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Optimization of Ginger (*Zingiber officinale*) Phenolics Extraction Conditions and its Antioxidant and Radical Scavenging Activities Using Response Surface Methodology

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Abstract: Response surface methodology was applied to optimize the water phenolics extraction conditions [ginger concentration (GC) and temperature (T)] to obtain the highest radical scavenging (RSA) and antioxidant activities (AA) effects. The extracts that prepared at 22°C had the highest phenolics content ranged between 43.9 to 89.5 μg gallic acid equivalent (GAE) /ml. Two-factor central composite design was employed to determine the effects of GC or T and radical scavenging reaction time as independent variables on RSA as dependent variable. The optimum GC and reaction time with maximum RSA were 0.72% for 24.5 min, with a predicted RSA of 94.1% (r^2=0.9731) compared to the BHT, which had a scavenging value was 95.2% at concentration 0.02% and reaction time 20.9 min (r^2= 0.9093). The optimum temperature of extraction and reaction time with maximum RSA were 56.12°C for 20.93 min., with a predicted RSA of 90.4% (r^2= 0.9869). The prepared extraction using GC 0.75% at 60°C had the highest AA in lenoleic acid emulsion system with lowest diene absorbance and highest protective factor with non-significant difference (P>0.05) compared to BHT.

Key words: Ginger · Phenolics · Radical scavenging activity · Antioxidant activity · Response surface methodology

INTRODUCTION

Ginger plant (*Zingiber officinale*) belong to family *Zingiberaceae* have been widely used as spice and flavoring agent in foods and beverages [1]. The rhizome of ginger (*Zingiber officinale*) is widely consumed as a common spice throughout the world and used in traditional oriental medicine [2]. Many herbs and spices, usually used to flavor dishes, are an excellent source of phenolic compounds, which have been reported to show good antioxidant activity [3]. Several active components are present in ginger [4]. Among these, the major active ingredients are gingerol and hexahydrocurcumin [5]. Atherosclerosis is the leading cause of death in modern societies. Oxidation of lipoproteins, especially low-density lipoprotein (LDL) cholesterol, plays a crucial role in the initiation and progression of atherosclerosis [6]. Antioxidants that prevent LDL oxidation *in vitro* also inhibit atherosclerosis in animals [7]. Therefore, reducing blood lipids and inhibiting lipid oxidation are both important for the prevention and treatment of atherosclerosis in rate. Inhibition of oxidative stress improves all disorders related to diabetic nephropathy [8]. Afshari *et al.*[9] reported that consumption of antioxidants such as ginger would be a useful addition to current treatment strategies e.g. with insulin and reduce the thiobarbituric acid reactive substance. Fresh ginger rhizome contains gingerol but it converts to zingerone, shogaol, etc. after drying. Zingerone also have antioxidant and anti-inflammatory effect and can prevent the growth of cancer. Gingerol and shogaol of ginger can protect heart from blood clotting [4, 10]. Ginger has been consumed since antiquity and is known to play diverse biological roles including antioxidation, anti-inflammation, hypolipidemia, anti-carcinogenesis, anti-nausea, antithrombosis and antibacterial properties [11-14].

Synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ) are used to depress rancidity of fats and oils, but the toxicity of synthetic antioxidants [15] as well as increasing consumer demand for natural products have directed our attention towards the edible plants as resources of safer and more effective natural antioxidants [16].

Water ginger extracts use as a traditional hot drink in the eastern region, which prepared by boil the dried ginger powder in the water. In the recent years, most of

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researchers studied the effect of ginger antioxidant either *in vitro* or *in vivo* using the ginger powder, water or non-water extracts. Nevertheless, the optimal conditions for water extracting effective compounds of ginger to give a high potential antioxidant and radical scavenging activities still are not studied.

The objectives of this study were prepared ginger water extracts at different ginger concentrations and temperatures. Determination the total phenolics content and evaluate the antioxidant and radical scavenging activities in prepared extracts compared to BHT as synthetic antioxidants. Application the response surface methodology to optimize the extraction conditions lead to the high antioxidant and radical scavenging activities extracts.

