In vitro Antioxidant Properties and Phenolic Content of Ginger (Zingiber officinale Rosc.) Root

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Abstract: Ginger root is one of the most widely used plants for medicinal aims in Anatolia. Determination of antioxidant properties and of phenolic contents traditionally used plants is important in respect to pharmacologic studies. In this study, antioxidant properties and phenolic content of ginger (*Zingiber officinale* Roscoe) root are investigated. Antioxidant activity of plant is measured with ferric thiocyanate method, reducing power and metal chelating assays. Also, antiradical activity of ginger (*Zingiber officinale* Roscoe) root is measured with 1,1-diphenyl-2-picryl-hydrazyl (DPPH$^{\bullet}$) radical scavenging activity. Additionally total phenolic content of plant are determined by the Folin-Ciocalteu reactive method. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), α-tocopherol and trolox were used as standard antioxidants for comparison. It has been show that ginger (*Zingiber officinale* Rosc.) root have gat antioxidant and antiradical properties and there is the correlation between these properties and phenolic and flavonoid contents of plant.

Introduction

Ginger (*Zingiber officinale* Rosc.) is a common additive in a number of commercial foods and beverages and is valued both for its aromatic volatile constituents and for its spicy, pungent constituents. Ginger (*Zingiber officinale* Rosc.) has been used as a spice for thousands of years. World production is estimated to be 100000 tyear^{-1} on a dry weight basis and it is cultivated in many tropical and subtropical countries (Bartley and Jacobs, 2000). Ginger (*Zingiber officinale* Rosc.), belonging to a tropical and sub-tropical family – Zingiberaceae, originating in South-East Asia and introduced to many parts of the globe, has been cultivated for thousands of years as a spice and for medicinal purposes (Park and Pizzuto, 2002).

Oxygen and nitrogen are very important elements for aerobe livings, but reactive oxygen and nitrogen species (RONS) generated in the living body can be very dangerous (Willet, 1994). Existing lifestyles cause free radicals and RONS to over-produce in human organism, and to decrease the physiological antioxidant capacity (Lopez, Akerreta, Casanova, Garcia-Mina, Cavero, & Calvo, 2007). RONS can cause many diseases such as atherosclerosis, coronary heart diseases, aging and cancer (Li, Wong, Cheng, & Chen, 2008). These diseases result from uncontrolled production of RONS and unbalanced mechanism of antioxidant protection. RONS contain superoxide anion radicals ($O_2^{\bullet-}$), hydroxyl radicals ($OH^{\bullet}$) and non free-radical species such as H_2O_2 and singlet oxygen ($^1O_2$) and these molecules are a class of highly reactive molecules generated on aerobic respiration in livings (Halliwel & Gutteridge, 1989). On the other hand, RONS can cause lipid peroxidation in foods that leads to the deterioration (Sasaki, Ohta, & Decker, 1996). Antioxidants can inhibit the outbreak or the advance of oxidative reactions and thus prevent cell

damage caused by RONS (Esmaeili & Sonboli, 2010). In order to decrease harmful effect of RONS, the antioxidants from plants can be used. Also, there are synthetic antioxidants such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT), but the usage of these molecules has some risks (Sun & Fukuhara, 1997). Therefore, in recent years, the use of synthetic antioxidants has been limited in many countries and the interest in natural antioxidants has increased more and more. The most important one of natural antioxidants is the medicinal plants on which many studies have been done so far. The medicinal plants have been used to treat many diseases in the Anatolia for a long time. Many researches have shown that many medicinal plants used in Anatolia have highly antioxidant activity. Also, these plants have rich phenolic content. Natural antioxidants in plants protect the human body from free radicals, oxidative stress and associated diseases. Hence, these antioxidants play a very important role in human health (Lopez et al., 2007). Plants are rich in biologically active compounds which have features such as antioxidant and radical scavenging activities. Many studies reveals that most of the foods contain phytochemicals such as phenolic compounds having potential protective effects (Rice-Evans, Miller, & Paganga, 1997), and increasing consumption of fruits and vegetables decrease degenerative diseases (Ames, Shigenaga, & Hagen, 1993; Reddy, Sreeramulu, & Raghunath, 2009).

Materials and methods

Chemicals

We obtained butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), nitroblue tetrazolium (NBT), the stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH•), linoleic acid, 3-(2-Pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ethylenediaminetetraacetic acid (EDTA), α-tocopherol, polyoxyethylenesorbitan monolaurate (Tween-20), 2,2'-bipyridine and trichloroacetic acid (TCA) from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany) and purchased ammonium thiocyanate from Merck.

