum phenolic contents were observed as 780.56 ± 32.78 GAE/100 g in leaves ethanolic extract.

Table 2 Means for TPC, Flavonoids and Flavonols of ginger extract

	Treatments	Ginger Part			Means
		Rhizome	Leaves	Flower	
TPC mg GAE/100 g	Methanol	430.72 ± 16.80	645.26 ± 25.17	585.60 ± 22.84	533.86 ± 21.60^b
	Ethanol	650.44 ± 27.32	780.56 ± 32.78	630.48 ± 26.45	687.16 ± 28.86^a
	Water	297.88 ± 10.43	421.52 ± 14.75	325.50 ± 11.39	348.30 ± 12.19^c
	Means	459.68 ± 18.39^c	615.78 ± 24.63^a	513.86 ± 20.55^b	
Flavonoids mg/100 g	Methanol	234.06 ± 9.13	246.98 ± 9.63	226.36 ± 8.83	235.80 ± 9.20^b
	Ethanol	239.52 ± 10.06	253.56 ± 10.65	243.12 ± 10.21	245.40 ± 10.31^a
	Water	218.34 ± 7.64	239.02 ± 8.37	228.68 ± 8.00	228.68 ± 8.00^c
	Means	230.64 ± 9.23^b	246.52 ± 9.86^a	232.72 ± 9.31^b	
Flavonols mg/100 g	Methanol	37.48 ± 1.56	42.96 ± 1.60	40.12 ± 1.58	40.18 ± 1.58^b
	Ethanol	43.38 ± 1.74	49.54 ± 1.74	45.98 ± 1.84	46.30 ± 1.73^a
	Water	32.24 ± 1.40	38.06 ± 1.43	36.38 ± 1.41	35.56 ± 1.39^c
	Means	37.70 ± 1.62^c	43.52 ± 1.52^a	40.82 ± 1.62^b	

Means carrying same letters do not differ significantly

The findings of current investigation were in line with the outcomes of Kaur and Kapoor, [19] who assessed the TPC level of ginger rhizome in water extract and reported that the value for the extract was 221.3 ± 9.4 mg GAE/100 g. The ginger parts showed maximum TPC in ethanol extract followed by methanol and water extracts as reported by Cai et al. [20] and Hinneburg et al. [21]. Another group of scientists, Stoilova et al. [22] worked on the different parts of ginger and concluded that different parts have different amounts of total phenolic contents. The maximum contents were found in green part of ginger that was 871 mg GAE/g of sample [23–28].

Meanwhile, Shirin and Prakash, [29] worked to determine the effect of different solvents namely, ethanol, methanol and acetone on ginger and reported that highest TPC were calculated in ethanol extract (800 ± 4.3 mg GAE/100 g) followed by methanol extract (780 ± 5.0 mg GAE/100 g) and acetone extract (325 ± 1.9 mg GAE/100 g). Numerous other researches also support the concept of present research work that ethanol has greater potential to extract phenolic compounds as compared to methanol and water. Furthermore, it was also suggested by literature that green parts of ginger have strong antioxidant power as compared to the rhizome [30–34].

Flavonoids

It has been proved from literature that flavonoids have strong antioxidant potential and have momentous impact on the human health as well as nutritional status. Flavonoids work on the principle to scavenge the free radicals. Purposely, flavonoids of different ginger parts i.e. flowers, leaves and rhizome were estimated by using methanol, ethanol and water as solvent. The statistical analysis revealed that both part and solvent have

significantly affected the flavonoid content however, the interaction showed non-momentous effect on total flavonoids. The flavonoids values (Table 2) suggested that the highest flavonoid contents were observed in ethanol extract (245.40 ± 10.31 mg/100 g) followed by methanol extract (235.80 ± 9.20 mg/100 g) and water extract (228.68 ± 8.00 mg/100 g). The interaction effect for flavonoid content concluded that ethanol was the best solvent among all and leaves were the best parts among all the ginger parts and ranked with highest flavonoid contents as 253.56 ± 10.65 mg/100 g. From part point, leaves showed maximum flavonoids content (246.52 ± 9.86 mg/100 g) and lowest were in rhizome (230.64 ± 9.23 mg/100 g).

