

Radical Scavenging Linked Antioxidant Comparison and Quantification of Conventional and Supercritical Fluid Ginger Extracts

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Abstract

Phytoceutics have been become increasingly famous with individuals anxious to mitigate the influence of unhealthy lifestyle as well as aging. Purposely, phytochemical analysis of ginger proved it as an excellent source of antioxidants and free radical scavengers. Regarding bioactive moieties especially gingerol and shogaol series extraction, ethanol showed maximum radical scavenging at 90 minutes than that of ethyl acetate and acetone. Ginger conventional extract showed maximum activity as $57.80 \pm 1.97\%$ superoxide anion radical scavenging, $38.23 \pm 1.30\%$ nitric oxide radical scavenging, $77.62 \pm 2.64\%$ hydroxyl radical scavenging and $52.61 \pm 1.79\%$ hydrogen peroxide scavenging activity in ethanol extract at 90 minute. Regarding to supercritical fluid extracts the maximum activity was observed at 3300 psi pressure and 40°C for 2 hr, followed by 3600 psi and 3000 psi pressure at same conditions. The maximum values were $78.13 \pm 2.50\%$, $54.83 \pm 1.75\%$, $86.71 \pm 2.81\%$ and $75.42 \pm 2.26\%$ for superoxide, nitric oxide, hydroxyl radical and hydrogen peroxide radical scavenging activity of supercritical fluid ginger extract. Afterwards, HPLC analysis portray that ginger extracts contain maximum amount of in gingerol (7.81 mg/g) at 3300 psi pressure followed by 3600 psi and 3000 psi pressure for supercritical and 5.74 mg/g in ethanolic extract among organic solvents.

Keywords: Supercritical fluid extract; Superoxide; Nitric oxide; Hydroxyl radical; Hydrogen peroxide; HPLC

Introduction

Plant food based bioactive moieties are the natural constituents that have the biological ability in addition to provide nutrition. These secondary metabolites have main role in the health of individuals via safe and confirmed position in prevention of disease, human growth as well as development. These isolates need the suitable and standard extraction method for their complete well-being profile [1]. Spices and herbs are rich sources of powerful antioxidants that have been used for flavor, color and aroma for more than 2000 years. They have also been used for preservation of foods and beverages primarily due to their phytochemicals. The antioxidants in spices and herbs are very effective because they possess excellent antioxidant activity either in the form of whole or ground spice/herb, extracts, encapsulated or as emulsions. Aside from their efficacy as antioxidants, spices and herbs are also classified as all natural and an attractive quality for consumers. Thus, spices and herbs may be used as a means to control lipid oxidation in foods [2].

Phenolic compounds are famous to have various functions in the plant such as structural, defense, attractants for pollinators and seed-dispersing animals. Although, plants also produce these substances to protect themselves against UV light for their survival and for adaptation to their environment. In this milieu, several studies have demonstrated that spices and herbs such as rosemary, ginger, sage and oregano with their high content of phenolic compounds serve as strong antioxidants. Furthermore, different hierarchical cluster *in vitro* analyses were employed to evaluate diverse classes of principle compounds in spices that are based on individual phenolic antioxidant activity [3].

Antioxidants are essential for health because it provides protection against oxidation as well as against the different factors which affect lipid oxidation include the presence of oxygen and transition metal ions, moisture, heat and light [4]. To prevent, minimize or slow down the rate of lipid oxidation, oxygen and metal catalysts must be

removed, or sequestered to render them unreactive. Therefore, foods that are prone to oxidation must be stored at low temperatures and/or shielded from light [2]. Antioxidants have the properties as red-ox system with enhanced potential than the drugs that are being used to protect from oxidation or inhibit the decomposition of cells due to free radicals. Overall, the impact of antioxidants is to decompose the chain of free radicals that is formed during the proliferation process either by giving an electron or hydrogen atom and in return getting excess of energy via activation of molecule. It has been recommended that natural plants, fruits and vegetables have a large variety of phytochemicals that is main source of antioxidant in diet from plants and have the potencies to reduce oxidative stress produced due to reactive oxygen species [5].