Samples and preparation of extract

We obtained dried ginger (*Zingiber officinale* Rosc.) from local market at Erzurum, Turkey. For ethanol extract ginger root (EEGR), 25 g dried ginger (*Zingiber officinale* Rosc.) root ground into in a mill, and the powdery ginger (*Zingiber officinale* Rosc.) root is mixed with 100 mL ethanol on a magnetic stirrer for 1 hour. The extracts are filtered and then filtrates are collected. The ethanol in mixture is removed using a rotary evaporator (RE 100 Bibby, Stone Staffordshire England) at 50^{o}C to dry the extract. The extract is placed in a dark plastic bottle and stored at -20^{o}C until used for experimental studies.

Total antioxidant activity determination by ferric thiocyanate method

The total antioxidant activity of EEGR and standard antioxidants is determined using the ferric thiocyanate method in linoleic acid emulsion (Mitsud, Yuasumoto & Iwami, 1996). The stock solutions are prepared by 10 mg of EEGR dissolved in 10 mL distilled water. The different concentrations of stock EEGR solution samples (10-20 μg/mL) are prepared in 2.5 mL of potassium phosphate buffer solution (0.04 M, pH 7.0) and then these are added to 2.5 mL of linoleic acid emulsion in potassium phosphate buffer solution (0.04 M, pH 7.0). The final solutions are incubated at 37^{o}C. During the incubation periodically, a 0.1 mL aliquot of the mixture is diluted with 3.7 mL of ethanol, and then it is added to the mixture of 0.1 mL of 30% ammonium thiocyanate and 0.1 mL of 20 mM ferrous chloride in hydrochloric acid (3.5%). The absorbance is measured at 500 nm for the determination of the peroxide level. The peroxides formed during linoleic acid oxidation oxidize Fe^{2+} to Fe^{3+} and the latter ions form a complex with thiocyanate. The complex has a maximum absorbance at 500 nm. The process is repeated every 6 h until the control reaches its maximum absorbance value. The amounts of inhibition are calculated by the following equation:

$$\text{Inhibition of lipid peroxidation (\%)} = 100 - \left(\frac{A_S}{A_C} \text{ x } 100 \right)$$

Where, A_S is the absorbance value of the control reaction and A_C is the absorbance value of working samples and standards. In the control, ethanol is used instead of the sample.

Fe^{3+} reducing power assay

The reducing activity of EEGR is determined according to the method of Oyaizu (1986). The capacity reducing of EEGR to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex of Prussian blue is measured by reading the absorbance at 700 nm. Shortly, different concentrations of EEGR (10-30 µg/mL) in 1 mL of distilled water are mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture is incubated at 50°C for 20 min. Then, 2.5 mL trichloroacetic acid (10%) was added to the mixture. Finally, 0.5 mL of $FeCl_3$ (0.1%) is added to this solution, and the absorbance is measured at 700 nm. Increased absorbance indicates greater reduction capability.

Cu^{2+} reducing power assay

In order to determinate of the reducing ability of EEGR, the cupric ions (Cu^{2+}) reducing method recommended by Apak et al. (2004) with slight modification is used. Shortly, 0.25 mL $CuCl_2$ solution (0.01M), 0.25 mL of ethanolic neocuproine solution (7.5×10^{-3} M) and 0.25 mL of CH_3COONH_4 buffer solution (1 M) are added to a test tube, followed by mixing with different concentrations of EEGR (10-30 µg/mL). Then, the final volume is increased to 2 mL with distilled water. The absorbance is read at 450 nm 30 minute later. The increased absorbance indicates the greater reduction capability.

Chelating activity on ferrous ion (Fe^{2+})

Ferrous ions (Fe^{2+}) chelating activity of EEGR is measured according to the method of Re and co-workers (1999). Briefly, the different concentrations (10-30 µg/mL) of ethanol extract from ginger (*Zingiber officinale* Rosc.) root in 0.25 mL ethanol, 0.25 mL FeSO4 solution (2 mM), 1 mL Tris-HCl buffer solution (pH 7.4), 1mL 2,2'-bipyridine solution (0.2% in 0.2 M HCl) and 2.5 mL ethanol solution are added to a test tube, respectively. Then, total volume is adjusted to 6 mL with distilled water, and stirred well. The absorbance is measured at 562 nm. Na_2EDTA is used as a standard ferrous ions (Fe^{2+}) chelator.