The results of current research work were in harmony with the findings of numerous scientists who agreed that the flavonoids exist in different concentrations in all the parts of ginger crop. The highest flavonoid contents were observed in leaves as compared to flowers and rhizome [10, 29, 35]. Moreover, Ghasemzadeh et al. [28] concluded that the polarity of solvent has greater influence on the flavonoid content of sample. By increasing the polarity, the rate of extraction for flavonoids increased. In their research work they used ethanol, methanol and acetone as solvent to determine the flavonoid contents in ginger flowers, leaves and rhizome. According to their outcomes, leaves have the highest flavonoid contents as 5.5 ± 0.54 to 7.05 ± 1.65 mg/g in ethanol, 4.70 ± 0.55 to 6.20 ± 1.7 mg/g in methanol and 4.54 ± 0.64 to 6.01 ± 1.65 mg/g in water acetone extract. For the ginger flower, the values of flavonoids were 3.60 ± 0.12 to 4.40 ± 0.57 mg/g, 3.40 ± 0.13 to 3.80 ± 0.12 mg/g and 3.23 ± 0.12 to 3.70 ± 0.15 mg/g in ethanol, methanol and acetone extract. The lowest flavonoids were observed in ginger rhizome that were 1.30 ± 0.12 to 1.70 ± 0.49 mg/g in ethanol, 0.83 ± 0.14 to 0.98 ± 0.14 m/g in methanol and

0.741.02 to 0.90 ± 0.16 mg/g in acetone extract. Recently, Amir et al. [34] depicted that ginger leaves have $0.84 \pm 0.03\%$ flavonoids (w/w) in dry form.

Flavonol contents

The statistical analysis revealed that the solvent as well as the part of ginger commodity have significant effect on the flavonol content however, the interaction of part and solvent proved non-momentous effect on flavonols. The values regarding the flavonols contents (Table 2) depicted that the maximum flavonols were observed in ethanol extract as 46.30 ± 1.73 mg/100 g however, the minimum value was observed in water based extracts as 35.56 ± 1.39 mg/100 g. similarly, the highest value of flavonols was calculated in ginger leaves (43.52 ± 1.52 mg/100 g) followed by ginger flowers (40.82 ± 1.62 mg/100 g) and ginger rhizome (37.70 ± 1.62 mg/100 g).

Amid all the herbs and spices, ginger has maximum antioxidant ability owing to the presence of a number of antioxidants that further comprised of flavonoids and flavonols. The results of present investigation were in harmony with the conclusions of Sultana and Anwar, [36] who suggested that the flavonol content of ginger 14.9 ± 0.6 mg/g among which the most abundant was kaempferol as 11.9 ± 0.4 mg/g. Following them, Ghazemzadeh et al. [28] worked on the flavonols content of ginger and reported that the ginger has a number of flavonols present in different parts of commodity. A few such as epicatechin and rutin are light dependent and their concentration increased with increased intensity of light on leaves as well as flowers. However, naringenin is present in very minute quantity in different parts of ginger.

Moreover, Naem et al. [37] depicted that the ethanol extract of ginger leaves possessed 3.36 ± 0.3 mg/g of

flavonols among which myricetin, quercetin and kaempferol were most important and present as 2.04 ± 0.3 , 0.97 ± 0.3 and 0.35 ± 0.3 mg/g accordingly. They further concluded that by increasing the concentration of ethanol from 50 to 80% the flavonol quantity gradually increased as the total flavonols increased to 46.18 ± 0.2 mg/g with increased ratio of myricetin (42.6 ± 2.3 mg/g), quercetin (4.9 ± 1.4 mg/g) and kaempferol (91.68 ± 0.9 mg/g). However, by further increasing the ratio to 90% the flavonols contents decreased.