The oxidative stress have great influence in the pathogenesis of ageing, inflammation and cancer. Free radicals such as superoxide, nitric oxide, hydroxyl radicals and hydrogen peroxide etc. commonly call as ROS (reactive oxygen species). These ROS are created through oxidation stress and have been implicated in etiology of various age and diet related diseases. Exposure to ionizing radiations also generates ROS, known as chief mediator that control signal transduction. These free radicals also affect mitochondrial membrane potential and thus causes apoptosis. The increased evidences indicate that these ROS are prime factor for protein oxidation induced via exercise along with contribution to mental over and above to muscle fatigue. It has been

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assumed that the only treatment to reverse the injuries generated by these species is the scavenging of these species with some specific mechanism [6].

Normally human body is exposed to 0.28 mg/kg/day of H₂O₂ (hydrogen peroxide) indirectly through environment with an average intake that is mostly composed of leafy vegetables. This H₂O₂ entered in human beings by the way of inhalation of mist or vapor and via skin and/or eye contact. However, hydrogen peroxide can easily breakdown into water and oxygen and produce hydroxyl radicals that have the perspective to start lipid peroxidation that further lead to degradation of DNA in body. In addition to hydrogen peroxide and hydroxyl radical, another radicals i.e., superoxide radical can also initiate lipid peroxidation, although these superoxide anion radicals are weak oxidants but can produce strong and hazardous singlet oxygen atoms and hydroxyl radicals, both of which cause oxidative stress. The most potent radical in biological system is hydroxyl radical that reacts with polyunsaturated fatty acids entities present in phospholipids of cell membrane and contribute to cell damage [7].

In this context, ginger root (*Zingiber officinale*) is the rhizome of Zingiber plant that can be consumed as spice, delicacy and/or medicine. It lends its name from its genus and family as *Zingiberaceae*. This family also includes cardamom, galangal and turmeric. The abstractedly linked dicots in Asarum genus have shared wild ginger owing to similarity in taste. *Zingiber officinale* is indigenous to Southern China from where it spreads to Asia, West Africa, Spice Island and Caribbean [8].

The main pungent constituents in fresh ginger (*Zingiber officinale*) are gingerols that are renowned for their role in nutrition and human health. The medicinal capability of ginger namely, alleviation of arthritis, pain, nausea and many other metabolic disorders, have been linked with gingerols. Another series of bioactive entities are shogaol that are gingerol analogues and are thermally labile. The gingerol can easily converted to shogaol via degradation reaction and it is main ingredient in dried ginger. Both these gingerol and shogaol have contribution to a number of biological activities such as antimicrobial, antioxidant, radical scavenger, anti-inflammatory, anticancer, anti-ulcer and anti-allergic to many central nervous system activities. Shogaols due to its diverse biological perspectives are recognized as chief biomarkers for the quality control of processed ginger commercial products [9].

Usually, bioactive compounds are recovered from different natural sources such as ginger by solvent extraction technique [10], in which the important factors in the process are solvent type, extraction time and temperature [11]. Both solvents and co-solvents with different polarities such as ethanol and water [12-14] and/or organic solvents [15-17] can be employed for solid-liquid extractions in heat reflux systems. Furthermore, non-conventional methods (supercritical fluids) that are extremely environmental friendly owing to less usage of organic as well as synthetic chemicals in addition to minimum operational duration and maximum extraction quality and yield [11]. SC-CO₂ can be thought to be sound clean technological pragmatic approach to isolate natural phytochemicals with an acknowledged environmental friendliness. All this is because of non-toxic fluids used as solvent and co-solvents in this techniques, whilst, the supercritical fluid extraction methods started from laboratory scale and broaden to industrial plants owing to pure and fast yield. Conclusively, supercritical fluid extraction have many advantages over classical organic solvent extraction methods such as low solvent volume, selectivity, rapidity, cleanliness and possible manipulation of composition of extract by selective precipitation of compounds [18].

Materials and Methods

Ginger variety (Suravi) was procured from the Ayub Agriculture Research Institute, Faisalabad. The reagents (analytical and HPLC grade) and standards were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan).

Sample preparation

Ginger was cut into small pieces in order to obtain desired size. Afterwards, slices were dried under vacuum and ground to a fine powder using grinder. Resultant ginger powder was used for further analyses.