DPPH free radical scavenging activity

DPPH free radical scavenging activity for EEGR is measured according to the method of Blois (1958). Briefly, a 0.1 mM ethanolic solution of DPPH· was prepared on daily bases. Then, 1 mL of this solution is added to 3 mL of EEGR solution in ethanol at different concentrations (10-20-30 µg/mL). After half an hour, the absorbance is measured at 517 nm for every sample. The DPPH· concentration (mM) in the reaction medium was calculated from the following calibration curve, determined by linear regression (R^2: 0.9974):

$$\text{Absorbance} = 5.869\text{x}10^{-4}[\text{DPPH}] + 0.0134$$

The capability to scavenge the DPPH· radical was calculated using the following equation:

$$\text{DPPH}\cdot \text{ scavenging effect (\%)} = \left(\frac{A_C - A_S}{A_C} \right) \text{x } 100$$

Where, A_C is the initial concentration of the stable DPPH free radical and A_S is the absorbance of the concentration of vestigial DPPH·in the presence of EEGR (Cristiane de Souza, Soares de Araujo, & Imbroisi 2004).

Determination of total phenolic content by Folin Ciocalteau assay

The total phenolic content in ginger (*Zingiber officinale* Rosc.) root is estimated by a colorimetric assay based on the procedure described by Slinkard et al. (1999) with slight modification. From EEGR 1 mg is added into a test tube and the final volume is increased to 23ml with distilled water. 3 minutes later Folin-Ciocalteu's reagent (0.5 mL) and 2% Na_2CO_3 (1.5 mL) are added. The samples are vortexed and then kept at room temperature for 30 minutes. The absorbance measurements are recorded at 760 nm. The distilled water is used either as blank or for control instead of sample. Gallic acid is used for comparison. The absorbance measurements of samples that contain 100, 200, 300, 400 and 500 µg gallic acid are recorded and standard gallic acid graph is drawn. The results are reported as µg gallic acid equivalents per mg extract.

Determination of total flavonoid content

Flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants (Spencer, 2008). The total flavonoid content in EEGR is estimated by a colorimetric assay based on the procedure described by Park et al. (1997). One mg EEGR samples are added into a test tube. Then 0.1 mL CH_3COOK (1 m) and 0.1 mL of 10% $Al(NO_3)_3$ in 4,3 mL ethanol solution is added and the samples are vortexed. Then the vortexed samples kept at room temperature for 40 minutes. The absorbance measurements are recorded at 415 nm. The distilled water is used either as blank or for control instead of sample. Quercetin is used for comparison. The absorbance measurements of samples that contain 20, 40, 60, 80 and 100 µg quercetin are recorded and then the standard graph is drawn. The results are reported as µg quercetin equivalents per mg extract.

Statistical Analysis

All the analyses on total antioxidant activity are carried out in duplicate sets. The other analyses were carried out in triplicate. The data are recorded as mean □ standard deviation and analysed by SPSS (version 11.5 for Windows 98, SPSS Inc.). One-way analysis of variance is performed by ANOVA procedures. The significant differences between means are determined by LSD tests. *P* values □ 0.05 and □ 0.01 are regarded as significant and very significant, respectively.

Result and discussion

Lipid peroxidation can cause hazardous effects in foods by forming complex mixture of secondary breakdown products of lipid peroxides. The further intake of these foods can cause a number of adverse effects including toxicity to mammalian cells. Lipid peroxidation is thought to proceed via radical mediated abstraction of hydrogen atoms from methylene carbons in polyunsaturated fatty acids (Rajapakse, Mendis, Byun, & Kim, 2005). Antioxidant activity is defined as the ability of a compound to inhibit oxidative degradation, such as lipid peroxidation (Roginsky & Lissi, 2005)

Natural antioxidants have biofunctionalities such as the reduction of chronic diseases, DNA damage, mutagenesis, carcinogenesis, etc. and inhibitions of pathogenic bacteria growth, which are often associated with the termination of free radical propagation in biological systems (Zhu, Hackman, Ensunsa, Holt, & Keen, 2002). Thus, for medicinal bioactive components, antioxidant capacity is widely used as a parameter. A number of assays have been introduced to measure the total antioxidant activity of pure compounds (Miller, Castelluccio, Tijburg, & Rice-Evans, 1996).

In this study, the antioxidant activity of the EEGR is compared to BHA, BHT, α-tocopherol and its water-soluble analogue trolox. The antioxidant activity of the EEGR, α-tocopherol, trolox, BHA and BHT is measured according to the total antioxidant activity by ferric thiocyanate method, DPPH free radical scavenging activity, metal chelating activity, reducing Fe^{3+} and Cu^{2+} activity. Besides, the total phenolic and flavonoid contents of these samples are determined.