In vitro study

DPPH scavenging capacity assay

DPPH is stable, non-reactive radical that can be potential to adopt hydrogen ion or an electron and can transform into stable free radical in the presence of ethanol or methanol solution of DPPH. The DPPH assay is mostly carried out to determine the antioxidant perspectives that valued up to the phytochemical profiling through free radical scavenging ability. In this context, ginger rhizome, leaves and flower extracts were used to determine their DPPH assay. The mean squares of DPPH assay concluded that the part as well as solvent has momentous effect on the DPPH assay however, their interaction depicted non-significant effect. The mean values for free radical scavenging ability of ginger (Table 3) demonstrated that the DPPH assay was maximum in ethanol extract ($65.30 \pm 2.74\%$) after that methanol extract ($60.56 \pm 2.36\%$) and water extract ($49.04 \pm 1.72\%$). Among the various ginger parts, leaves depicted maximum DPPH assay potential as $65.64 \pm 2.63\%$ and minimum were reported for ginger rhizome $52.76 \pm 2.11\%$.

The outcomes of present investigation were in line with the conclusions of Hinneburg et al. [21] and

Table 3 Means for DPPH, Antioxidant activity and FRAP assay of ginger extract

	Treatments	Ginger Part			Means
		Rhizome	Leaves	Flower	
DPPH %	Methanol	57.82 ± 2.25	68.16 ± 2.66	55.70 ± 2.17	60.56 ± 2.36^a
	Ethanol	51.10 ± 2.15	75.54 ± 3.17	69.26 ± 2.91	65.30 ± 2.74^b
	Water	49.36 ± 1.73	53.22 ± 1.86	44.54 ± 1.56	49.04 ± 1.72^c
	Means	52.76 ± 2.11^c	65.64 ± 2.63^a	56.50 ± 2.26^b	
Antioxidant Activity %	Methanol	58.38 ± 2.28	70.42 ± 2.75	61.22 ± 2.39	63.34 ± 2.22^b
	Ethanol	72.46 ± 3.04	77.88 ± 3.27	69.56 ± 2.92	73.30 ± 3.08^a
	Water	51.32 ± 1.80	56.60 ± 1.98	53.84 ± 1.88	53.92 ± 2.10^c
	Means	60.72 ± 2.43^b	68.32 ± 2.73^a	61.54 ± 2.46^b	
FRAP (μ mole TE/g)	Methanol	95.40 ± 3.72	100.18 ± 3.91	98.84 ± 3.85	98.14 ± 3.83^{ab}
	Ethanol	99.52 ± 4.18	105.72 ± 4.44	102.62 ± 4.31	102.62 ± 4.28^a
	Water	92.54 ± 3.24	97.46 ± 3.41	94.58 ± 3.32	94.86 ± 3.32^b
	Means	95.82 ± 3.83^b	101.12 ± 4.04^a	98.68 ± 3.95^{ab}	

Means carrying same letters do not differ significantly

Stoilova et al. [22] who reported that the DPPH assay of different parts of ginger resulted as 90.1% when 9 mg/mL of DPPH solution was used. Similarly, Wei and Shibamoto, [38] reported that the ginger oil extracted from ginger flowers concluded as 50% inhibition at the concentration of 200 µg/mL. Moreover, Qusti et al. [25] suggested that moisture content of ginger has strong effect on the DPPH assay as the value increased in dried form as compared to fresh ginger due to the intermediate bonding of water molecules with antioxidant moieties.

One of their peers, El-Ghorab et al. [27] determined that the ginger essential oil extracted from green ginger parts exhibits 83.03% DPPH assay @ 240 µg/mL. Furthermore, Ghasemzadeh et al. [28] did their research work on the DPPH assay of different parts of ginger and concluded that the ginger flowers depicted free radical scavenging activity in the range of 48.22 ± 1.19 to $41.41 \pm 0.51\%$ however, in ginger leaves the DPPH assay was observed between 56.36 ± 0.97 to $51.12 \pm 1.65\%$ and 32.85 ± 0.57 to $31.45 \pm 1.49\%$ for ginger rhizome. They further reported that after maturity level, the DPPH assay of ginger rhizome increased and the free radical scavenging ability of leaves decreased because of molecules movements from leaves to rhizome as main edible part of ginger crop.

