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RESEARCH ARTICLE

Pistachio (*Pistacia vera* L.) Gum: a potent inhibitor of reactive oxygen species

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Abstract

In the present study, in order to evaluate antioxidant and radical scavenging properties of Pistachio gum (P-Gum), different bioanalytical methods such as DPPH$^{\bullet}$ scavenging activity, DMPD$^{\bullet+}$ radical scavenging activity, total antioxidant activity determination by ferric thiocyanate, reducing ability Fe^{3+}–Fe^{2+} transformation, Cuprac and FRAP assays, $O_2^{\bullet-}$ scavenging by riboflavin-methionine-illuminate system and ferrous ions (Fe^{2+}) chelating activities by 2,2′-bipyridyl reagent were performed separately. P-Gum inhibited 54.2% linoleic acid peroxidation at 10 μg/ml concentration. On the other hand, BHA, BHT, α-tocopherol and trolox, pure antioxidant compounds, indicated inhibition of 80.3%, 73.5%, 36.2% and 72.0% on peroxidation of linoleic acid emulsion at the same concentration, respectively. In addition, all of sample had an effective DPPH$^{\bullet}$, DMPD$^{\bullet+}$ and $O_2^{\bullet-}$ scavenging, Fe^{3+} reducing power by Fe^{3+}–Fe^{2+} transformation and FRAP assay, Cu^{2+} reducing ability by Cuprac method and Fe^{2+} chelating activities.

Keywords

Antioxidant activity, metal chelating, Pistachio (*Pistacia vera*) gum, radical scavenging

History

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Introduction

Pistachio (*Pistacia vera* L.), a member of *Anacardiaceae* family, the only 1 of the 11 species of the genus *Pistacia*, having an edible green kernel enclosed in a woody shell, is native to Asia minor and widely distributed in the Mediterranean region including Turkey as well as USA. Pistachio, a common desert plant, is highly tolerant of saline soil. It grows well when irrigated with water having 3–4 mm of soluble salts[1]. The shell of the pistachio is naturally a beige color, but it is sometimes dyed red or green in commercial pistachios[2]. Pistachios are rich fat source and contain essential fatty acids including oleic, linoleic and linolenic acids[3].

Pistachioh as an economic value, as it is the source of a traditional medicinal agent, an oleoresin, gum, which is seeping out of some parts of this plant[4]. Pistachio gum is obtained from *Pistacia vera* L., as an exudate after ''hurting'' the trunk and branches. This resin is custom scented, pale yellow, in the viscosity of honey while it is fresh. It hardens over time. According to Alma et al.[5], gum of *Pistacia vera* showed antibacterial activity.

Oxidation is defined as electrons transferring from one atom to another atom. It represents an essential part of aerobic life and human metabolism. Oxygen is the ultimate electron acceptor in the electron flow system that produces energy in the form of ATP[6]. It constitutes most of the mass of living organisms, because water is their major constituent. It is very important for the continuity of vital functions and has unpaired two electrons and tends to form oxygen-centered free radicals, known as reactive oxygen species (ROS) including superoxide anion radical ($O_2^{\bullet-}$), hydroxyl radical (HO·), peroxyl radical (ROO·), alkoxyl radical (RO·) and nitric oxide radical (NO·)[7–9]. They are recognized to play a dual role as both beneficial and deleterious species. At physiological concentrations, ROS may be required for normal cell functions. ROS are important for intracellular messenger molecules. But when the antioxidant defense systems are in case of inadequate, ROS cause the cell damaging and oxidative stress occurs. As a result of damage to biomolecules such as carbohydrates, lipids, proteins, nucleic acids and DNA, free-radical chain reactions can stimulate subsequently and some disease conditions such as cancer, aging, coronary heart disease, neurodegenerative disease can be occur[10–12].

