Reconnoitring the impact of different extraction techniques on ginger bioactive moieties extraction, antioxidant characterization and physicochemical properties for their therapeutic effect

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Abstract: The core directive of current study was to compare the different extraction techniques for their extraction yield of ginger bioactive moieties. Purposely, ultrasonic assisted and supercritical fluid extraction techniques were adapted alongside conventional solvent extraction for comparison. The variables targeted for the extraction process in different modules were time (20, 30, 40 minutes), temperature (30, 40, 50^{o}C), solvent to sample ratio (4:6, 6:4, 8:2), pressure (2000, 3000, 4000 psi) and amplitude (20, 30, 40 %). All variables were found to be momentously ($P>0.05$) effecting the extraction rates. The antioxidant potential of bioactive moieties was evaluated through FRAP, DPPH and ABTS. The outcomes of the optimization process suggested that the total phenolic content and total flavonoids content extracted through 80% ethanol at 50^{o}C for 40 minutes showed maximum antioxidant activity. However, ultrasound assisted extraction, by using 80% ethanol, at 50^{o}C for 40 minutes and at ultrasonic amplitude of 40% exhibited best results. Moreover, supercritical fluid extraction at 50^{o}C for 40 minutes at5000 psi pressure, showed maximum extraction potential. All the extracts gathered through conventional, ultrasound and supercritical techniques were further quantified through HPLC protocols. The statistical interpretation of the results from all the analytical findings revealed highest concentration of polyphenols in supercritical fluids extracts followed by ultrasound and conventional extracts. One best treatment on the basis of superior nutritional profile as depicted by HPLC quantification was selected for the formulation of ginger drink. Physicochemical analysis elucidated momentous upshot on color, pH and acidity with progressive storage period whereas TSS followed a non-significant trend. Moreover, storage interval and treatments significantly affected the antioxidant potential of drinks.

Keywords: Polyphenols, ginger, conventional solvent extraction (CSE), supercritical fluid extraction (SFE), ultrasound assisted extraction (UAE), functional drink.

INTRODUCTION

Ginger (Zingiber officinale) belongs to the family Zingiberaceae and is a Perennial herb with thick tuberous rhizomes. Ginger is originated in South-East Asia and then became widespread in many ecological zones. It produces a pungent aromatic rhizome that has been used both as a spice and medicinal herb since several years across the globe. Ginger is one of the classic examples of an herb used for not only culinary preparations but also for unique therapeutic applications due to presences of polyphenols. (Jyotsna *et al.,* 2017: Masood and Tauseef, 2011). Polyphenols are bioactive moieties occurring widely in plant kingdom that are eaten regularly by substantial number of people. These compounds have been found effective to cure various ailments such as cancer insurgence, diabetes & cardiovascular complications, obesity, aging, inflammatory disorders, microbial contaminations, liver cirrhosis, and allergic response, respectively (Gupta *et al*., 2013). In past few decades the extraction of phenolic compounds from natural products has attracted many researchers for their promising impact in improving the health status. Researchers are striving for reconnoitering more innovating strategies to maximize the extraction rates from different plant materials (Herrero *et al*., 2006;

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Farias-Campomanes *et al*., 2013). In phenolic compounds extraction, factors like extraction method, chemical nature of compounds extracted, solvent, presence of interfering substances as well as storage conditions are the potential extraction yield determining variables (Mokrani and Madani, 2016; Da Porto and Natolino, 2017). Solvent extraction method is an outmoded method for extraction and is more commonly used for the segregation of bioactive compounds. In this method, extraction return of bioactive compounds is dependent on circumstances of Extraction and the solvent polarity (Wu *et al*., 2001; Hromadkova, 2003). Ultrasound assisted extraction is a innovative method to effectively extract chemical constituents from plant materials. Ultrasound has high capacity to extract phenolic compounds and that is the purpose it is being used frequently (Shirsath *et al*., 2012; Idris *et al.*, 2019). Supercritical fluid extraction methodology is yet another modern approach towards the extraction of an array of bioactive phytochemicals and has remained curious horizons for researchers for decades. Supercritical state is achieved when a substance acquires dual attributes of liquids as well as gases at certain temperature and pressure above its critical limits (Wang and Weller, 2006). It is the most preferred approach practiced in food and pharmaceutical sector, owing to its environmentally benign nature. The application of ultrasound during such supercritical extraction methods has been suggested recently as an appliance both for rate acceleration and yield improvement. The extraction of polyphenols from natural sources using supercritical fluids has significant benefits over old methods (Palmer and Ting, 1995; Unger and Hung, 1994). Higher solubility, mass transfer rates and selectivity make supercritical fluid extraction (SFE) more gorgeous approach. The selectivity of the compound to be extracted is reliant on the density of the supercritical fluid, which can be reformed by varying process situations. The low latent heat of evaporation and high volatility of the solvent makes the extract free of residual solvent. Moreover, extraction can be practiced at low temperatures, which aids to preserve thermally degradable food entities (Heidi, 1982; McHugh and KruKoins, 1994; Ali *et al.*, 2018). The bioactive moieties from natural sources are thermally instable and prone to higher degradation during all types of processing stages (Liu *et al*., 2001). As far as the phenolic compounds are apprehensive, a numeral of altered extraction techniques have been presented, with the most frequent being with the use of water and organic solvents (e.g., ethanol, methanol) (Latoui *et al*., 2012; Da Porto and Natolino, 2017). Considering the importance of different extraction techniques, the recent study was intended to evaluate the impact of different extraction techniques on bioactive moieties extraction from ginger. Moreover, antioxidant profiling and therapeutic intervention development is also a matter of prime consideration.

MATERIALS AND METHODS

Procurement of Raw Material

For experimenting, ginger was procured from (Vegetable section) Ayub Agriculture Research Institute (AARI), Faisalabad. Whereas, the HPLC standards and analytical chemicals were procured from Merck (Germany) and sigma-Aldrich (Japan).