Preparation of ginger extract

Soxhlet extraction of ginger: Ginger extracts were prepared using three different solvents at varying time intervals as mention (Table 1) [19].

The ginger powder was successively extracted using soxhlet apparatus with ethanol, ethyl acetate and acetone as solvent. 100 g sample was extracted from 250 mL of respective solvent. Afterwards the resultant extracts were subjected to rotary evaporator (Eyela, Japan) to remove solvent and stored for further analysis.

Supercritical fluid extraction (SFE): For comparing the efficiency of conventional and supercritical fluid extraction (SFE) technique, ginger extracts was obtained by using supercritical fluid extractor (SC-CO₂), model SFT-150 (supercritical fluid extractor incorporation USA) as depicted (Table 2) [20].

Extraction yield: Extraction yield of ginger extract obtained by soxhlet and supercritical fluid extraction was calculated [21]. The extraction yield of extracts was measured by following formula.

$$\text{Extraction yield} = \text{Mass of extract per mass of dried material} \times 100$$

In vitro studies

Superoxide anion radical scavenging activity: Although superoxide anion is a weak oxidant, it ultimately produces powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress. Estimation of superoxide anion radical scavenging activity of ginger extracts was carried [22]. The superoxide anion radicals are generated in 3 mL of Tris-HCl buffer (16

Extraction method	Solvent	Treatment	Time (min)
Soxhlet apparatus	Ethanol	T ₁	60
		T ₂	90
		T ₃	120
	Acetone	T ₄	60
		T ₅	90
		T ₆	120
	Ethyl acetate	T ₇	60
		T ₈	90
		T ₉	120

Table 1: Treatments for solvent conventional extraction (SCE).

Extraction method	Solvent	Treatment	Pressure (psi)
Supercritical	CO ₂	T _{1SFE}	3000
		T _{2SFE}	3300
		T _{3SFE}	3600

Table 2: Treatment for the supercritical fluid extraction (SFE).

mM, pH 8.0), containing 0.5 mL of nitroblue tetrazolium (NBT) (0.3 mM), 0.5 mL NADH (0.936 mM) solution, 1 mL extract and 0.5 mL Tris-HCl buffer (16 mM, pH 8). The reaction is initiated by adding 0.5 mL phenazine methosulfate (PMS) solution (0.12 mM) to the mixture, incubated at 25°C for 5 min and then the absorbance was measured at 325 nm using UV/visible light Spectrophotometer (CECIL CE7200) against a blank sample along with ascorbic acid as standard.

The superoxide anion radical scavenging ability was expressed as percentage inhibition (%) relative to the control by following equation.

$$\text{Inhibition (\%)} = \frac{(\Delta A_{325\text{nm, Control}}/T) - (\Delta A_{325\text{nm, ginger extract}}/T)}{(\Delta A_{325\text{nm, ginger extract}}/T)} \times 100$$

Nitric oxide radical scavenging activity: Nitric oxide radical scavenging activity of ginger extracts was determined [23]. Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacted with oxygen to produce nitrite ions which were measured using Griess reaction method. For this purpose, 3 mL of 10 mM sodium nitroprusside in phosphate buffer 0.2 M, pH 7.4 was added to 2 mL of ginger extract. A similar procedure was repeated with methanol as a blank, which served as control in which only 5 mL Griess reagent was added. The absorbance of the chromophore formed was measured at 546 nm. All tests were performed in triplicate. Percent inhibition of the nitric oxide generated was measured by comparing the absorbance values of control and test preparation.

$$\text{Inhibition (\%)} = \frac{\text{Control absorbance} - \text{Ginger extract absorbance}}{\text{Control absorbance}} \times 100$$

Hydroxyl radical scavenging activity: Hydroxyl radical scavenging activity of ginger extracts was determined [24]. The hydroxyl radical scavenging activity was measured by evaluating the competition between methanol and ginger extract for hydroxyl radicals generated from the Fe³⁺/ascorbate/EDTA/H₂O₂ system. The reaction mixture and 10 µL of ginger extract along with control were incubated for 1 hr at 37°C. Hydroxyl radical degradation was measured as percentage inhibition calculated. The percentage inhibition was calculated according to the formula:

$$\text{Inhibition \%} = [(AB - AA)/AB] \times 100$$

Where:

AB=Absorbance of blank sample.