An antioxidant molecule has been defined as any substance that when present in low concentrations compared to that of an oxidizable substrate significantly delays or inhibits the oxidation of that substrate. Oxidizable substrate encompasses almost everything except H_2O, found in foods and in living tissues[10,13–17]. On the other hand, free radicals are the primary reason for cancer and that the risk of disease. They can be reduced by increased consumption of food-borne antioxidants has prompted an enormous growth of interest in antioxidant nutrients and other antioxidants substances in food[18,19]. Plant foods are potential sources of natural antioxidants, such as vitamin C, α-tocopherol, carotenoids, flavonoid and phenolic acids, which prevent free radical damage. They can provide phenolic hydroxyl group to react with free radicals. The synthetic antioxidants used

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are phenolic compounds such as BHA, BHT, TBHQ and propyl gallate (PG), which is an ester of gallic acid[20,21] have been very thoroughly tested for their toxicological behaviors. However, some of them are coming, after a long period of use, under heavy pressure as new toxicological data impose some caution in their use. In this context, natural products appear as healthier as and safer than synthetic antioxidants[22–24]. Consequently, there is a growing interest in exploring natural sources of natural and safer antioxidants for food and pharmaceutical applications[25,26].

The aim of this study was to investigate ferric ions (Fe^{3+}) reducing antioxidant power assay, FRAP assay, cupric ions (Cu^{2+}) reducing antioxidant power assay, DPPH· radical scavenging, $DMPD^{\bullet+}$ radical scavenging, superoxide anion radical scavenging, ferrous ions (Fe^{2+}) chelating activities and the inhibition of lipid peroxidation in linoleic acid system of pistachio (*Pistacia vera* L.) gum (P-Gum).

Materials and methods

Chemicals

2,4,6-Tripyridyl-*s*-triazine (TPTZ), butylated hydroxyanisole (BHA), 1,1-diphenyl-2-picryl-hydrazyl (DPPH·), butylated hydroxytoluene (BHT), *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD), linoleic acid, methionine, riboflavin and 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine) were purchased from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany). All other chemicals used were analytical grade and obtained from either Sigma–Aldrich or Merck (Darmstadt, Germany).

Preparation of pistachio (*Pistacia vera* L.) P-gum

P-Gum of was obtained from pistachio trees from Gaziantep province of Turkey. About 10 mg P-Gum dissolved in 10 ml ethanol, is used directly, without extraction.

Determination of total phenolic contents

The total phenolics in P-Gum were determined according to a modified version of the procedure described by Slinkard and Singleton[27] using by Folin–Ciocalteu phenolic reagent[28]. Gallic acid (GA) was used as a positive standard phenolic compound. The quantity of phenolic compounds in P-Gum was determined as microgram of gallic acid equivalent (GAE) using an equation that was obtained from standard gallic acid graph (r^2: 0.9849).

$$\text{Absorbance } (\lambda_{760}) = 0.0006 \times \text{Total phenols } (\mu g)$$

The total phenolics in P-Gum were calculated by employing a standard above curve prepared using GA and expressed as micrograms of GAE[26,28].

Determination of total flavonoids

Total flavonoid determination of P-Gum was performed according to Gülçin et al.[29,30]. Total flavonoids quantity was calculated using quercetin as standard(r^2: 0.9872):

$$\text{Absorbance } (\lambda_{415}) = 0.0107 \times \text{Total flavonoids } (\mu g)$$

The content of flavonoids in P-Gum was calculated from above standard curve prepared using quercetin and expressed as micrograms of quercetin equivalents (QE)[26,29].

Ferriccyanide (Fe^{3+}) reducing power assay

The ferric reducing antioxidant power was carried out by slight modification of method of Oyaizu[30]. Reducing power of P-Gum was calculated by the direct $Fe^{3+}(CN^-)_6$–$Fe^{2+}(CN^-)_6$ reduction and determined by absorbance measurement at 700 nm of the formation of the Perl's Prussian Blue complex following the addition of excess Fe^{3+}, as described previously[31–34]. Increased absorbance of the reaction mixture indicates grater reduction capability[35].