Raw material handling

In first step, ginger was through washed with clean water to clear it from any impurities. Afterwards, the sample was dried through hot air oven at 50^{o}C for 48 hrs. Lastly, the dried sample was turned into powder through small laboratory grinder (Panasonic, Japan, Model MJ-W176P) and filled in jars for further utilization.

Proximate analysis

The proximate estimation of ginger powder was evaluated through determination of moisture, fat, protein, fat, fiber and ash by adapting the guidelines of AACC (2000). Whereas, NFE was estimated through subtraction method.

Minerals analysis

The ginger sample was evaluated for minerals like sodium (Na), potassium (K), calcium (Ca), zinc (Zn), magnesium (Mg) and iron (Fe) through standard protocol of AOAC (2006). The first two were estimated through Flame Photometer-410 (Sherwood Scientific Ltd., Cambridge) however, remaining by Atomic Absorption Spectrophotometer (Varian AA240, Australia).

Preparation of extracts

Conventional solvent extraction

The ginger powder extracts were prepared by using the protocols devised by (Rusak *et al*., 2008). Purposely, ethanol, methanol alongside water were adapted at three different time, temperature and solvent to sample ratios 20, 30, 40 minutes, 30°C, 40°C, 50°C and 40:60, 60:40, 80:20, respectively. The obtained extracts were further concentrated and turn into powder form by using freeze drying and stored at optimum condition.

Supercritical fluid extraction (SFE)

The extraction of ginger polyphenols from Supercritical fluid extraction was carried out by using three parameters of temperature, time and pressure of 30 ,40 ,50 ^{o}C, 20, 30 40 minutes and 2000, 3000, 4000 psi, respectively (Ahn *et al*., 2013).

Ultrasonic assisted extraction (UAE)

The UAE of bioactive moieties was conceded out following the procedures of (Albu *et al*., 2004) with some modifications. For extraction, methanol, ethanol and water were utilized as extraction solvent three different parameters of time, temperature, solvent to sample ratio and amplitude were 20, 30, 40 minutes, 30°C, 40°C, 50°C, 40:60, 60:40, 80:20 and 20, 30, 40%, respectively.

Phytochemical screening assays

Different antioxidant assays were executed through total phenolics, Total flavonoids, DPPH, ABTS and FRAP assay estimation.

Determination of total phenolics (TP)

The total polyphenols (TP) were the most common assay to estimate the antioxidant potential of the tested compound. Purposely, the equal amount of FC reagent and sample was taken alongside 500µL of distled water and provide stay for 5 minutes afterwards, 4.5mL of 7% Na_2CO_3 was added and stay for 90 minutes. Lastly, the absorbance was measured through spectrophotometer (IRMECO, U2020) at 760nm. The total phenolics were calculated as gallic acid equivalent (mg gallic acid/g) following the procedure of (Sengul, 2009).

Determination of Total Flavonoid (TF)

Flavonoids are the largest class of polyphenols and elucidated the compounds ability to impart therapeutic potential. Briefly, a mixture of sample, water, $NaNO_2$, & 10% $AlCl_3$ was added in ratio of 0.1, 4 0.3ml & 5%, respectively and rested for 6 minutes and added 1.0M NaOH. The final absorbance was carried out at 430 nm by adapting the guidelines of Ghasemzadeh and Jaafar (2013).

Table 1: Nutritional Analysis of Ginger

Constituents	Observed Concentration
Moisture	80±1.1 %
Protein	3.2±0.21 %
Fat	1.00±0.01%
Fiber	3.00±0.04%
Ash	2.60±0.01%
NFE	10.2±0.02 %
Na	24±0.14 mg/kg
K	1200±5.25 mg/kg
Ca	82±0.94 mg/kg
Zn	20.5±0.05 mg/kg
Fe	17.5±0.90 mg/kg
Mg	198±1.01 mg/kg
Mn	21±0.47 mg/kg

Values are mean ± SEM (n = 03)

Antioxidant potential

DPPH Radical Scavenging Assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging ability estimation is the most common test to apprehend the antioxidant potential of tested compound. In short, sample and DPPH solution (0.12mM) was added in test tube in ratio of 4 & 1Ml, respectively and placed for 30 minutes in dark place. Afterwards, absorbance was r at 520 recorded by using UV/Visible Spectrophotometer alongside control and blank (Tomsone *et al*., 2012).

ABTS (2,2'-azino-bis, 3-ethylbenzothiazoline-6-sulfonic acid) Assay

ABTS assay of ginger extract was estimated conferring to the method outlined by (Hossain *et al*., 2008). For the preparation of ABTS radicals, %mL freshly prepared ABTS solution (7mM) was mixed with 5mL potassium per sulfate solution (2.45mM) to make 10mL total volume. The mixture was transferred to opaque bottle and allowed to stand for 16 hrs in a dark place to achieve stable oxidized state. The mixture was diluted with ethanol and was adjusted to give 0.7 absorbance at 734 nm. Additionally, 10µL ginger extract was added 1mL ABTS solution, mixed thoroughly and subjected to spectrophotometery to measure the absorbance at 734 nm after 30 min stay time. The antioxidant activity using the trolox standard curve was reported in µmol Trolox /g sample extract.

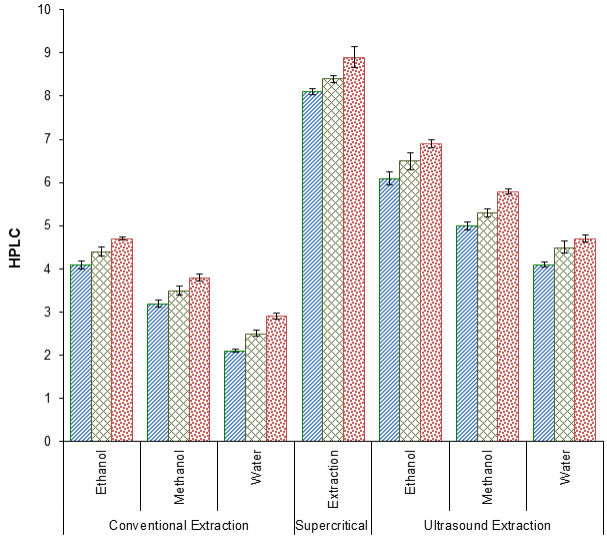

Fig. 1: Absolute values for gingerol characterize through reverse phase HPLC in mg/g. Three solvents and extraction techniques were applied, and comparisons were made between solvents and extraction techniques. Values are mean ± SEM (n = 03) and level of significance were determined at ($p \leq 0.05$).