MATERIALS AND METHODS

Materials: 1,1-Diphenyl-2-picrylhydrazyl (DPPH˙) and Linoleic acid were obtained from Fluka Chemical Company (Buchs, Switzerland). The powder ginger was purchased from the local market in Cairo, Egypt.

Methods

Preparation of Ginger Extracts: The powder ginger extracts were prepared at three concentrations of 0.5, 0.75 and 1% (w/v) using distilled water at three different temperatures. Weight 1 g of ginger in three beakers, then add 200 ml of distilled water to the first beaker, mix well and leave the suspension at room temperature (22°C ±2) over night. Add to the second and third beakers 200 ml of hot distilled water at 60 and 100°C, respectively. Thereafter, leave the beakers contain the hot extract until take the room temperature. Repeat the above steps with the weights 1.5 and 2 g of ginger powder.

Total Phenolics Content: Total phenolics content was determined spectrophotometrically using the modified Folin-Ciocalteau colorimetric method [17]. A 125 µl of the ginger extract mixed with 0.5 ml of distilled water in a test tube; followed by addition of 125 µl of Folin-Ciocalteau reagent and allowed to stand for 6 min. Then, add 1.25 ml of 7% sodium carbonate solution. The final volume was adjusted to 3 ml with distilled water. Each sample was allowed to stand for 90 min. at room temperature and the absorbance was measured at 760 nm using spectrophotometer Shimadzu UN-1201 (Shimadzu Co., Ltd., Kyoto, Japan). The total phenolics content was expressed as a micrograms gallic acid equivalent/ ml extract (µg GAE/ml) by reference to the gallic acid standard calibration curve using the following equation:

$$Y = 11.06 + 368.57\ X \quad r^2 = 0.9927$$

Radical Scavenging Ability: Radical-scavenging ability of the prepared ginger extracts was tested by the method of Paiva-Martins and Gordon [18]. Two milliliters of different ginger extracts or BHT solution (20 mg/100 ml) were added to one ml of methanolic DPPH˙ solution (0.128 g/l methanol). The decrease in absorbance was determined at 515 nm after 1, 5 up to 30 min using spectrophotometer Shimadzu UN-1201 (Shimadzu Co., Ltd., Kyoto, Japan). The scavenged percent of DPPH˙ in the reaction was calculated from the calibration curve using the following equation:

$$Y = 0.073199 + 7159.73X \quad r^2 = 0.9995$$

Antioxidant Activity: Antioxidant activity was determined using a diene conjugated formation method according to Lingnert *et al.* [19]. The substrate consisted of linoleic acid emulsified with an equal amount of Tween 20 in different ginger extracts (0.1mol/l). Then, the mixture was homogenized at high speed for 1 min. The emulsions were incubated at 50°C for 6 h. Absorbance was measured at 234 nm during the incubation period.

Statistical Analysis: The compared between means was exposed by Duncan multiple range at significance 5%. Results followed by different alphabetical letters significantly differed. ANOVA and Regression analysis (using PROC REG procedure) were carrying out by Statistical Analysis System (SAS)[20]. Three-dimension contour plot were used as a methods to study the response surface of radical scavenging ability as dependent variable with ginger concentration, temperature of extraction and reaction time as independent variables. The response surface methods was applied using Sigma Plot [21] to locate the optimum conditions to prepare water ginger extract with high anti-radical and antioxidative activities.

RESULTS AND DISCUSSION

Numerous studies have indicated the positive association between consumption of foods rich in phenolic phytochemicals and health. Studies in vegetables confirm that thermal processing significantly alters the physical and bio-chemical composition [22]. The potential health benefit of phenolics is mainly attributed to its antioxidant activity by donating a hydrogen atom from the aromatic hydroxyl group to free radicals [23].