Cupric ions (Cu^{2+}) reducing power – Cuprac assay

Cu^{2+} reducing ability of P-Gum was determined by slight modification of the cupric ions (Cu^{2+}) reducing power method[36,37]. Cuprac assay is a chromogenic redox reaction, carried out at close to physiological pH (pH 7) and 2,9-dimethyl-1,10-phenanthroline (Neocuproine) is used as chromogenic agent. Absorbance was measured at 450 nm after 30 min against a reagent blank. Increased absorbance indicates increased reduction capability of P-Gum or standards[38].

Fe^{3+}–Fe^{2+} reducing capacity – FRAP assay

The FRAP assay measures the ability of antioxidants to reduce the ferric $[Fe^{3+}–(TPTZ)_2]^{3+}$ to the intensely blue colored ferrous complex $[Fe^{2+}–(TPTZ)_2]^{2+}$ in acidic medium[39,40]. $[Fe^{3+}–(TPTZ)_2]^{3+}$ reducing values of P-Gum are estimated by measuring the absorbance increase at 593 nm and relating it to a ferrous ions standard solution or to an antioxidant standard solution. The change in absorbance is proportional to the combined $[Fe^{3+}–(TPTZ)_2]^{3+}$ reducing value of the antioxidants in P-Gum[41,42]. Increased absorbance of the reaction mixture of P-Gum shows increased reduction capability.

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

Ferrous ions (Fe^{2+}) chelating activity was evaluated by following method of Dinis[35] described previously[43]. Accordingly, the inhibition of the formation of Fe^{2+}–ferrozine complex by P-Gum screened with decreased absorbance at 562 nm as Fe^{2+}-chelating ability of sample. The IC_{50} value of inhibition of ferrozine–Fe^{2+} complex formation was calculated by equation obtained from the graphic of P-Gum or standards. The control sample contains only $FeCl_2$ and ferrozine[16,44].

DPPH$^{\bullet}$ free radical scavenging activity

In DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow colored diphenyl-picrylhydrazine[45]. This method is based on the reduction of DPPH in alcoholic solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H in the reaction. This spectrophotometric assay uses the stable radical, DPPH·, as a reagent. The method of Blois[46] previously detailed described by Gülçin[16] with slight modifications in order to assess the DPPH· free radical scavenging capacity of P-Gum. The DPPH· scavenging capacity P-Gum and standards was expressed as mM in the reaction medium and calculated from the calibration curve determined by linear regression (r^2: 0.9966):

$$\text{Absorbance } (\lambda_{517}) = 0.0026 \times [\text{DPPH} \cdot]$$

Measurement of $DMPD^{\bullet+}$scavenging ability

$DMPD^{\bullet+}$ scavenging ability of P-Gum was performed according to Fogliano et al.[47] and reported previously[48]. The scavenging capability of $ABTS^{\bullet+}$ radical of the sample was monitored in spectrophotometer at 505 nm. The $DMPD^{\bullet+}$ concentration (mM) in the reaction medium was calculated from the below calibration curve, determined by linear regression (r^2: 0.9725):

$$\text{Absorbance } (\lambda_{505}) = 0.0008 \times [DMPD^{\bullet+}]$$

Superoxide anion radical scavenging activity

Superoxide radical scavenging activity of P-Gum was determined by spectrophotometric measurements of nitrobluetetrazolium (NBT). To this end, superoxide radicals were generated by method of Beauchamp and Fridovich[49] with slight modification[50]. They are generated in riboflavin-methionine-illuminate system and may reduce NBT into formazan. This reduction was spectrophotometrically monitored at 560 nm. The un-illuminated reaction mixture was used as a blank sample. P-Gum was added to the reaction mixture, in which $O_2^{\bullet -}$ was scavenged, thereby inhibiting the NBT reduction.