Ferric reducing antioxidant power (FRAP)

The metal ion chelation ability is another parameter to access the antioxidant capacity of the tested compounds under different challenge and for this ferric reducing antioxidant power is the most adapted test. For estimation of FRAP, 0.5mL of sample was added in 125 mL of phosphate buffer prepared 0.2M and Ph of 6.6 and potassium ferricyanide solution (1%), placed in water bath at 50°C for a duration of 15 min. in resulted sample, equal amount of 1.25mL Trichloroacetic acid (10%) and distilled water alongside 0.25mL of ferric chloride (1%) were added and provide stay time for 10 min and record the reading at 700 nm. (Baek *et al*., 2008).

HPLC quantification of bioactive compounds
Different prepared ginger extracts were exposed to HPLC quantification to evaluate the comparative abundance of bioactive molecules.

Polyphenol quantification
The gingerol is the most abundant antioxidant in ginger and its estimation is important to impart positive health benefits to the ginger. Purposely, HPLC quantification was carried out for gingerol contents estimation in ginger extracts obtained through all extraction techniques. Briefly, reverse phase HPLC (PerkinElmer, Series 200, USA) with C18 column. The mobile phase was comprised of methanol/water, 65:35 (v/v), with sample size of 1mL, with 1.0 mL/min flow rate. For estimation, UV detection was made at 282 nm. The calculation were made by comparing the peak time and height with the gingerol standard (Yeh *et al*., 2014)

Product development
In product development four treatments of polyphenol drink were formulated by incorporating different levels of selected conventional (1%), supercritical (1%) and ultrasound extract (1%). A control deprived of extract was also formulated for comparison. Raw materials used for drink preparation were table sugar, aspartame, citric acid, CMC, sodium benzoate, food grade color and flavor.

Physicochemical analysis of functional drink
Total soluble solid, pH and total acidity of the ginger drink was carried out by their standard procedures as defined by (AOAC, 2006) during 0, 20, 40 and 60 days storage periods. The color of ginger-based drink was assessed by estimating the different color values like a*, b*, L*, through CIE-Lab Color Meter (CIELAB SPACE, Color Tech-PCM, USA). Whereas, chroma (C*) and hue angle were calculated by obtained a and b values (Lara *et al*., 2010).

Antioxidant potential of drink
All the prepared drinks were exposed to in vitro phytochemical screening and antioxidant potential proves methods as per protocol described in section 3.6 and 3.7 (AOAC, 2006).

Sensory evaluation
Sensory evaluation of ginger-based drink was conceded out by following the guidelines of (Meilgaard *et al*., 2007).

STATISTICAL ANALYSIS

The data for each parameter was analyzed statistically to check the level of significance. For analysis of variance one way and two way ANOVA was performed and for the estimation of significance among the means Tukey's HSD test was applied. The values were represented in mean ± S.D.

RESULTS

Proximate and mineral analysis
The mean values for moisture, protein, fat, fiber, ash and carbohydrate content were found to be 80%, 3.2%, 1%, 3%, 2.6%, and 10.2 %, respectively. Likewise, the values for observed minerals were recorded as Na (24.08±1.46mg/100g), K (1200.19±20.04mg/100g), Ca (82.22±6.22mg/Kg), Zn (20.5±0.93 mg/Kg), Fe(17.51±0.72 mg/Kg), Mg (198.43±2.94 mg/Kg), Mn (21.67±0.82 mg/Kg) and P (166.41±4.31 mg/Kg), respectively (table 1).

In vitro characterization
Means regarding the total phenolic contents, total flavonoids and antioxidant activity for ginger extracted through different extraction techniques showed significant differences due to treatments and extraction parameters. Solvents, temperature, pressure, amplitude and time significantly affected the total phenols, total flavonoids and antioxidant potential (DPPH, FRAP, ABTS) of different extracts. Highest recovery was found in supercritical fluid extraction trailed by ultrasound assisted extraction and conventional solvent extraction respectively. Highest values were found at pressure 4000 psi, time 40 minutes and temperature 50^{o}C in supercritical fluid extraction while in ultrasound assisted extraction highest recovery was at time 40 minutes, temperature 50^{0}C, ethanol 80% and amplitude 40%. Likewise, in conventional solvent extraction same tendency was noticed in which highest figs. were at time 40 minutes, temperature 50^{0}C and ethanol 80%. Ethanol extraction showed best recovery than methanol and water in ultrasound and conventional extraction (table 3).

HPLC quantification
Statistical analysis of ginger bioactive components described significant differences in gingerol content of conventional, ultrasound and supercritical fluid extraction. Means concerning the effect of different solvents and their ratios elucidated highest recovery of gingerol in 80% ethanolic extract among conventional and ultrasound extractions as a function of treatment. Generally, ethanolic extracts showed higher gingerol recovery which increased with increase in solvent concentration, time, temperature and amplitude. Among supercritical fluid extracts, the recovery of bioactive component increased with increased in pressure, time and temperature (fig. 1).