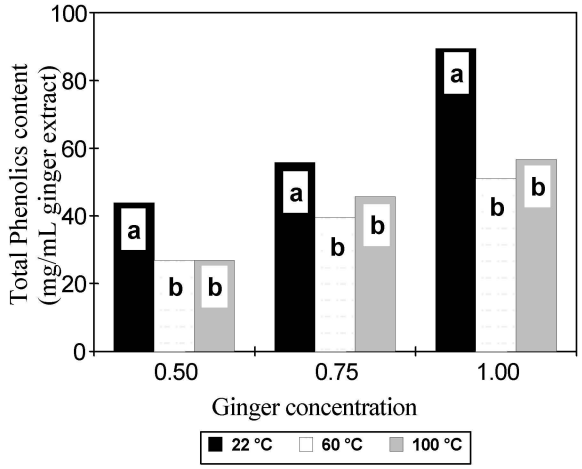

Fig. 1: Total phenolics content (µg GAE mL^{-1}) in ginger powder extracts at different concentrations and temperatures

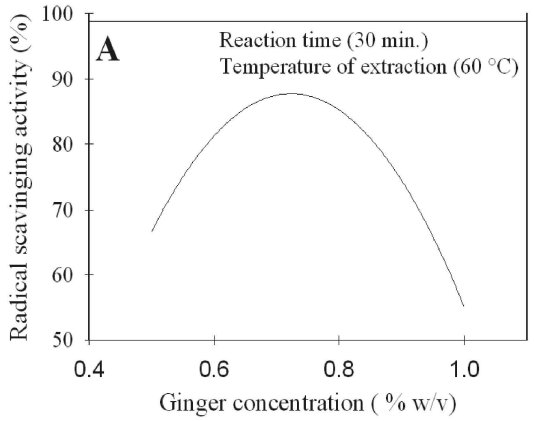

Fig. 2: Polynomial quadratic trend of ginger concentration and radical scavenging activity (%)

Total Phenolics Content: Total phenolics content in different ginger water extracts presented in Fig. 1. These extracts were prepared at different concentrations (0.5, 0.75 and 1%) and temperatures (22, 60 and 100°C). Generally, the extractable phenolics increased in parallel with increasing the ginger concentration at all used temperatures. On the other hand, the extracts, which prepared at 22°C, had significantly ($p < 0.05$) the highest phenolics content ranging between 43.9 to 89.5 µg GAE/ml extract compared to other extracts that prepared at 60 or 100°C. This finding was regarding with Cai *et al*. [24]. While, total phenolics content in the extracts were prepared at 60 or 100°C came in the second order with non-significant ($p > 0.05$) difference between each other ranging between 26.9 to 56.7 µg GAE/ml extract. Roy *et al*. [25] and Turkmen *et al*. [26] reported that cooking of some vegetable at 100°C for 10 or 30 min. decreased phenolics content below 60%. Extractable phenolics content was decreased at high temperature or long thermal treatment period. This could be due to phenolics compounds breakdown during thermal treatment [27].

Effect of Ginger Concentration: The effect of ginger concentration on the radical scavenging activity was presented as a polynomial trend in Fig. 2. The ginger concentration had a significant ($p<0.05$) effect on the DPPH• scavenging activity at reaction time 30 min. and temperature of extraction 60°C. According to the polynomial quadratic regression, (Eq. 1) the radical scavenging activity increased with increasing the ginger concentration from 0.5 to 0.75%, whereas dramatically decreased at concentration 1.0% with correlation coefficient ($r^2= 0.9999$).

$$Y= -134.3 + 614.2X - 424.8 X^2 \text{ [Eq. 1]}$$

The predicted radical scavenging activity increased from 66.6 to 87.7% with increasing the ginger concentration from 0.5 to 0.72% (w/v), then decreased gradually to 55.1% with increasing ginger concentration up to 1% (w/v). Sun *et al*. [28] reported that free-radical scavenging effects related to its affinity to the radical in the specific site. The radical-scavenging ability decreased at high phenolic concentrations in the peroxyl radical system. The biomolecular matrices may be attacked by derivatives from sample components, especially the phenolic compounds, resulting in secondary oxidation damage [29].