AA=Absorbance of ginger extract.

Hydrogen peroxide potential: Human beings are exposed to H₂O₂ indirectly via the environment along with intake mostly from herbs and spices. The ability of plant extracts to scavenge hydrogen peroxide can be estimated [5]. A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (pH 7.4), different concentrations of ginger extract and standard L-Ascorbic acid solution (10 µg/mL) in methanol (1 mL) were added to hydrogen peroxide solution (2 mL). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The antioxidant activity of the extract was expressed as percentage. All the tests were performed in triplicate. The percentage inhibition activity was calculated from:

$$\text{Percent inhibition: } [(A_0 - A_1)/A_0] \times 100$$

Where:

A₀ is the absorbance of the control

A₁ is the absorbance of extract/standard

Selection of best treatment for HPLC analysis

Out of nine treatments (Table 1) three best treatments, one from each extracts (ethanol, ethyl acetate and acetone) were selected on the basis of phytochemical screening test and *in vitro* studies for HPLC analysis.

Quantification of active ingredient: The HPLC quantification of nutraceutical_{CSE} and nutraceutical_{SEE} fractions was carried out depending on gingerol contents [25]. According to this method, 1 mL of ginger extract was re-dissolved in deionized water to a final volume of 10 mL. The aqueous extract was filtered and injected onto HPLC (PerkinElmer, Series 200, USA) containing shim-pack CLC-ODS C₁₈ column (15 cm × 4.6 mm, 5.0 µm particle size) by an auto sampler. The mobile phase for gingerols was methanol/water, 65:35 (v/v), at a flow rate of 1.0 mL/min; UV detection was at 282 nm and column temperature at 40°C were kept during the whole analysis. Quantification of active ingredient was achieved by comparing the retention time of peaks in samples to those of gingerol standard.

Results and Discussion

Extraction yield

Natural plant extracts have been extracted from many years for various purposes by numerous methods. Nowadays these extracts have been evaluated as an alternative for remedies as well as preservatives. To evaluate the extraction yield of natural plant material, the experimental soxhlet extraction conditions were applied on the ginger by using ethanol, acetone and ethyl acetate as solvents along with supercritical fluid extraction by using 3000, 3300 and 3600 psi pressure at 40°C for 2 hours. The different in extraction yield is given below in Table 3. The yield was calculated for each treatment as the mass of extract divided by the mass of material used for extraction. The extraction yield increased with the time due to more availability of solvent as well as with pressure due to more incorporation of carbon dioxide into dry material. The highest extraction was observed for ethanol 2.39% followed by ethyl acetate 2.16% and acetone 1.84% at 120 minutes whilst, the lowest was for at 60 minutes for ethanol, ethyl acetate and acetone, 1.91%, 1.27% and 1.64%, respectively. For supercritical extraction treatments maximum extraction was observed at 3600 psi (4.52%) and lowest at 3000 psi (4.08%).

The outcomes of this experiment are in line with the findings of Imm et al. [26] that determined the extraction yield of ethyl acetate ginger extract and concluded that extraction yield was 2.5% for 3.23 kg of ginger in one liter of ethyl acetate. Nonetheless, Naghia [27] evaluated the extraction yield of supercritical extract of ginger and suggested that the extraction yield increased by the addition of ethanol as co-solvent that was 4.5% without co-solvent and upsurge to 4.8%, 5.9% and 6% by the addition of 6%, 8%, 10% of ethanol at 45°C temperature and 250 bar pressure for 2.5 hours. He also observed that by increasing flow rate, extraction yield increased from 3.5%-4.5% by varying the flow rate from 5-15g/mL.