Physicochemical analysis of functional drink
The physicochemical attributes of ginger-based drinks were analyzed including color, pH, acidity and TSS. For color analysis, CIELB color system was used which is based on L*, a* and b* values. L* value is indication of lightness and darkness, a* value represents greenish and reddish tone, b* value indicates yellowish and bluish

color. Means related to pH depicted in values from 4.49±0.03 at initial day to 4.30±0.03 after 60 days. Mean square regarding acidity exhibited significant effect of treatments while same variation was observed during storage. Mean value of acidity was higher in solvent extracts as compared to control. Moreover, a significant elevation in acidity was noted during 60 days of storage and treatments. Means squares concerning TSS elucidated non-significant effect of storage intervals and treatments on TSS value. Minor increase in brix value was observed during 60 days storage study. Chroma value represents color saturation, more the chroma value more will be the intensity of the color. Means concerning chroma values exhibited highest chroma value in control T_0 whereas, minimum value for this character was noted in drink containing conventional solvent extraction. Moreover, significant decline in chroma value was observed during storage. Similarly, means regarding hue angle were 79.75±0.70, 82.84±0.78, 82.95±0.82 and 83.62±0.73 for T_0, T_1, T_2 and T_3 respectively. As a role of storage interval, the recorded value at 0, 20, 40 and 60 days were 81.97±1.02, 82.00±0.94, 82.84±0.81 and 82.71±0.73, respectively showed increasing trend (table 3). Conclusively, the color tone of functional drinks changed from yellowish towards brownish during two months of storage.

Antioxidant potential of ginger drink

Means regarding effect of treatment on TPC, TF, DPPH, FRAP, ABTS assays are shown in table. For treatments observed value for TPC in ginger drink were highest in supercritical extract followed by ultrasound extract, conventional extract and control respectively. Similarly, same trend was observed in TF. For DPPH free radical scavenging activity, maximum activity was noted in T_2 (supercritical) followed by T_3 (ultrasound), T_1 (conventional) and T_0 (control). Likewise, same trend was observed for FRAP value. Moreover, significant difference in ABTS value was detected as 3.85±0.18, 4.39±0.17, 4.82±0.22, 4.52±0.20. Means regarding effect of storage interval on TPC, TF, ABTS, DPPH, FRAP assays are presented in table. A substantial decline in TPC was detected during 60 days storage study. The observed figs. for TF also showed declined trend at 0, 20, 40 and 60 days. Similarly, DPPH free radical scavenging activity of polyphenols supplemented drink also exhibited decreasing trend at initiation of study to the end of 60 days storage trail. Moreover, significant decline in FRAP and ABTS value was observed from 67.64±0.74 to 64.02±0.83 and 5.28±0.15 to 3.60±0.09, respectively.

Sensory analysis

Means for color delineated non-significant behavior among treatments and storage of sensorial attributes. It is evident from mean for flavor highest values were observed in T_2 (Supercritical extract) and T_3 (Ultrasound extract) followed by T_1 (Conventional extract) while lower was in T_0 (Control) whereas storage showed non-significant behavior. Mean of taste showed no off taste was observed among treatment and storage. Likewise, same non-significant trend was observed in sensorial attribute of sweetness among treatment and storage interval. Moreover, significant drop was observed in overall acceptability during storage but non-significant pattern showed among treatments.

DISCUSSION

Outcomes presented that ethanol was more operative solvent than water. The consequences of this study are in line with the conclusions of Shirin and Jamuna (2010) who delineated ethanolic extract of ginger root is more efficient while methanol was less efficient. The current consequences are in accordance with the results of (Ismail *et al.,* 2011) that methanol soluble fraction of dried ginger extract contained highest quantities polyphenols compared to aqueous fraction. (Suprabhat *et al*., 2014) investigated that maximization the extraction of polyphenols from ginger can be achieved by using highest quantity of ethanol. (Rina *et al*., 2015) and (Offei-Okyne *et al*., 2015) on the basis of their research outcomes from zinger rhizome extract using water and ethanol as solvent proposed ethanolic extracts elucidated best result for TF, TPC and antioxidant activity. Another finding reported that solvent exhibits higher antioxidant potential when compared to aqueous soluble fractions was highest in ethanol followed by methanol (Faez and Muhd Taha, 2016). The results of this investigation are in concord with the conclusions of (Sohail *et al*., 2017) who reported that the ethanolic extract revealed higher radical-scavenging activity while aqueous extract presented lower inhibition. Likewise, (Tohma and Gulçin, 2010) suggested the same that ethanolic extract delineated 95.8% free radical scavenging activity in contrast to the aqueous extract that exhibited 81.7% inhibition. As (Jaffery *et al.,* 2003) specified in his study, the composition and quantity of the phenolics differ significantly owing to different intrinsic and extrinsic factors, including plant genetics and cultivars, soil and growing situations, maturity state and harvest conditions. Extraction method also has a momentous effect on the composition and properties of the ultimate extract (Gallardo *et al*., 2006). Antioxidant components and activity are highly reliant on extracting solvent and concentration of solvent (Turkmen *et al*., 2006), but they also differ with the nature of samples. The extraction of phenolic compounds from diverse plant resources with the use of carbon dioxide in a supercritical state has been studied by several authors in the temperature range 25-120^{o}C and pressure of CO_2 range from 1200-9600 psi. Optimal conditions for recovery by different researchers were pressure from 2400-4300 psi and temperature 40-60^{o}C (Michele *et al.,* 2012; Barroso *et al*., 2011; Rodrigo *et al.,* 2012; Wu *et al.,* 2001). Researchers compared the

Table 2: Comparison of different extraction techniques on antioxidant potential of ginger