Following them, Lu et al. [39] proved that the DPPH assay of dried ginger is up to $32.38 \pm 1.42\%$. At the same moment, Ali, [40] counted that the DPPH assay of ginger in ethanol extract ranged up to 79%. Furthermore, Mariutti et al. [41] reported that ginger rhizome showed 7.8% DPPH assay however, for ginger flower the value was 19%. Recently, Kubra et al. [42] anticipated that the aqueous extract of ginger has 42.80% free radical scavenging ability that has quite resemblances with the findings of current investigation.

Antioxidant activity (AA)

The statistical analysis proved that the antioxidant activity for ginger extracts has significantly affected by the type of solvent and type of ginger part whilst, their interaction proved non-momentous effect on the antioxidant activity. The mean values regarding the antioxidant potential of ginger extracts (Table 3) demonstrated that the maximum antioxidant activity was observed in ethanol extract ($73.30 \pm 3.08\%$) followed by methanol extract ($63.34 \pm 2.22\%$) and water extract ($53.92 \pm 2.10\%$). Regarding the part of ginger crop, the leaves revealed highest antioxidant activity as $68.32 \pm 2.73\%$ tackled by flower ($61.54 \pm 2.46\%$) and rhizome ($60.72 \pm 2.43\%$).

The findings of current investigation were in agreement with the research work of Stoilova et al. [22] who

suggested that the antioxidant activity of ginger is temperature dependent. Purposely, they prepared the extracts at two different temperatures 37 °C and 80 °C and incubated for 4 days. After the given time period, they concluded that the extract prepared at 37 °C showed 62.5% antioxidant activity even @ 0.02% however, the extract prepared at 80 °C didn't impart any effect on the antioxidant activity. Following them, El-Baroty et al. [43] concluded that the antioxidant activity of ginger essential oil ranged up to 66.5%.

Nevertheless, Kaur and Kapoor, [19] determined the antioxidant activity of ginger leaves and concluded that the ethanol extract of ginger leaves showed 71.8% ability to bleach beta-carotene however, in methanol the antioxidant activity of ginger leaves reduced to 65.0%. Another group of researchers, Eleazu et al. [31] determined the antioxidant activity of ginger and reported that the ethanol extract of ginger has the ability to bleach the beta-carotene and alpha-linolenic acid in the range of 75.22 to 94.28%, with condition that the extracts should be prepared with 250 g of ginger.

Ferric reducing antioxidant potential (FRAP)

The ferric reducing antioxidant potential is the ability of any compound to reduce ferric ion via the addition of hydrogen removed from phenolic compound. The availability of reducing agents along with the position as well as number of the hydroxyl groups also have influence in the reduction procedure to enhance the antioxidant procedure. For FRAP assay, the statistical analysis proved that both, the type of solvent and part of ginger crop have momentous effect however, their interaction depicted non-significant effect on the FRAP assay. The mean values of FRAP (Table 3) illustrated that ginger leaves revealed maximum FRAP potential as 101.12 ± 4.04 µmole TE/g followed by flower as 98.68 ± 3.95 µmole TE/g and rhizome extract (95.82 ± 3.83 µmole TE/g). From solvent type, maximum FRAP ability was observed in ethanol extract (102.62 ± 4.28 µmole TE/g) as compared to methanol extract (98.14 ± 3.3 µmole TE/g) and water extract (94.86 ± 3.32 µmole TE/g).

The results of present research work were in line with the findings of Liu et al. [44] who reported that the antioxidant FRAP potential of ethanol extract of ginger was 0.806 mmole of ferric (g). Similarly, El-Ghorab et al. [27] did their research work on the antioxidant potential of ginger and concluded that the FRAP assay is dependent on the concentration of ginger in extract either in fresh or dry form and increased the presence of phenolic compounds hence, increased the absorbance for FRAP assay. Moreover, Sanwal et al. [45] concluded that diploid and tetraploid ginger clones have different FRAP potential. They further reported that the diploid has higher ferric

reducing antioxidant power s compared to tetraploids. According to their findings, the diploid ginger has FRAP assay between 4.28 to 4.86 $\mu\text{g/g}$ however, FRAP assay of tetraploid ginger clone ranged from 4.60 to 5.19 $\mu\text{g/g}$.