Furthermore, Mesomo with his colleagues [21], assessed the effect of

	60	90	120
Ethanol	1.91	2.18	2.39
Acetone	1.27	1.43	1.84
Ethyl acetate	1.64	1.9	2.16
Supercritical	3000	3300	3600
	4.08	4.36	4.52

Table 3: Extraction yield (%).

pressure and temperature at the extraction yield of ginger supercritical fluid extract and recommended that by increasing the pressure the extraction yield increased up to a limit and after that it decreased owing to acceleration in vibrational motion of molecules and the bioactive entities started to decompose. By their research work they suggested that by changing the pressure from 10 to 25 MPa the extraction yield changed from 1.68 to 1.90 at 293.15 K however, at 313.15 K the yield was 0.24%, 1.88% and 2.62% by using 10, 7.5 and 25 MPa pressure. Afterwards, Marrelli et al. [28] worked on the phytochemical screening of ginger pulp and peel extracts and concluded that the extraction yield was 2.3% in both ginger peel along with pulp. One of their peers, Gan et al. [29] performed their research work on separation and purification of 6-gingerol from ginger supercritical extract and proposed that extraction yield of supercritical fluid was 3.7% without co-solvent.

Phytochemical screening test

Phytodrugs are the secondary metabolites acting as antioxidants that prevent and decelerate oxidation reaction both as *in vitro* and *in vivo* by eliminating the chain reaction of oxidation. Many crude plant extracts along with some pure extracted compounds are stated to have antioxidant and free radical scavenging activity. The higher accumulation and/or formation of free radicals in the body have the ability to accelerate the rate of lipids oxidation that results in bad health. To overcome these complications herbs and spices are used in food to provide health benefits along with flavor. In this context, ginger proved its phytoentities having moderate antioxidant capability as the pharmacological drugs to enhance the treatment for disorders [30].

Superoxide anion free radical scavenging activity: Ginger based bioactive compounds, in addition to other phenolic antioxidant tests have the ability to reduce oxidative stress markers. Regarding superoxide, the means for solvents (Table 4) i.e., ethanol, acetone and ethyl acetate were $54.20 \pm 1.84\%$, $32.07 \pm 1.03\%$ and $46.22 \pm 1.39\%$, correspondingly whilst means regarding to time effect were $42.17 \pm 1.35\%$, $49.33 \pm 1.58\%$ and $40.98 \pm 1.32\%$ at 60, 90 and 120 min, respectively. Maximum superoxide anion radical scavenging 57.80 ± 1.97 was noted in ethanol extract at 90 min. In addition to this solvents, the supercritical ginger extract showed maximum activity at 3300 psi ($78.13 \pm 2.50\%$) followed by 3600 psi ($72.96 \pm 2.19\%$) and 3000 psi ($69.24 \pm 2.35\%$) (Table 5).

Nitric oxide radical scavenging activity: Nitric oxide radicals are produced by specific nitric oxide synthases in biological tissues which have the ability to metabolize arginine to citrulline via the formation of nitric oxide radical by involving five electron based oxidative reaction. Due to this reaction, at physiological pH i.e., 7.2 a compound namely sodium nitroprusside decomposed itself into aqueous solution and enhance the production of nitric oxide radicals. Although, under aerobic environment these radicals react with oxygen and convert into nitrate and nitrite, the stable compounds [7]. It is mostly used to assess the anti-oxidative markers that valued the antioxidant indices through free radical scavenging perspective of ginger. Means for nitric oxide (Table 6) depicted that free radical scavenging activity of ethanol

Treatments	Time			Means
	60 min	90 min	120 min	
Ethanol	50.61 ± 1.72	57.80 ± 1.97	54.18 ± 1.84	54.20 ± 1.84^a
Acetone	31.56 ± 1.01	36.92 ± 1.18	27.73 ± 0.89	32.07 ± 1.03^c
Ethyl acetate	44.35 ± 1.33	53.27 ± 1.60	41.04 ± 1.23	46.22 ± 1.39^b
Means	42.17 ± 1.35^{ab}	49.33 ± 1.58^a	40.98 ± 1.32^b	-

Table 4: Means for superoxide anion radical scavenging activity (%) of ginger extracts. Values having different superscripts differ significantly.