Effect of Temperature of Extraction: Effect of different temperatures were used to preparation of ginger water extracts at the ginger concentration 0.75% and reaction time 30 min. on DPPH• scavenging activity presented in Fig. 3. Polynomial quadratic regression (Eq. 2) was used to predict the optimal temperature of extraction.

$$Y= 40.81 + 1.74X - 0.016X^2 \text{ [Eq. 2]}$$

Output data indicated that the temperature of extraction was a very effective factor to produce an extract with high radical scavenging efficiency with correlation coefficient ($r^2= 0.9999$). The predicted radical scavenging activity gradually increased from 71.3 to 87.9% with increasing the temperature of extraction from 22 to 54.3°C. Some researches indicated that processing not effect in antioxidant potential of fruit and vegetables or enhanced it due to improvement of antioxidant properties of

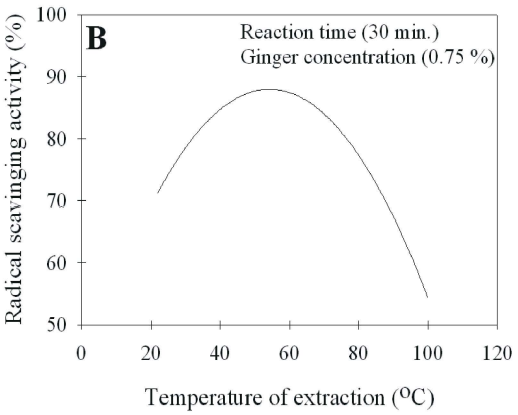

Fig. 3: Polynomial quadratic trend of temperature of extraction and radical scavenging activity (%)

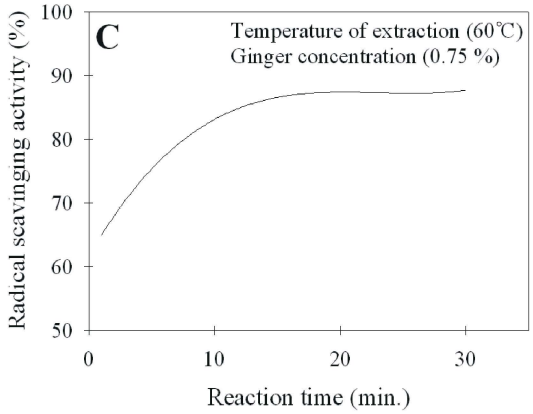

Fig. 4: Polynomial cubic trend of reaction time and radical scavenging activity (%)

naturally occurring compounds or formation of novel compounds having antioxidant activity [30, 31]. Manzocco *et al*. [32] found that pasteurization of tea extracts cause an increased in antioxidant activity of extracts, which was attributed to the formation of compounds having antioxidant activity during heat treatment. On the other hand, with increased the temperature of extraction the radical scavenging activity decreased gradually to 54.3% at temperature 100°C. Although the phenolics content in prepared ginger extract at 60°C were less than that prepared at room temperature, however it had a higher radical scavenging activity. Manzocco *et al*. [32] reported that radical scavenging activity decreased with percent of 20% in the prepared tea extract at 100°C. Zhang and Hamauzu [22] reported that raw broccoli florets had total antioxidant activity measured by DPPH with 60.5% but after cooking for 5 min by boiling the florets retained 35% of total antioxidant activity.

Effect of Reaction Time: Effect of reaction time on radical scavenging activity at ginger concentration 0.75% and temperature of extraction 60°C presented in Fig. 4. Increase the percentage of scavenging with low reaction time reflects the efficiency of the ginger extract as a radical scavenger. Polynomial cubic regression (Eq. 3) appeared the correlation between the reaction time and radical scavenging activity with r^2= 9933.

$$Y = 61.67 + 3.43X - 0.15X^2 + 0.0021X^3 \text{ [Eq. 3]}$$

With increasing the reaction time, the radical scavenging activity was increased. At reaction time 1 min. the radical scavenging activity was 64.9%. However, increasing the reaction time to 30 min. the radical scavenging activity was increased gradually up to 87.7%.