	Treatments	Parameters				
		TPC (mg GAE/100g)	TF (mg CE/100g)	DPPH (%)	FRAP (µM Fe^{+2}/g)	ABTS (µM TE/g)
Conventional (CSE)	$T_{CSE1}E_1$	173.55±2.92	20.11±0.24	50.24±1.75	49.84±1.69	0.340±0.006
	$T_{CSE2}E_2$	196.58±5.10	22.66±0.62	59.12±1.82	52.78±1.17	0.370±0.002
	$T_{CSE3}E_3$	225.66±5.26	28.54±0.52	66.16±2.20	55.09±1.41	0.390±0.005
	$T_{CSE4}M_4$	141.54±3.29	15.33±0.20	44.08±0.38	44.66±0.80	0.310±0.007
	$T_{CSE5}M_5$	161.88±4.36	17.89±0.39	45.98±1.44	46.11±1.08	0.330±0.004
	$T_{CSE6}M_6$	170.56±3.69	19.44±0.31	48.55±2.18	49.02±1.90	0.350±0.005
	$T_{CSE7}W_7$	127.11±2.57	8.87±0.22	31.67±0.27	33.12±0.78	0.240±0.008
	$T_{CSE8}W_8$	142.67±3.71	10.34±0.08	35.66±0.84	36.17±0.79	0.260±0.002
	$T_{CSE9}W_9$	150.44±2.04	11.45±0.19	40.44±1.55	39.23±0.51	0.290±0.005
Ultrasound (UAE)	$T_{UAE10}E_{10}$	260.12±5.03	186.12±4.43	60.11±0.49	24.34±0.58	9.96±0.20
	$T_{UAE11}E_{11}$	277.23±6.57	199.45±1.44	63.14±0.76	26.14±0.79	10.45±0.13
	$T_{UAE12}E_{12}$	300.12±7.01	210.19±1.47	65.44±0.96	28.13±0.72	11.10±0.14
	$T_{UAE13}M_{13}$	236.11±4.52	171.15±5.01	52.88±1.71	20.12±0.41	9.28±0.17
	$T_{UAE14}M_{14}$	242.34±8.46	176.34±3.26	56.17±0.43	22.34±0.64	9.37±0.21
	$T_{UAE15}M_{15}$	250.31±7.18	182.12±1.76	59.33±1.02	24.10±0.28	9.56±0.27
	$T_{UAE16}M_{16}$	200.33±1.45	161.33±1.75	40.22±1.15	11.44±0.30	7.56±0.08
	$T_{UAE17}M_{17}$	210.11±4.13	167.34±1.71	46.33±1.14	15.33±0.43	8.10±0.22
	$T_{UAE18}M_{18}$	220.11±3.52	170.23±1.52	50.34±0.96	18.44±0.23	8.67±0.25
Supercritical (SFE)	T_{SFE19}	312.44±1.94	218.72±5.81	67.23±1.15	28.67±0.66	11.45±0.20
	T_{SFE20}	366.12±10.53	246.24±7.89	70.54±2.48	31.33±0.51	12.98±0.28
	T_{SFE21}	456.11±10.19	286.85±7.18	77.66±1.50	35.87±0.82	14.65±0.24

Values are mean ± SEM (n = 03) and level of significance were determined at ($p \leq 0.05$).

$T_{CSE1}E_{1=}$Time 20 min, Temp 30^{0}C, Ethanol40%	$T_{UAE11}E_{11=}$ Time 30 min, Temp 40^{0}C, Ethanol 60%, amplitude 30%
$T_{CSE2}E_{2=}$ Time 30 min, Tempe 40^{0}C, Ethanol 60%	$T_{UAE12}E_{12=}$= Time 40 min, Temp 50^{0}C, Ethanol 80%, amplitude 40%
$T_{CSE3}E_{3=}$ Time 40 min, Temp 50^{0}C, Ethanol 80%	$T_{UAE13}M_{13=}$ Time 20 min, Temp 30^{0}C, Methanol 40%, amplitude 20%
$T_{CSE4}M_{4=}$ Time 20 min, Temp 30^{0}C, Methanol40%	$T_{UAE14}M_{14=}$ Time 30 min, Temp 40^{0}C, Methanol 60%, amplitude 30%
$T_{CSE5}M_{5=}$ Time 30 min, Tempe 40^{0}C, Methanol 60%	$T_{UAE15}M_{15=}$ Time 40 min, Temp 50^{0}C, Methanol 80%, amplitude 40%
$T_{CSE6}M_{6=}$ Time 40 min, Temp 50^{0}C, Methanol 80%	$T_{UAE16}M_{16=}$ Time 20 min, Temp 30^{0}C, Water 40%, amplitude 20%
$T_{CSE7}W_{7=}$ Time 20 min, Temp 30^{0}C, Water 40%	$T_{UAE17}M_{17=}$ Time 30 min, Temp 40^{0}C, Water 60%, amplitude 30%
$T_{CSE8}W_{8=}$ Time 30 min, Temp 40^{0}C, Water 60%	$T_{UAE18}M_{18=}$ Time 40 min, Temp 50^{0}C, Water 80%, amplitude 40%
$T_{CSE9}W_{9=}$ Time 40 min, Temp 50^{0}C, Water 80%	$T_{SFE19=}$ Time 20 min, Temp 30^{0}C, Pressure 2000 psi
$T_{UAE10}E_{10=}$ Time 20 min, Temp 30^{0}C, Ethanol 40%, amplitude 20%	$T_{SFE20=}$ Time 30 min, Temp 40^{0}C, Pressure 3000 psi
	$T_{SFE21=}$ Time 40 min, Temp 50^{0}C, Pressure 4000 psi

radical scavenging linked antioxidant of ginger extracts by conventional solvent extraction and supercritical CO_2 extraction at different time, temperature and pressure combinations. Results elucidated high gingerol and maximum radical scavenging in supercritical rather than conventional (Tanveer *et al*., 2016). Additionally Adina *et al*. (2015) performed three techniques (conventional, ultrasound and supercritical) in order to recover the polyphenols. Results showed highest recovery in SFE followed by UAE and CSE.