Another group of scientists, Ghasemzadeh et al. [28] worked on the FRAP assay of different parts of ginger and reported that the maximum FRAP assay was observed in ginger leaves as 680.68 ± 18.38 to $767.2 \pm 41.53 \mu\text{mol Fe (II)/g}$. The yellowish green part of ginger crop (flowers) has lower ferric reducing antioxidant power and ranged between 537.94 ± 37.30 to $579.6 \pm 61. \mu\text{mol Fe (II)/g}$ however, the minimum FRAP assay was observed in ginger rhizome that varied from 368.27 ± 23.43 to $376.94 \pm 50.97 \mu\text{mol Fe (II)/g}$. Similarly, Pawar et al. [35] supported the findings of Ghasemzadeh et al. [28] and concluded that the ferric reducing antioxidant power of different parts of ginger crop changed with the changings in environmental conditions, age and variety. Moreover, Lu et al. [39] investigated the FRAP assay of ginger and concluded that the FRAP value was $157.95 \pm 2.2 \mu\text{mole TEAC/g}$. Nonetheless, Maizura et al. [33] determined the FRAP assay of fresh ginger juice and reported that the value was $26.2 \mu\text{mole Fe/g}$. One of their peers, Kruawan and Kangsadalampai, [46] who elaborated that the aqueous extract of fresh ginger has strongest FRAP ability as $1030.5 \pm 11.49 \mu\text{mol/g}$.

ABTS assay

ABTS is a reaction that take place between the ABTS reagent and persulphate that in the end produce blue color. For the sample extract prepared with any organic solvent, it produces a pre-formed radical that further reduced to ABTS depending upon the concentration of sample used to prepare that extract. Following the same principle for the ABTS assay of ginger extracts, the results proved that both the type of solvent and part of ginger imparted significant effect on the ABTS assay however, their interaction didn't affect ABTS assay

significantly. The mean values (Table 4) of ABTS assay suggested that maximum antioxidant potential in the form of ABTS was observed by ethanol extract of ginger leaves ($118.43 \pm 4.97 \mu\text{mol TE/g}$). The ginger leaves proved more antioxidant power ($101.02 \pm 4.04 \mu\text{mol TE/g}$) over and above to flowers ($88.30 \pm 3.53 \mu\text{mol TE/g}$) and rhizome ($83.47 \pm 3.34 \mu\text{mol TE/g}$). From the solvent side, ethanol proved maximum ABTS assay as $105.90 \pm 4.45 \mu\text{mol TE/g}$ however, minimum was observed in water extract as $80.68 \pm 2.84 \mu\text{mol TE/g}$.

The results of present investigation were in harmony with the findings of Puengphian and Sirichote, [23] who performed their research work on the ABTS assay of fresh as well as dried ginger and concluded that the extract prepared with dried ginger showed maximum ABTS assay ($169.06 \pm 3.96 \mu\text{mol TE/g}$) as compared to fresh ginger extract in which the ABTS assay were depicted as $403.71 \pm 7.24 \mu\text{mol TE/g}$. Moreover, Hossain et al. [47] worked on the methanol extract of ginger prepared with 80% methanol and centrifuge for 15 min @ 3000 rpm and reported that the ABTS assay in ginger extract were $406.29 \pm 17.35 \text{ g TE/100 g}$. One of their peers, Lu et al. [39] performed their research work on the methanol extract of ginger (60%) prepared at 35°C for 15 min @ 1500 rpm and then investigated its ABTS assay. According to their findings, the dried ginger methanol extract showed $75.66 \pm 1.15 \mu\text{mol TE/g}$ antioxidant potential. Recently, Mariutti et al. [41] determined the ABTS potential of ginger in ethanol extract and concluded that the ABTS assay of ginger ethanol extract was $23.0 \pm 0.3 \text{ mM/g ABTS reagent}$.