Total antioxidant activity determination by ferric thiocyanate method

The ferric thiocyanate method determines the amount of peroxide produced during the initial stages of oxidation. 20 □g/mL concentrations of EEGR on lipid peroxidation of linoleic acid emulsion are shown in Figure 1 and are found to be 57.4%. Otherwise, α-tocopherol and trolox display 61.5 and 81.5% inhibition on peroxidation of linoleic acid emulsion, respectively at the 20 □g/mL concentration. As a result, Ginger have potent antioxidant activity in the ferric thiocyanate assays (Figure 1).

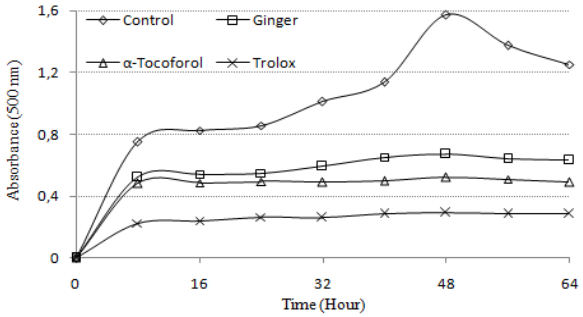

Figure 1. Total antioxidant activity.

Reducing power

Reducing power of EEGR and standards (BHT, α-tocopherol and trolox) are determined by using the potassium ferricyanide reduction and cupric ions (Cu^{2+}) reducing methods. For the measurements of the reductive activity, the Fe^{3+}-Fe^{2+} transformation is investigated in the presence of EEGR using the method of Oyaizu (1986). The reducing activity of EEGR, and standard compounds increases with increasing their concentrations. Ferric ions (Fe^{3+}) reducing ability of samples is as follows: Trolox > BHT > Ginger > α-Tocopherol (Figure 2A). The results indicate that EEGR has notable ferric ions (Fe^{3+}) reducing ability and electron donor properties for neutralizing free radicals. Also, Cu^{2+} reducing capability of EEGR by Cuprac method is found to be concentration dependent. Cupric ion (Cu^{2+}) reducing ability of EEGR is shown in Fig. 2B and there is a correlation between the cupric ions reducing ability and concentrations of studied samples. Results are as follows: BHA > Ginger > Trolox > α- Tocopherol (Figure 2B).

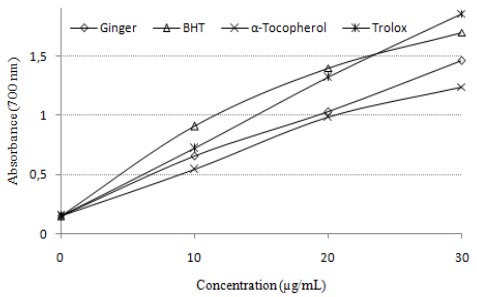

Figure 2A. The Fe^{3+} reducing activity.

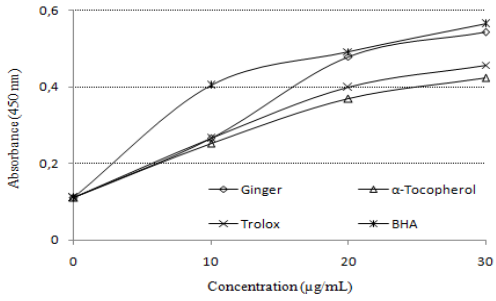

Figure 2B. The Cu^{2+} reducing activity

Chelating capacity

Metal ions can cause lipid peroxidation that can produce free radicals and lipid peroxides. Therefore, metal chelating activity indicates antioxidant and antiradical properties. The decreased absorbance of the reaction mixture indicates higher metal chelating capability. EDTA is used as a standard metal chelating agent at the

method firstly used by Re et al. (1999). According to the results, EEGR indicates less metal chelating from EDTA (Figure 3). In this study 2,2'-bipyridine is used as a metal chelating agent.