Table 3: Effect of storage and treatment on L*, a*, b* Values, Chroma and Hue angle of functional drinks

L* Value					
Treatments	Days				Means
	0	20	40	60	
T0	80.65±1.55	80.40±2.62	79.37±2.22	78.77±2.22	79.80±0.96A
T1	67.73±0.44	66.10±1.15	66.05±1.51	64.46±0.29	66.08±0.58C
T2	65.17±1.52	64.96±0.09	63.57±1.15	62.88±0.79	64.15±0.53C
T3	70.44±0.33	70.07±1.57	69.11±2.97	67.96±1.32	69.42±0.70B
Means	71.00±1.83A	70.38±1.96AB	69.27±2.00AB	68.52±1.96B	

a* Value					
Treatments	Days				Means
	0	20	40	60	
T0	6.24±0.20gh	6.14±0.18gh	6.17±0.15gh	6.04±0.14h	6.15±0.08D
T1	8.78±0.16a	8.39±0.06ab	7.91±0.26ad	7.85±0.24be	8.21±0.14A
T2	7.40±0.10cf	7.10±0.20ef	6.84±0.14fg	6.18±0.09gh	6.88±0.15C
T3	8.12±0.16abc	7.84±0.17be	7.33±0.19def	6.81±0.19fgh	7.54±0.18B
Means	7.64±0.29A	7.37±0.26AB	7.11±0.22B	6.72±0.23C	

b* Value					
Treatments	Days				Means
	0	20	40	60	
T0	65.45±1.85	64.82±0.85	63.21±1.21	62.96±0.77	64.11±0.62A
T1	62.72±0.99	62.10±1.99	61.33±0.77	60.82±1.17	61.71±0.57A
T2	50.32±0.89	49.34±1.15	49.10±0.79	48.28±1.09	49.26±0.48B
T3	63.85±2.06	62.25±0.36	61.49±2.16	59.14±1.54	61.70±0.88A
Means	60.59±1.93A	59.63±1.90AB	58.77±1.76AB	57.80±1.78B	

Chroma Angle					
Treatments	Days				Means
	0	20	40	60	
T0	63.65±0.77	63.45±0.89	63.14±0.89	62.16±1.48	63.10±0.48A
T1	60.22±1.58	59.60±1.81	59.65±0.97	58.24±1.03	59.45±0.61B
T2	64.36±1.98	62.34±0.59	61.56±1.82	58.54±0.39	61.70±0.87AB
T3	62.69±0.94	61.96±0.78	61.25±3.14	60.67±2.04	61.68±0.74AB
Means	62.73±0.76A	61.84±0.64AB	61.27±0.76AB	59.90±0.76B	

Hue Angle

Treatments	Means			
	0	20	60	
T0	79.25±1.84	79.65±1.47	80.24±0.96	79.75±0.70B
T1	82.69±2.52	82.44±1.21	83.10±2.30	82.84±0.78AB
T2	82.77±2.34	82.46±2.44	83.35±0.92	82.95±0.82AB
T3	83.17±1.61	83.46±2.40	84.14±0.61	83.62±0.73A
Means	81.97±1.02A	82.00±0.94A	82.71±0.73A	

Values are mean ± SEM (n = 03). Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05)
T0=Control T1= Conventional extract T2= Supercritical extract T3= Ultrasound extract

Table 4: Effect of storage and treatment on pH, TSS and acidity of functional drinks

Ph					
Treatments	Days				Means
	0	20	40	60	
T0	4.50±0.07	4.45±0.09	4.40±0.04	4.27±0.03	4.41±0.04A
T1	4.46±0.08	4.42±0.05	4.39±0.07	4.31±0.09	4.40±0.04A
T2	4.45±0.10	4.41±0.13	4.37±0.07	4.28±0.07	4.38±0.05A
T3	4.53±0.03	4.49±0.12	4.43±0.11	4.33±0.06	4.45±0.04A
Means	4.49±0.03A	4.49±0.03A	4.40±0.03AB	4.30±0.03B	

TSS					
Treatments	Days				Means
	0	20	40	60	
T0	12.10±0.28	12.15±0.08	12.24±0.35	12.39±0.31	12.22±0.12A
T1	12.18±0.27	12.25±0.38	12.39±0.25	12.49±0.32	12.33±0.13A
T2	12.15±0.26	12.23±0.27	12.35±0.19	12.50±0.35	12.31±0.12A
T3	12.24±0.08	12.37±0.18	12.45±0.27	12.49±0.12	12.38±0.07A
Means	12.17±0.10A	12.25±0.11A	12.35±0.12A	12.47±0.12A	

Acidity

Treatments	Means		
	0	60	
T0	0.13±0.000c	0.14±0.000bc	0.135±0.002C
T1	0.14±0.000bc	0.15±0.006ab	0.143±0.002B
T2	0.14±0.000bc	0.16±0.000a	0.153±0.003A
T3	0.15±0.000ab	0.16±0.000a	0.153±0.001A
Means	0.140±0.002C	0.153±0.003A	

Values are mean ± SEM (n = 03) Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05) according to Tukey's HSD test.
T0=Control T1= Conventional extract T2= Supercritical extract T3= Ultrasound extract

Table 5: Effect of storage and treatment on antioxidant potential of functional drinks

TPC					
Treatments	Days				Means
	0	20	40	60	
T0	23.15± 0.55	22.67± 0.65	21.83± 0.27	19.68± 0.17	21.83± 0.45D
T1	24.66± 0.74	23.66± 0.14	22.25± 0.21	21.10± 0.28	22.92± 0.45C
T2	30.71± 0.68	29.56± 0.90	28.65± 0.59	27.87± 0.38	29.20± 0.43A
T3	27.45± 0.73	26.45± 0.74	25.67± 0.48	24.33± 0.43	25.98± 0.43B
Means	26.49± 0.92A	25.59± 0.86AB	24.60± 0.85B	23.25± 0.96C	

TF					
Treatments	Days				Means
	0	20	40	60	
T0	6.14± 0.10de	5.77± 0.13def	5.14±0. 12fg	4.82± 0.05gh	5.47± 0.16B
T1	6.95± 0.10bc	5.63± 0.09ef	4.66± 0.15gh	4.22± 0.08h	5.37± 0.32B
T2	7.75± 0.15a	6.97± 0.10b	6.17± 0.10de	5.75± 0.17def	6.66± 0.24A
T3	7.10± 0.18ab	6.92± 0.20bc	6.30± 0.09cd	5.66± 0.12def	6.50± 0.18A
Means	6.99± 0.18A	6.32± 0.20B	5.57± 0.21C	5.11± 0.20D	