Effect of Ginger Concentration and Reaction Time on Radical Scavenging Ability: The three-dimension response surface plot in Fig. 5 is explaining the relationships between the radical scavenging activity and both ginger concentrations and reaction time for prepared ginger extracts at temperatures of 22, 60 and 100°C. Scavenging activity was increased with increasing both ginger concentration from 0.50 to 0.75% and reaction time from 1 to 30 min. at all extraction temperatures. On contrary, scavenging activity was decreased with increasing both temperature of extraction to 100°C and ginger concentration to 1%. Response surface analysis showed significant (p<0.05) regression relationships between both of ginger concentration (GC) and reaction time (t) as independent variables and radical scavenging activity (RSA) as response variable.

Radical scavenging activity for the prepared ginger extracts at room temperature presented in Fig. 5 A. The predicted model (Eq. 4) had a high correlation coefficient (r^2=0.9741). From output data, it could be noticed that the best predicted radical scavenging activity for that equation was 74.7% at ginger concentration 0.72% and reaction time 24.5 min.

$$RSA = -201.70 + 2.73t + 685.4GC - 0.056\,t^2 - 482.5\,GC^2 \text{ [Eq. 4]}$$

Fig. 5 B showed that the effect of prepared ginger extracts at 60°C on radical scavenging activity. Predicted model (Eq. 5) cleared that effect of reaction time and concentration of ginger extract, which prepared at 60°C on RSA as follows:

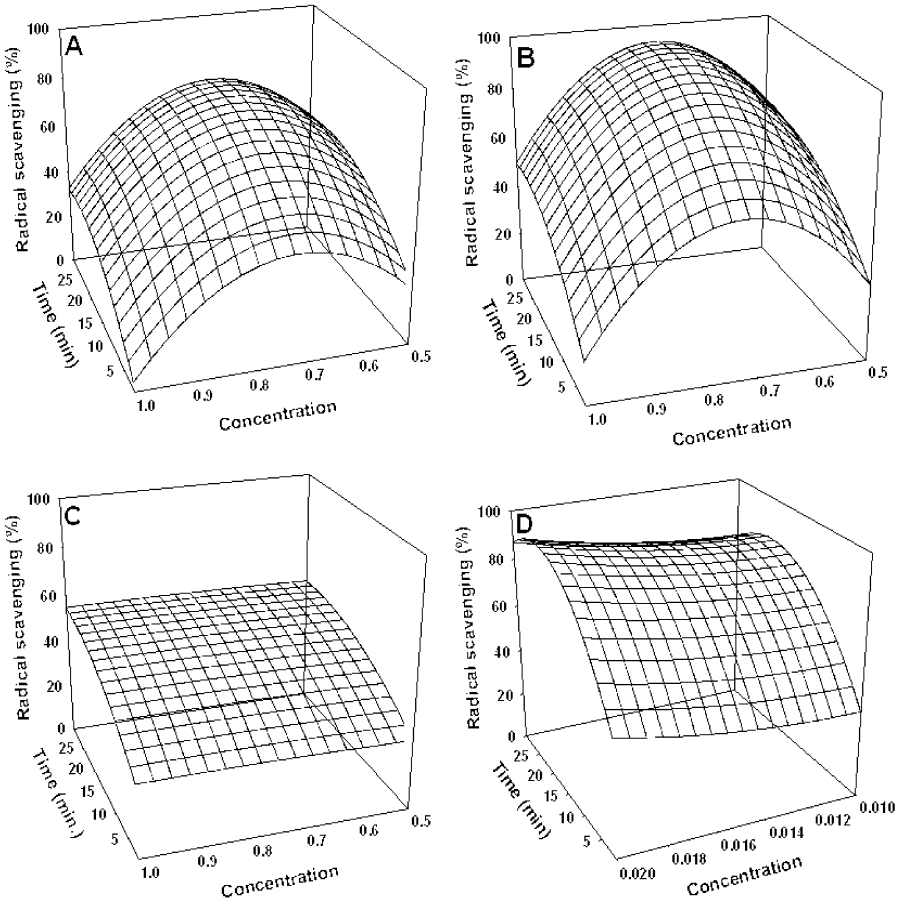

Fig. 5: Response surface plot showing the effect of effect of ginger water extracts which prepared at different temperature (A: 22°C; B: 60°C and C: 100°C) on radical scavenging (%) at different concentrations and times

$$RSA = -254.3 + 2.78t + 858.1GC - 0.053\ t^2 - 589.5\ GC^2 \quad [Eq.\ 5]$$

The highest scavenging value was 94.1% at ginger concentration 0.72% and reaction time 24.5 min. with $r^2 = 0.9731$. On the other hand, prepared ginger extracts at 100°C gave the lowest predicted radical scavenging with percent of 54.7% at ginger concentration 0.90% and reaction time 28.1 min. as shown in Fig. 5 C. Predicted model (Eq. 6) appeared that the weakness of prepared ginger extracts at 100°C. The correlation coefficient of this equation was 0.9428.