Metal chelating potential

The lipid peroxidation is initiated in the body by the presence of metal ions that further increased the process of lipid peroxidation by forming free radicals. The metal chelating potential provides the results

Table 4 Means for ABTS and metal chelating assay of ginger extract

	Treatments	Ginger Part			Means
		Rhizome	Leaves	Flower	
ABTS $\mu\text{mol TE/g}$	Methanol	81.54 ± 3.18	92.88 ± 3.62	84.24 ± 3.29	86.22 ± 3.36^b
	Ethanol	96.26 ± 4.04	118.43 ± 4.97	103.00 ± 4.33	105.90 ± 4.45^a
	Water	72.64 ± 2.54	91.74 ± 3.21	77.66 ± 2.72	80.68 ± 2.84^c
	Means	83.47 ± 3.34^c	101.02 ± 4.04^a	88.30 ± 3.53^b	
Metal Chelating %	Methanol	15.02 ± 0.53	31.36 ± 1.10	18.48 ± 0.65	21.62 ± 0.76^b
	Ethanol	17.98 ± 0.76	35.16 ± 1.48	21.32 ± 0.90	24.82 ± 1.04^a
	Water	13.86 ± 0.54	29.54 ± 1.15	16.30 ± 0.64	19.90 ± 0.78^c
	Means	15.62 ± 0.62^b	32.02 ± 1.28^a	18.70 ± 0.78^b	

Means carrying same letters do not differ significantly

about the antioxidant potential of any compound along with the anti-radical properties. The absorbance for the metal chelating potential is inversely proportional to the metal chelating power as high absorbance indicated low metal chelating potential and vice versa. The ability of free radicals to start the process can be delayed by the chelation of metal. Therefore, metal chelating potential is considered as a mandatory test to determine the antioxidant potential of any bioactive compound. The statistical analysis regarding the metal chelating potential proved that type of solvent and part of crop both have significantly affected the metal chelating potential however, the aspect remained non-momentous for their interaction. The values (Table 4) relating to metal chelating potential of ginger revealed that the ethanol extract showed maximum metal chelating potential ($24.82 \pm 1.04\%$) followed by methanol extract ($21.62 \pm 0.76\%$) and water extract ($19.90 \pm 0.78\%$). From the part of ginger commodity, ginger leaves showed maximum metal chelating ability $32.02 \pm 1.28\%$ as compared to ginger flower and ginger rhizome as 18.70 ± 0.78 and $15.62 \pm 0.62\%$, respectively.

The findings of current investigation were in line with the results of Hinneburg et al. [21] who performed their research work on the metal chelating potential of ginger extract and concluded that the ginger extracts prepared with different concentrations have different ferric ion chelation potential that varied from 16.0 ± 0.17 to $21.6 \pm 0.51\%$. They further reported that the metal chelating potential of ginger is dependent upon the concentration of ginger taken to produce extract. Following them, Lee et al. [48] resulted that the metal chelating potential of ginger extract is near about 52% when the extract is prepared with g/mL concentration. Moreover, Zhang et al. [49] used three different type of solvent for the preparation of ginger extracts as alkaline, acid and water extract. According to their outcomes, the metal chelating potential was maximum in alkaline extract ($57.1 \pm 1.83\%$) tracked by water extract ($56.8 \pm 0.12\%$) and acid extract ($35.4 \pm 0.56\%$). Likewise, Chen et al. [50] worked on dried ginger leaves and reported that the gingerol content of leaves have the ability to withstand with temperature however, in ginger rhizome gingerol transformed into shogaol by the application of heat during drying process as a result of thermal degradation. They advocated that the metal chelating potential of dried ginger leaves was 73% however, in dried ginger rhizome this metal chelating potential was less than 10% against EDTA as standard. They further suggested that moisture content of ginger have effect on the metal chelating potential of bioactive moieties of ginger.

HPLC assessment

HPLC quantification is a mandatory step for the quantification of resultant extracts to further categorize them according to the presence of bioactive compounds. Depending upon the results of phytochemical screening and in vitro antioxidant assay, ethanol extract of all three parts, rhizome, leaves and flower were selected for the qualitative as well as quantitative analysis of phytoceutics as gingerol. The HPLC graded gingerol standard was used and the presence of gingerol in different ginger parts was ensured by comparing the peak area of sample and standard along with the retention time.