Total antioxidant activity determination by ferric thiocyanate method

In order to measure the preventing effects of peroxidation of linoleic acid emulsion of P-Gum and reference antioxidants, the ferric thiocyanate method was performed[36,51]. This method was used to measure the peroxide level during the initial stage of linoleic acid oxidation. The peroxide levels were determined by reading the absorbance at 500 nm in a spectrophotometer (Shimadzu, UV-1208 UV-VIS Spectrophotometer, Kyoto, Japan) after reaction with $FeCl_2$ and thiocyanate at intervals during incubation. The assay steps were repeated every 12 h until absorbance reached a maximum value. For this purpose, the percent inhibition was calculated at this point (96 h). The solution without P-Gum was used as blank samples. Linoleic acid mixture without the addition of sample was used as a control. The percent inhibition of lipid peroxidation in linoleic acid emulsion was calculated by following equation:

$$\text{ILP}\ (\%) = \left(1 - \frac{\lambda_S}{\lambda_C}\right) \times 100$$

where ILP is inhibition of lipid peroxidation, λ_C is the absorbance of the control reaction, which contains only linoleic acid emulsion and sodium phosphate buffer. λ_S is the absorbance of sample in the presence P-Gum or other tests compounds[48,52].

Statistical analysis

The experimental results were performed in triplicate. The data were recorded as mean ± SD and analyzed by SPSS (version 11.5 for Windows 2000, SPSS Inc., San Francisco, CA). One-way analysis of variance (ANOVA) was performed by procedures. Significant differences between means were determined by Duncan's Multiple Range tests, and $p < 0.05$ was regarded as significant and $p < 0.01$ was very significant[53].

Results and discussion

The human diet contains different compounds that possess antioxidant activities. These compounds have been suggested to scavenge ROS based on their structural properties. Phenolic compounds are a class of chemical compounds consisting of a hydroxyl group (–OH) bonded directly to an aromatic hydrocarbon group. They are secondary plant metabolites and naturally present in almost all plant materials, including food and pharmaceutical products of plant origin. Phenolic compounds are thought to be an integral part of both human and animal diets[24,25].

P-Gum exhibited effective potassium ferricyanide reduction, FRAP and cupric ions (Cu^{2+}) reducing methods when compared to the standards such as BHA, BHT, α-tocopherol and trolox. For evaluation of the reduction ability of P-Gum, the Fe^{3+}–Fe^{2+} transformation was investigated by the method of Oyaizu[31]. The reducing capacity of sample was measured as direct $Fe[(CN)_6]_3$–$Fe[(CN)_6]_2$ reduction. As can be seen in Table 1, P-Gum showed potent Fe^{3+} reducing ability and these differences were statistically very significant ($p < 0.01$). Increase $Fe[(CN)_6]_2$ absorbance of the reaction mixture indicate increased reducing capacity due to an increase in the formation of the complex. So, reducing power of P-Gum and standard compounds exhibited the following order: BHA (2.098 ± 0.04) > BHT (1.591 ± 0.19) > α-tocopherol (1.052 ± 0.10) > trolox (0.567 ± 0.03) > P-Gum (0.223 ± 0.03). The results demonstrated that P-Gum changed yellow color of the test solution to green or blue depending on their ferric ions (Fe^{3+}) reducing ability. On the other hand, according to Fe^{3+} to Fe^{2+} reducing capacity-FRAP assay results, at 30 μg/ml concentration of P-Gum and standard antioxidants were shown in Table 1, showed the following order: BHA (2.261 ± 0.02) > α-tocopherol (1.764 ± 0.05) > BHT (1.283 ± 0.09) > trolox (1.024 ± 0.07) > P-Gum (0.530 ± 0.07).

Table 1. Determination of reducing power of P-Gum by potassium ferricyanide reduction and FRAP methods, cupric ions (Cu^{2+}) reduction capacity by Cuprac method.