DPPH					
Treatments	Days				Means
	0	20	40	60	
T0	41.47± 0.89	40.55± 0.68	39.65± 1.09	38.10± 0.30	39.94± 0.51C
T1	43.25± 1.15	42.44± 1.13	41.55± 0.41	40.07± 1.28	41.83± 0.57B
T2	46.11± 1.15	45.34± 1.33	42.66± 0.95	40.56± 1.00	43.67± 0.82A
T3	44.56± 0.71	43.88± 0.33	43.10± 0.32	42.55± 1.20	43.52± 0.39AB
Means	43.85± 0.67A	43.05± 0.67AB	41.74± 0.52BC	40.32± 0.65C	

FRAP					
Treatments	Days				Means
	0	20	40	60	
T0	65.87± 0.65	64.33± 0.95	63.56± 2.01	61.44± 0.81	63.80± 0.71C
T1	66.12± 1.85	65.55± 1.26	63.66± 1.44	62.44± 0.87	64.44± 0.74BC
T2	70.12± 0.59	68.33± 1.73	67.87± 1.53	66.44± 1.51	68.19± 0.72A
T3	68.43± 1.35	67.12± 1.45	66.55± 0.64	65.77± 1.58	66.97± 0.63AB
Means	67.64± 0.74A	66.33± 0.74AB	65.41± 0.85AB	64.02± 0.83B	

ABTS					
Treatments	Days				Means
	0	20	40	60	
T0	4.66± 0.10	4.10± 0.05	3.54± 0.08	3.10± 0.08	3.85± 0.18C
T1	5.10± 0.14	4.67± 0.04	4.13± 0.12	3.65± 0.05	4.39± 0.17B
T2	5.87± 0.14	4.98± 0.08	4.54± 0.09	3.87± 0.07	4.82± 0.22A
T3	5.47± 0.12	4.68± 0.12	4.14± 0.06	3.77± 0.06	4.52± 0.20B
Means	5.28± 0.15A	4.61± 0.10B	4.09± 0.11C	3.60± 0.09D	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05) according to Tukey's HSD test.
T0=Control T1= Conventional extract T2= Supercritical extract T3= Ultrasound extract

Table 6: Effect of treatments and storage on sensorics attributes on functional drinks

Color						Flavor					
Treatments	Days				Means	Treatments	Days				Means
	0	20	40	60			0	20	40	60	
T0	7.85± 0.22	7.80± 0.10	7.73± 0.17	7.70± 0.16	7.77± 0.07A	T0	7.50± 0.09	7.40± 0.11	7.35± 0.20	7.33± 0.11	7.40± 0.06B
T1	7.83± 0.21	7.81± 0.20	7.78± 0.18	7.74± 0.09	7.79± 0.08A	T1	7.60± 0.20	7.56± 0.14	7.52± 0.11	7.48± 0.22	7.54± 0.07AB
T2	7.86± 0.25	7.83± 0.14	7.79± 0.12	7.75± 0.18	7.81± 0.08A	T2	7.65± 0.13	7.61± 0.16	7.57± 0.10	7.51± 0.14	7.59± 0.06A
T3	7.87± 0.08	7.85± 0.09	7.80± 0.21	7.78± 0.07	7.83± 0.04A	T3	7.68± 0.12	7.60± 0.21	7.55± 0.27	7.51± 0.24	7.59± 0.09A
Means	7.85± 0.09A	7.82± 0.06A	7.77± 0.08A	7.74± 0.06A		Means	7.61± 0.06A	7.54± 0.07A	7.50± 0.07A	7.46± 0.08A	
Taste						Sweetness					
Treatments	Days				Means	Treatments	Days				Means
	0	20	40	60			0	20	40	60	
T0	7.65± 0.24	7.57± 0.14	7.52± 0.15	7.47± 0.15	7.55± 0.08A	T0	7.53± 0.25	7.45± 0.13	7.42± 0.11	7.34± 0.11	7.44± 0.07A
T1	7.70± 0.13	7.65± 0.13	7.60± 0.07	7.55± 0.19	7.62± 0.06A	T1	7.50± 0.13	7.54± 0.18	7.49± 0.09	7.44± 0.19	7.49± 0.06A
T2	7.68± 0.25	7.63± 0.16	7.54± 0.13	7.50± 0.17	7.59± 0.08A	T2	7.51± 0.12	7.47± 0.08	7.41± 0.17	7.35± 0.23	7.44± 0.07A
T3	7.70± 0.21	7.64± 0.22	7.58± 0.38	7.53± 0.09	7.62± 0.09A	T3	7.54± 0.14	7.50± 0.20	7.44± 0.20	7.39± 0.11	7.47± 0.07A
Means	7.68± 0.09A	7.62± 0.07A	7.56± 0.07A	7.51± 0.07A		Means	7.52± 0.07A	7.49± 0.07A	7.44± 0.06A	7.38± 0.07A	

Overall acceptability					
Treatments	Days				Means
	0	20	40	60	
T0	7.65± 0.16	7.59± 0.21	7.53± 0.13	7.50± 0.19	7.57± 0.08A
T1	7.67± 0.21	7.60± 0.25	7.55± 0.07	7.50± 0.06	7.58± 0.07A
T2	7.69± 0.12	7.55± 0.17	7.50± 0.15	7.46± 0.16	7.55± 0.07A
T3	7.70± 0.10	7.63± 0.08	7.57± 0.09	7.51± 0.16	7.61± 0.06A
Means	7.68± 0.07A	7.59± 0.08AB	7.54± 0.05AB	7.49± 0.06B	

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05) according to Tukey's HSD test.
T0=Control T1= Conventional extract T2= Supercritical extract T3= Ultrasound extrac