HPLC quantification of 6-gingerol

The quantification of 6-gingerol through High performance liquid chromatography (HPLC) revealed that ginger parts have 4 times more concentration of gingerol as compared to other bioactive moieties. The resultant peaks of ginger rhizome, leaves and flower obtained by HPLC were inferred with the peaks of standard for the peak area, retention time and spectral exploration. The HPLC quantification of ginger parts (rhizome, leaves and flower) in Table 5, proved that highest gingerol content were present in ethanol extract of ginger leaves (4.19 mg/g) followed by ethanol extract of ginger flower (2.87 mg/g) and ethanol extract of ginger rhizome (1.03 mg/g). It was also discovered from phytochemical profiling that ethanol extract was the most efficient solvent to solubilize the essential oils of ginger crop owing to its polar and organic nature as compared to methanol and water.

Gingerol provides the highly purified essence to the ginger and it can be extracted by using many protocols such as solvent extraction and steam distillation [6, 51, 52]. Ginger extract isolated by ether technique is then purified and quantified by HPLC and being used in pharmacy, aromatherapy along with seasoning and beverages. Purposely, Schwertner and Rios, [53] depicted the amount of gingerol after characterization and quantification of gingerol via HPLC in beverage with special reference to tea. According to their findings, the dried ginger parts have more than 9.5% gingerol as compared to fresh portions. They further suggested that the dried green part of ginger has more gingerol as compared to

Table 5 HPLC quantification for gingerol (mg/g) in ginger parts ethanol extracts

Part of ginger crop	Concentration (mg/g of dry matter)
Rhizome	1.03
Leaves	4.19
Flower	2.87

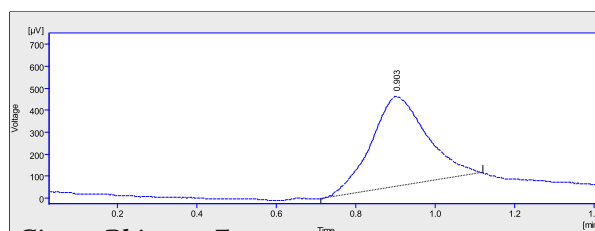
yellow part owing to the presence of heat stable bioactive compounds.

Following them, Puengphian and Sirichote, [23] did their research work on the antioxidant profiling and quantification of ginger and concluded that the dried ginger has 18.81 mg/g of gingerol however, in fresh ginger the content ranged up to 104 to 965 $\mu\text{g/g}$. The dried part has lesser amount of gingerol with higher antioxidant properties [54]. The ginger in fresh form almost contain 92% of bioactive moieties however, the major part is contributed towards zingiberone, arcurmene, β -bisabolene and subsequently geraniol in the range of 28.6, 5.6, 8.5 and 5.8%, accordingly [55]. According to literature, 6-gingerol has highest rank in ginger ranging from 1.030 to 3.046 mg/g of ginger rhizome. Over and above to 6-gingerol, 8-gingerol also exist in ginger whilst in lesser quantity ranging from 0.078–0.0425 mg/g [45]. Their findings were further elaborated by another group of scientists, Pawar et al. [35] who suggested that the gingerol concentration of water extract of dried ginger varied from 1.17 to 2.08 mg/g.

At the same moment, Wohlmuth et al. [56] investigated the gingerol content from 12 different clone of ginger crop. During the quantification of gingerol, the used acetonitrile and water combination as mobile phase for the verification of methanolic extract of ginger and concluded that the 6-gingerol content in methanol extract were 2.10 mg/g however, in minutes quantities 8-gingerol and 10-gingerol were also present. Overall the quantity of bioactive compound in dried ginger was 29.2% [57]. After that, Hasan et al. [58] worked on the preparation and quantification of methanol and hexane-based ginger extracts and reported that the methanol extract gave higher peak as 25% however, it was 235 for hexane extract whilst, the retention time was same 738 min. Similarly, Silva et al. [59] did their research work on HPLC quantification of methanolic extract of dried ginger crop powder by using water and acetonitrile as mobile phase in C_{18} column having flow rate @1 mL/min and claimed that the amount of gingerol in methanolic extract of ginger was 30 mg/g. one of their peers, Rafi et al. [60] worked on different parts of ginger crop. In their research work, they extracted the ginger extracts via ultra-sonication and then quantified through HPLC. According to their results, the 6-gingerol content in ginger rhizome were 2.98 ± 0.06 mg/g although, in ginger leaves the gingerol content were 18.83 ± 0.28 mg/g when the mobile phase used was comprised of acetonitrile. In this context, Jiang et al. [61] quantified the ginger profile and reported that the isolated ginger extract has 1.93–3.57 mg/g of gingerol content that is responsible for its strong and unique pungency required in spices and food products to enhance taste as well as flavor.