Pressure (psi)	Superoxide	Nitric oxide	Hydroxyl	Hydrogen peroxide
3000	69.24 ± 2.35	47.14 ± 1.60	82.57 ± 2.81	69.36 ± 2.36
3300	78.13 ± 2.50	54.83 ± 1.75	86.71 ± 2.77	83.19 ± 2.66
3600	72.96 ± 2.19	49.61 ± 1.49	77.30 ± 2.32	75.42 ± 2.26

Table 5: *In vitro* antioxidant indices (%) of supercritical ginger extracts.

Treatments	Time			Means
	60 min	90 min	120 min	
Ethanol	30.81 ± 1.05	38.23 ± 1.30	33.46 ± 1.14	34.17 ± 1.16^a
Acetone	18.64 ± 0.60	22.15 ± 0.71	19.94 ± 0.64	20.24 ± 0.65^c
Ethyl acetate	29.53 ± 0.89	31.78 ± 0.95	25.12 ± 0.75	28.81 ± 0.86^b
Means	26.33 ± 0.84^b	30.72 ± 0.99^a	26.17 ± 0.84^b	-

Table 6: Means for nitric oxide radical scavenging activity (%) of ginger extracts. Values having different superscripts differ significantly.

extract was maximum $34.17 \pm 1.16\%$ followed by ethyl acetate and acetone extracts $28.81 \pm 0.86\%$ and $20.24 \pm 0.65\%$, respectively. Among various ginger extracts, antioxidant activity of ethanol ginger extract was highest $38.23 \pm 1.30\%$ at 90 min whilst, lowest at 120 min $33.46 \pm 1.14\%$. In supercritical fluid extraction of ginger maximum nitric oxide inhibition was 54.83 ± 1.75 at 3300 psi and lowest was $47.14 \pm 1.68\%$ at 3000 psi pressure (Table 5).

Hydroxyl radical scavenging activity: Mean hydroxyl radical scavenging activity (Table 7) regarding three solvents i.e., ethanol, acetone and ethyl acetate has revealed maximum activity $73.83 \pm 2.51\%$ in ethanol followed by ethyl acetate ($64.56 \pm 1.94\%$) and acetone ($56.94 \pm 1.82\%$). In the same way, time factor has also influenced hydroxyl radical scavenging rate of extracts that was highest at 90 min $67.80 \pm 2.18\%$ and lowest at 120 min $61.10 \pm 1.96\%$. For hydroxyl radical scavenging of ginger maximum reduction was observed at 3300 psi $86.71 \pm 2.77\%$ and lowest at 3600 psi $77.30 \pm 2.32\%$ (Table 5).

Hydrogen peroxide: The presence of reducing agents accomplished with number and position of hydroxyl group also matter in this reduction mechanism to enhance antioxidant perception. Results (Table 8) have illustrated that ethanol extract has maximum H_2O_2 reducing power 47.09 ± 1.60 that was low in acetone extract $33.74 \pm 1.12\%$. In the same way, significant effect was noted in time factor for each extract that was higher $45.01 \pm 1.48\%$ at 90 min while lowest $37.72 \pm 1.21\%$ at 120 min. Besides this the supercritical extract of ginger showed $69.36 \pm 2.36\%$ inhibition in hydrogen peroxide at 3000, 83.19 ± 2.66 at 3300 and $75.42 \pm 2.26\%$ at 3600 psi as depicted in Table 5.

The finding of current research work are in accordance with the results of Dugasani et al. [31] who conducted the comparative study of antioxidant and anti-inflammatory effects of 6, 8, 10 gingerol along with 6 shogaol and suggested that superoxide anion radical scavenging activity of ginger extract was 5-40% by the addition of 1-6 μM ginger as a sample. Moreover, the hydroxyl radical scavenging activity and nitric oxide scavenging activity of ginger was 30-80 and 45-80% by using the same concentration of ginger sample. Moreover, another group of scientists, Amir et al. [32] investigated the phytochemical analysis and *in vitro* antioxidant analysis of ginger and depicted that the superoxide radical, hydroxyl radical, nitric oxide radical and hydrogen peroxide scavenging activity of ginger was 30-60% by using 10-50 $\mu\text{g/mL}$ of ginger extract, 41-50% by addition of 5-25 $\mu\text{g/mL}$ of ginger extract, 35-50% by supplementation of 10-50 $\mu\text{g/mL}$ of ginger extract and 35-65% by adding $\mu\text{g/mL}$ of ginger extract, correspondingly.