It has been testified that ultrasound-assisted extraction is far more superior to practice for higher reclamation of phenolic compounds compared to conventional extraction methods (Vázquez *et al*., 2014; Cai *et al*., 2016; Rodríguez-Pérez *et al*., 2015). In the presence of ultrasound-assisted extraction both extraction rate and yield increases. The greater extraction rate is endorsed to disruption of cell structures and rise in the accessibility of the solvent to internal particle structure that improves the intra-particle diffusivity (Balachandran *et al*., 2006). Phenolic compounds are dispersed in the cell on the basis of solubility that is due to their polarity. Solvents such as methanol or ethanol have a significantly lesser polarity than water and this favors the solubility and diffusion of the phenolic compounds by reducing the dielectric constant of the solvent (Nelly *et al*., 2017). The solute/solvent ratio is one of the most acute factors during mass transfer, because a greater volume of solvent aids to rush the diffusion process (Muñiz-márquez *et al*., 2013). An increase in the concentration of phenolic compounds is detected as the solute/solvent ratio increases (Galvan *et al*., 2012). Moreover, extraction efficiency associated to the time factor is also proven. Scientists reported that ultrasonic waves reduced the extraction time of phenolic compounds by 87% (Pan *et al*., 2013). Other scientists also stated a reduction in the ime of extraction for phenolic compounds with ultrasound assisted extraction requiring 10 times less extraction time than that of maceration. All revisions agreed that this development in extraction is due to the fact that the use of ultrasound favors the rupture of the cell wall, with the ensuing increase in the penetration of the solvent (Carrera *et al*., 2012). Studied showed that the optimal conditions for phenolic extraction and antioxidant activity were 80% ethanol, 40^{o}C temperature, time 25 minutes and 40% ultrasonic amplitude (Ammar *et al*., 2016; Seyed *et al*., 2016; Safdar *et al*., 2017).

The outcomes of present study are well supported by the early works of Jo *et al*. (2003) who estimated the effect of storage on color parameters and functional properties of licorice root extract at different temperatures. They reported decrease in L* value of irradiated licorice extract during two weeks storage. Moreover, a decrease in a* and b* value of licorice extract was also observed during storage. Likewise, the result regarding color are also in adjacent agreement with the research outcomes of Jayashree *et al.* (2012) who investigated the qualitative variations during storage of altered ginger-based spice sauces. They reported considerable decreased in L*, a*, b* values of sauces during 135 days storage study. Earlier Marti *et al*. (2002) notice similar trend and reported decrease in L* value and increased in hue angle during storage of pomegranate.

Results regarding change in pH and acidity are in agreement with the previous work of Kausar *et al*. (2012). They investigated the storage stability of cucumber-melon based functional drink and stated a decline in pH value whereas an increase in acidity was noted. Likewise El-Faki *et al.* (2010) estimated the effect of storage on physicochemical attribute of soft drinks and observed a declining tendency in pH during 6 months storage. Similarly, results of Fasoyiro *et al*. (2005) were also in closed agreement with current study who reported an increased in acidity with a subsequent decrease in pH during storage of fruit drink. Likewise, Ahmed *at al*. (2008) and González-Molina *et al*. (2009) also observed the same trend in pH and acidity as in present case. The results regarding TSS pertaining to increase in TSS during storage are in contour with the research outcomes of Singh *et al*. (2014); Harsha and Aarti (2016) and Sasi *et al*. (2013) they prepared functional based drinks and concluded that TSS increased during storage. Polyphenols are prone to degradation with the course of time due to certain factors including oxidation, pH change, enzymatic degradation and reactions with other substances. Polymerization is another major contributing factor in the loss of bioactive moieties during storage. Resultantly, the TPC and antioxidant potential extract decreased with time (Choi *et al.,* 2002). In such a study, (Alighourchi and Barzegar, 2009) estimated the effect of storage on degradation kinetics of anthocyanin in pomegranate juice. They reported a substantial decreased in total anthocyanin content of juice during 210 days of storage. They concluded that the loss of anthocyanin was attributed to oxidation and condensation to ascorbic acid. Later, (Fang and Bhandari, 2011) assessed the storage stability of bayberry polyphenols at different temperatures during 6 months of study. They reported a significant decreased in TPC and anthocyanin by 6-8% and 7-27% respectively at 4^{o}C. The results obtained in present investigation are in close arrangement with the previous work of Jo *et al*. (2003). They investigated the effect of storage on electron donating capacity of extract through DPPH assay and reported a significant decrease in free radical scavenging potential during 2 weeks storage at refrigeration temperature. Likewise, Chen *et al*. (2003) reported the antioxidant activities of different herbal drinks prepared from Chinese medicinal herbs. They noticed that extract exhibited highest DPPH radical exhibition among all 29 herbs selected for preparation of drink. Conclusively, polyphenols-based drink has considerable antioxidant activity owing to the rich photochemistry of ginger extract. More over drink containing SFE had greater phytochemicals content and antioxidant capacity as compared to drink with UAE and CSE, even at lower concentration. The total phenols content decreased significantly in all treatments and during storage period (Singh *et al*., 2014). The phenol compounds display significant part to defining the color and flavor of a product, but its loss might be due to these compounds are extremely volatile and easily oxidizable, which condensed in to brown pigments (Siddiqui *et al*., 2013). This may

probably be due to greater movement of oxygen, water vapor and oxidation of ascorbic acid, organic acid and polyphenols during storage. Other workers reported that the antioxidant potential slowly decreased during the storage period from 54.2 in 10% to 60.1 in 25% RTS (Harsha and Aarti, 2016). Alike outcomes were described by Gao and Rupasinghe (2012) on apple carrot juice blends.

CONCLUSION

The fallouts of the current study suggest that SFE is confirmed to be a more efficient process for highest recovery of total polyphenols from ginger with promising antioxidant potential followed by UAE and conventional extraction. This study further revealed that increasing the concentration of solvent, time, temperature, pressure and ultrasonic amplitude resulted in higher concentration of polyphenols and antioxidant potential. As far as the effect of solvent is concerned the outcomes of this study are strongly recommending ethanol as the most effective solvent for polyphenols extraction than methanol and water. Carbon dioxide is non-polar entity while organic solvent is polar in nature, and their combined utilization in SFE delineated superior outcomes than other extraction strategies. A momentous impact of storage period was observed on color, pH and acidity but no effect of storage interval was observed on TSS. Likewise, storage interval and treatments significantly affected the antioxidant potential of drinks. The organoleptic evaluation of drink unveiled that the impact of storage interval was non-significant for all parameters except for flavor and overall acceptability.

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