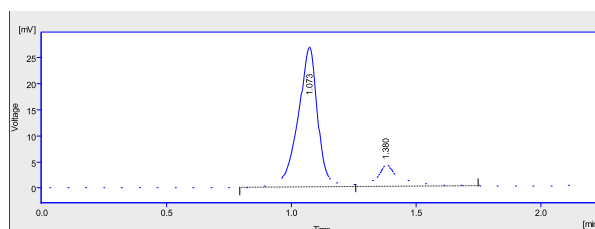
HPLC Peaks: Gingerol Std 500 ppm

Gingerol Std 500ppm



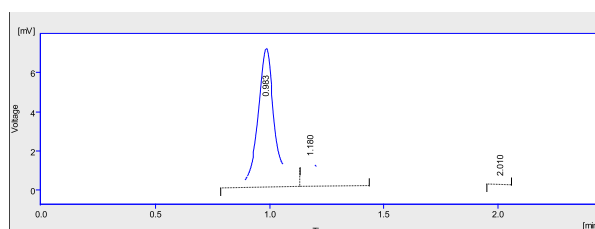
Ginger Rhizome Extract

Ginger Rhizome Extract



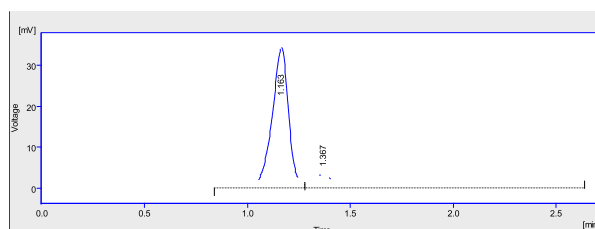
Ginger Leaves Extract

Ginger Leaves Extract



Ginger Flower Extract

Ginger Flower Extract



Conclusion

The finding of present investigation concluded that ginger leaves have maximum antioxidant potential as compared to ginger flowers and rhizome. The gingerol present in ginger leaves have heat sensitive properties and did not decompose into shogaol on heat treatment during the process of drying. Furthermore, among the solvents, ethanol has the strongest ability to hold the bioactive ingredients as compared to methanol and water. Contemporary, it is the need of current era to develop designer food products by the addition of ginger leaves. It is also anticipating that green leaves and flowers have the strong potential to be used in daily dietary regime along with development of novel food products.

Abbreviations

ABTS: 2, 2-azino-bis, 3-ethylbenzothiazoline-6-sulphonic acid; DPPH: 1,1-diphenyl-1-picrylhydrazyl; EDTA: Ethylenediaminetetraacetic acid; FRAP: Ferric reducing antioxidant power; HPLC: High Pressure Liquid Chromatography; TE: Trolox Equivalent; TEAC: μmol trolox equivalent antioxidant capacity; TPC: Total phenolic contents; TPTZ: 2,4,6-Tripyridyl-s-triazine 2,4,6-Tri (2-pyridyl)-s-triazine

Acknowledgements

The authors are thankful to Functional and Nutraceutical Food Research Section, National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

ST, TM and SZ designed the project under the supervision of AS, however, ZA helped in the extraction method. SA, TM and SZ performed all the tests and prepared the manuscript. The author(s) read and approved the final manuscript.

Funding

This research was partially supported by Higher Education Commission, Pakistan under Pak-US Science and Technology Cooperation Program Phase. IV (Project Grant No. 10/01/10-09/30/12), project entitled "Establishment of Functional and Nutraceutical Food Research Section at the National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan".

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Ethics approval was provided by the head of the NIFSAT-UAF, Pakistan, by reviewing the plans of Environmental Ethics Committee, UAF. The care of environment during experimentation were as per the instructions provided by the committee and the university.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 13 August 2019 Accepted: 11 February 2020

Published online: 24 February 2020

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