Afterwards, Padamanabhan and Jangle [23], worked on the *in vitro* antioxidant potential of herbal blend by the addition of *Aloe vera*, *Bacopa*

Treatments	Time			Means
	60 min	90 min	120 min	
Ethanol	73.58 ± 2.50	77.62 ± 2.64	70.30 ± 2.39	73.83 ± 2.51 ^a
Acetone	61.26 ± 1.96	56.75 ± 1.82	52.81 ± 1.69	56.94 ± 1.82 ^c
Ethyl acetate	64.45 ± 1.93	69.03 ± 2.07	60.19 ± 1.81	64.56 ± 1.94 ^b
Means	66.43 ± 2.13 ^a	67.80 ± 2.18 ^a	61.10 ± 1.96 ^b	-

Table 7: Means for hydroxyl radical scavenging activity (%) of ginger extracts. Values having different superscripts differ significantly.

Treatments	Time			Means
	60 min	90 min	120 min	
Ethanol	45.37 ± 1.54	52.61 ± 1.79	43.29 ± 1.47	47.09 ± 1.60 ^a
Acetone	34.53 ± 1.10	36.94 ± 1.18	29.76 ± 0.95	33.74 ± 1.12 ^c
Ethyl acetate	38.60 ± 1.16	45.48 ± 1.36	40.12 ± 1.20	41.40 ± 1.24 ^b
Means	39.50 ± 1.27 ^{ab}	45.01 ± 1.48 ^a	37.72 ± 1.21 ^b	-

Table 8: Means for hydrogen peroxide (%) of ginger extracts. Values having different superscripts differ significantly.

monniera, *Moringa oleifera* and *Zingiber officinale* and suggested that the superoxide, nitric oxide and hydroxyl radical scavenging activity of this blend was 19-66, 30-60 and 80-88% by the addition of 200-1000 µg/mL of that extract. At the same time, Oboh and his colleagues [33], worked on the antioxidant and inhibitory effect of red and white ginger and proposed that hydrogen peroxide and hydroxyl radical scavenging activity of ginger extract ranges from 10-40 and 10-70%, accordingly. At that juncture, Morakinyo et al. [34] insinuated that the superoxide anion radical scavenging of ginger extract ranges from 20-80% by addition of 10-500 µg/mL of ginger aqueous and ethanol extracts. Then and there another group of scientists, Kazeem et al. [35] determined the antioxidant and toxicological potential of ginger by using ethyl acetate, water and hexane as solvent and recommended the superoxide anion radical scavenging value of ginger ranges from 20-55%.

Nonetheless, Oranusi et al. [36] evaluated the antioxidant perspectives of some herbs and spices from Nigeria, amongst they concluded that hydrogen peroxide value as well as the nitric oxide value of ginger extract was 72.5-75.6 and 18.78-32.14% respectively by using different concentrations from 3-25 mg of ginger powder per mL of ethanol. Similarly, Jeena et al. [24] worked on the antioxidant potential of ginger essential oil and depicted that superoxide and hydroxyl radical scavenging activity of ginger extract was 25-65 and 3-40% by the addendum of 50-200 µg/mL of ginger extract. One of their peer, Ghosh et al. [5] assessed the *in vitro* antioxidant potential of ginger and believed that the superoxide and hydroxyl radical scavenging activity of ginger aqueous extract was 10-60 and 15-56% by the supplementation of 1-6 µM of ginger extract.

At the moment, Lin with his colleagues [22], conducted a chemical study of [6] gingerol for its protective effect against hydrogen radical and superoxide anion radical scavenging activity and depicted that IC₅₀ for hydrogen peroxide was 70.39 whilst, for superoxide it was 228.40. Recently, Nile et al. [37], evaluated the chromatographic, antioxidant and anti-inflammatory activities of ginger extracts and reported that by using 1 µg/mL of ethanol and ethyl acetate extracts the IC₅₀ for hydrogen peroxide was 20 for ethyl acetate and 34 for ethanol extract. Nevertheless, Marrelli et al. [28] performed a research work on ginger pulp and peel for its phytochemical analyses and advocated that by the application of 1 mg/mL of ginger extract the nitric oxide radical scavenging activity was 36.74% for pulp however the ginger peel did not show any activity against nitric oxide scavenging.