$$RSA = 21.11 + 1.87t + 15.85GC - 0.033\ t^2 - 8.60\ GC^2 \quad [Eq.\ 6]$$

Radical scavenging activity of BHT presented in Fig. 5 D. The best scavenging efficiency for that synthetic antioxidant was 95.2% at concentration 0.02% and reaction time 20.9 min. The quadratic model (Eq. 7) appeared the best conditions to obtained the high radical scavenging activity with $r^2 = 0.9093$.

$$RSA = 42.20 + 4.75t - 2181.4GC - 0.111\ t^2 + 114841\ GC^2 \quad [Eq.\ 7]$$

Chen and Yen [29] reported that the polyphenon 60 and guava leaf extracts showed weaker effects, at high concentrations, in antioxidant activity and peroxyl radical scavenging assays. Hanasaki *et al*. [33] illustrated that the multiple hydroxyflavonoids, especially with OH in the B-ring, significantly increased production of hydroxyl radicals in a Fenton system. Liu *et al*. [34] demonstrated that 4-hydroxyquinoline derivatives could inhibit the free radical-induced peroxidation, but also play a prooxidative role in the vesicle of dipalmitoyl phosphatidylcholine.

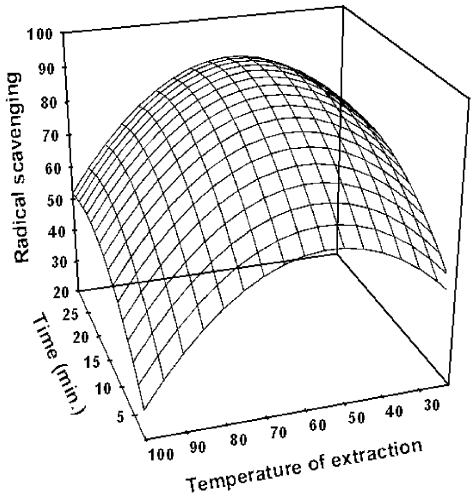

Fig. 6: Response surface plot showing the effect of ginger water extracts at concentration of 0.75% on radical scavenging (%) at different temperatures of extraction and times.

This could be due to the electron-attracting group at the ortho position to hydroxyl group in the phenoxy radical of quinoline derivatives. At high concentrations, the phenoxy radical initiated additional propagation of lipid peroxidation.

According to the predictive models, it could be Saied that the ginger extract that prepared using 0.72% ginger and temperature 60°C to competitive the BHT with high effect as a scavenger.

Effect of Extraction Temperature and Reaction Time on Radical Scavenging Ability: Three-dimension response surface method was used to study the relationship between temperature of extraction and reaction time on radical scavenging ability for ginger water extracts at concentration 0.75%. According to Fig. 6 the radical scavenging ability was increased with increasing both of extraction temperature (up to 60°C) and reaction time. On the other hand, at high extraction temperature the radical scavenging ability was decreased. Output data of response surface study showed significant (p<0.05) relationships between both of extraction temperature and reaction time as independent variables and radical scavenging activity as response variable. The predictive equation (Eq. 8) shows the effect of extraction temperature (T) and reaction time (t) on radical scavenging activity (RSA).

$$RSA= 10.08+2.42t+1.93T-0.0524t^2-0.0177T^2 \quad [Eq. 8]$$

The highest observed predictive radical scavenging ability was 90.4% at 56.12°C and 20.93 min. as extraction temperature and reaction time, respectively with r^2= 0.9869. Roy *et al.* [25] found that relationship (r^2 = 0.94) between total phenolics content and anti-DPPH$^{\bullet}$ radical activity in vegetable juices. The degradation of phenolic compounds in response to thermal treatment shows somewhat resemblance with the behavior of the components responsible for antioxidant activity, in the tested vegetable juices.