Radical scavenging activity

DPPH has been extensively used to measure the free radical-scavenging ability of various antioxidant substances. DPPH• assay is used in the this study for a primary screening of the EEGR free radical-scavenging activity, because this assay can accommodate a large number of samples in a short period and is sensitive enough to detect natural compounds at low concentrations. DPPH• scavenging method provides information on the reactivity of test compounds with a stable free radical. Besides, this method is simple and fast. Antioxidants react with DPPH•, which is a free radical, and convert it to 1,1-diphenyl-2-picryl hydrazine. In the meantime the discolouration degree at the test tube indicates the radical-scavenging capability of the antioxidant (Singh, Murthy, & Jayaprakasha, 2002). In this study, antioxidant activities of EEGR and standards are measured. DPPH• gives a strong absorption at 517 nm because of its odd electron. As this electron becomes paired off in the existence of a free radical scavenger, the absorption vanishes. Consequently, EEGS exhibits remarkable DPPH free radical scavenging activity. The decrease (p 0.05) in the concentration of DPPH radical due to the scavenging ability of EEGR and standards is shown in figure 5. BHA and BHT were used as references radical scavengers in this study. The scavenging effect of EEGR and standards on the DPPH radical decreased in that order: BHA Ginger BHT (Figure 4).

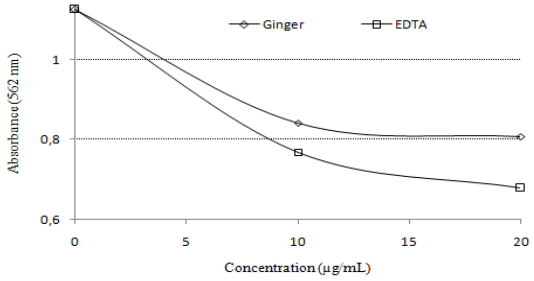

Figure 3. The ferrous ion (Fe^{+2}) chelating activity.

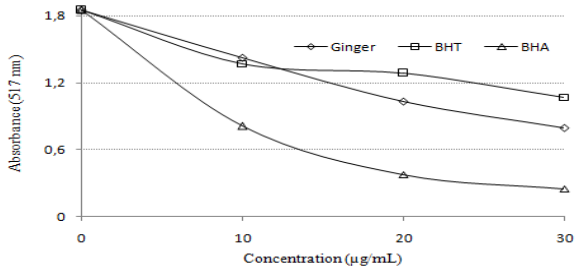

Figure 4. The DPPH scavenging effect.

Total phenolic content

The total phenolic contents of ethanolic extract ofginger (*Zingiber officinale* Rosc.) root is determined with Folin Ciocalteu reagent. The standard graph of gallic acid is drawn (r^2: 0.994). The amount of total phenolic is determined from the standard graph equation as gallic acid equivalents per one mg of extract (GAE/mg extract). As can be seen in Table 1, 136 µg/mg of gallic acid equivalent of phenolic content is calculated in 1 mg of EEGR. The correlation between the antioxidant capacities of EEGR and the standard graph of gallic acid is determined. According to this result, it says that the phenolic compounds contribute significantly to the antioxidant capacities of the root parts of of ginger (*Zingiber officinale* Rosc.) plants.

Total flavonoid content

Flavonoids, including flavones, flavanols and tannins, are a class of secondary metabolites in plants. The consumption of the flavonoid containing fruits and vegetables has been linked to the protection against cancer and heart disease (Hertog, Hollman & Venema, 1992). Flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants. Quercetin is a well known plant-derived flavonoid; studies show that it may have antioxidant properties (Davis et al 2009). The standard graph of quercetin is drawn. The amount of total flavonoid is determined by this standard graph equation as quercetin equivalents per one mg of extract. The result of EEGR is found to be 15.4 µg QE/mg extract (Table 1).

	EEG (µg/mg)
Total phenolic content (GAE)	136
Total flavonoid content (QE)	15.4

Table 1: Total phenolic and flavonoid contents of ginger (*Zingiber officinale* Rosc.) Root

Conclusions

This study pointed out comparatively the potential antioxidant properties of ginger. According to the obtained data, ginger is found to be effective antioxidants in different in vitro assays including ferric thiocyanate method, reducing power and DPPH• scavenging activities when compared to standard antioxidant compounds such as BHA and BHT, synthetic antioxidants, □-tocopherol, a natural antioxidant, and trolox which is water-soluble analogue of tocopherol. Besides, phenolic and flavonoid contents of ginger are determined as gallic acid and quercetin equivalent, standard phenolic and flavonoid compounds respectively. Also, according to the result obtained, ethanol extract of ginger (*Zingiber officinale* Rosc.) root has effective antioxidant and antiradical capabilities, compared to standard antioxidant compounds. However, this extract doesn't has good chelating power. The inhibition of lipid peroxidation in linoleic acid emulsion of EEGR is found to be higher than α-tocopherol, a standard antioxidant. The amount of total phenolic is approximately nine times of the amounts of total flavonoid in this extract.

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