Quantification by high pressure liquid chromatography

HPLC (High Performance Liquid Chromatography) quantification of the powdered ginger revealed that among all the pungent ingredients 6-gingerol was four times more intense. The resultant peaks obtained from HPLC regarding to 6-gingerol were inferred with the help of standard spectral exploration, peak area and retention time. HPLC assessment for ginger (Table 9) proved that the highest gingerol concentration was present in supercritical extracts as 7.81 mg/g in 3300 psi pressure, 6.19 mg/g at 3600 and 5.63 mg/g in 3000 psi followed by ethanol, ethyl acetate and acetone 5.74 mg/g, 4.56 mg/g and 3.20 mg/g respectively.

The current findings are in harmony with the outcomes of Aly et al. [38] who characterized ginger by high performance liquid chromatography (HPLC) and concluded about the quantity of 6-gingerol. They suggested that the quantity of gingerol varies in rhizome, roots and shoots as the highest quantity of 6-gingerol was found in rhizome (42.64 mg/g) followed by shoots (7.46 mg/g) and roots (6.40 mg/g). Furthermore, Naghia [27] worked on the supercritical extraction of ginger oleoresin by using specific flow rate and co-solvent and depicted that the by quantifying the supercritical extract of ginger by HPLC ranged from 179 mg/100g-221 mg/100g. After that, another group of scientists, Anisa et al. [39], evaluated the quantity of 6-gingerol and 6-shogaol in ethanolic extract obtained by soxhlet apparatus by HPLC and resolved that the quantity of 6-gingerol was 8406.996 µg/g as well as 716.760 µg/g for 6-shogaol.

Nonetheless, Sonale et al. [40] did research work on the characterization of gingerol and its analogues in supercritical fluid extracts and recommended that the HPLC percentage portion of 6-gingerol was 30-35% in crude ginger extract and 75-79% in purified supercritical extract. One of their peers, Cho with his colleagues, [41] performed analysis of 6-gingerol in Zingiber fresh, powder and in different commercial products by using HPLC and concluded that the 6-gingerol content in root of *Zingiber officinale* ranged from 13.24 ± 0.55-17.09 ± 0.10 mg/g. Moreover, Guo et al. [42] conduct a research to evaluate the decomposition of 6-gingerol during processing and concluded that during processing gingerol decreased and shogaol content increased. Amongst all gingerol series, 6-gingerol decreased to 2.11 ± 0.2 mg/g from 9.72 ± 0.03 mg/g that was the concentration of 6-gingerol in unprocessed fresh ginger.

Even so, Murthy et al. [43] determined the composition of ginger oleoresin along with its bioactivity and usage as bio-preservative. In this research work they concluded after soxhlet apparatus extracting of ginger oleoresin by using acetone, 6-gingerol is the chief bioactive ingredient that accounts up to 12.80% in ginger extract. Recently, Ito et al. [44] determined the composition of ginger extract and quantified it through RP-HPLC by using water and acetonitrile as mobile phase in gradient system under UV detector (280 nm) against standards of 6-gingerol and 6-shogaol. Conclusively, they suggested that in fresh ginger extract the main ingredient was 6-ginger (110 mg/g) followed by a lesser amount of shogaol (5 mg/g).

Solvent	Concentration (mg/g of dry matter)
Ethanol	5.74
Acetone	3.2
Ethyl acetate	4.56
SFE 3000	5.63
SFE 3300	7.81
SFE 3600	6.19

Table 9: HPLC quantification for gingerol (mg/g) in ginger extracts.

Practical Application

Conclusively, supercritical extract of ginger showed maximum health boosting properties by highest inhibition of free radical. In previous literature many papers are present related to redox potential of conventional extract however it is the first paper describing redox potential of supercritical extract. The quantity of bioactive moiety decreased at higher pressure due to increase in vibrational motion within molecule. This paper will ensure the usage of supercritical based nutraceutical in routine diet.

Conflict of Interest

No external funding was applied to this project.

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