Antioxidant Activity: The antioxidant activity of ginger aqueous extracts, which prepared using concentrations 0.5, 0.75 or 1.0% at temperatures 22, 60 or 100°C, was determined. Antioxidant activity data of studied ginger extracts presented in Table 1. The antioxidant activity after 6 h of the different ginger extracts significantly (P < 0.05) increased with increasing ginger concentration from 0.5 to 0.75%. On the contrary, the diene absorbance increased with raising the ginger concentration to 1.0%.

Table 1: Effect of ginger concentration and temperatures of extraction on antioxidant activity in linoleic acid emulsion system at 50°C for 6 hr compared to BHT at concentration 0.02%

Conc. (%)	Temp. of extraction	O.D at 234 nm. after 6 hr	Slope	PF	CV	R^2
0.50	22	0.314^{cd}	0.0199	0.75	3.6	0.9584
	60	0.314^{cd}	0.0189	0.80	8.5	0.7555
	100	0.307^{de}	0.0190	0.79	3.3	0.9644
0.75	22	0.292^{ef}	0.0167	0.90	5.6	0.8689
	60	0.293^{ef}	0.0160	0.94	7.4	0.7734
	100	0.292^{ef}	0.0175	0.86	7.3	0.8096
1.0	22	0.325^{be}	0.0229	0.65	5.4	0.9228
	60	0.335^{b}	0.0248	0.60	6.7	0.8971
	100	0.364^{a}	0.0292	0.51	5.5	0.9456
BHT		0.276^{f}	0.0150	1.00	6.1	0.8313

PF, protective factor; CV, coefficient of variation; R^2, correlation coefficient

It has indicated that high ginger concentration promoted the lenoleic acid oxidation. That oxidation due to formation of diene conjugated double bond in linoleic acid leading to increase the absorbance at 234 nm. Cao *et al.* [35] reported that most plant polyphenol compounds possess both antioxidant and prooxidant properties, depending on concentration and environmental factors. On the other hand, the obtained antioxidant activity data, which collected during incubation period ranged between 0 to 6 h, for all prepared extracts and BHT solution were analyzed by regression. The output slops were used to calculate the protective factor compared to BHT, which set at protective factor 1.0 (100%) as a synthetic antioxidant. The correlation between temperature of extraction and efficiency of the ginger extracts antioxidant activity was observed. The regression for data were highly significant ($p < 0.05$) with correlation coefficient (r^2) ranged "between" 0.9644 to 0.7555. The coefficients of variation (CV) were <10%. At concentrations 0.75% the extract which, prepared at 60°C had a high protective factor 0.94 compared to BHT 1. While, increased the temperature of extraction to 100°C lead to decrease the protective factor to the minimum value 0.51 at concentration of 1.0%. Weak the antioxidant activity of ginger extract, which prepared by soaking over night at room temperature may be due to the oxidation in the phenolics compounds by the available oxygen during that long period. A possible mechanism of polyphenol cytotoxicity may be related to their prooxidant properties. Tea extracts showed both antioxidant and prooxidant activities in oxidative damage of biomolecules [36]. In addition, decline in antioxidant activity of ginger extracts, which prepared at 100°C due to the effect of thermal treatment. Thermal conditions that occur during air drying and home cooking are known to affect in the phenolic content of some fruits and vegetable [37, 38]. Ginger extract, which prepared at concentration of 0.75% and temperature 60°C give the best antioxidant parameters ($OD_{234\ nm}$, slop and protective factor) compared to BHT.

CONCLUSION

It was found that using of 0.72% powder ginger and water at 56.12°C was the most efficient set of conditions for the preparation of ginger water extract with non-significant difference compared to BHT. Prepared ginger extract using ginger concentration higher than 0.72% lead to give a prooxidant effect. In addition, using water with temperature higher than 56.12°C gave an extract with damaged phenolics had a low radical scavenging and antioxidant activity.

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