Antioxidants	Fe^{3+}–Fe^{3+} reducing power (700 nm)*	Cu^{2+}–Cu^{+} reducing power (450 nm)*	FRAP Assay (593 nm)*
BHA	2.098 ± 0.04	0.598 ± 0.04	2.261 ± 0.02
BHT	1.591 ± 0.19	0.550 ± 0.04	1.283 ± 0.09
α-Tocopherol	1.052 ± 0.10	0.546 ± 0.13	1.764 ± 0.05
Trolox	0.567 ± 0.03	0.486 ± 0.11	1.024 ± 0.07
P-Gum	0.223 ± 0.03	0.158 ± 0.03	0.530 ± 0.07

P-Gum, pistachio (*Pistacia vera* L.) gum; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene.
*Expressed as absorbance values.

The Cuprac is a reduction method based on reduction Cu^{2+} to Cu^{+} in the presence of neocuproine[53]. Cu^{2+} reducing ability of P-Gum and standard compounds were shown in Table 1. Between Cu^{2+} reducing ability of P-Gum and its concentration (30 μg/ml) was observed a correlation. Cupric ions reducing power of P-Gum and standard compounds at the same concentration (30 μg/ml) exhibited the following order: BHA (0.598 ± 0.04) ≥ BHT (0.550 ± 0.04) ≈ α-tocopherol (0.546 ± 0.13) > trolox (0.486 ± 0.11) > P-Gum (0.158 ± 0.03). Also, embedded thiols (–SH groups) of proteins release in urea buffer at pH 7 and react with reagent. So, this method can be used for measurement of activity of antioxidants, which include thiol groups such as glutathione[37,53].

Due to the fact that, the ionic species as ferrous (Fe^{2+}) ions facilitate the production of ROS in organism, iron-chelating capacity is quite important. Metal chelating activity is an important antioxidant activity method, is used to prevent or delay the oxidation reactions catalyzed by metal ions. Iron is an essential mineral for organisms but more than necessary can cause cell damage[54]. Owing to the high activity, it is known as the most important oxidizing metal among the transition metals. Ferrous ions (Fe^{2+}) are the most significant prooxidative ions. Ferrozine can form a complex with divalent metal ions such as ferrous ions (Fe^{2+}) in even the quantitative amount. As a result that colored ferrozine–metal complex show the maximum absorbance at 562 nm. P-Gum was assessed for its ability to compete with ferrozine for ferrous ion in the solution[16].

P-Gum had effective chelating effect on ferrous ions (Fe^{2+}). P-Gum had effective IC_{50} values of Fe^{2+} chelating activity than that of standard antioxidants (BHA, BHT, α-tocopherol and trolox). In addition, P-Gum exhibited 5.3 μg/ml as IC_{50} value. On the other hand, the Fe^{2+} chelating capacity of BHA, BHT, α-tocopherol and trolox were found to be 28.9, 8.5, 16.5 and 7.5 μg/ml, respectively. These results clearly show that the Fe^{2+}

Table 2. Total antioxidant activity by ferric thiocyanate method, IC_{50} values of $DPPH^{\bullet}$ free radical scavenging activity, DMPD radical scavenging activity, superoxide anion radical ($O_2^{\bullet-}$) scavenging activity and metal chelating activity of P-Gum.

Antioxidants	Metal chelating activity*	$O_2^{\bullet-}$ scavenging activity*	$DPPH^{\bullet}$ scavenging activity*	$DMPD^{\bullet+}$ scavenging activity*	Total antioxidant activity†
BHA	28.9	19.8	10.2	12.6	80.3
BHT	8.5	20.4	27.7	14.4	73.5
α-Tocopherol	16.5	17.8	11.8	15.4	36.2
Trolox	7.5	21.0	10.9	12.0	72.0
P-Gum	5.3	16.5	23.9	15.4	54.2

P-Gum, pistachio (*Pistacia vera* L.) gum; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DPPH, 1,1-diphenyl-2-picryl-hydrazyl radicals; $DMPD^{\bullet+}$, *N*,*N*-dimethyl-*p*-phenylenediamine radicals; $O_2^{\bullet-}$, superoxide anion radicals.
*Expressed as IC_{50} values (μg/ml).
†Expressed as percent (%) inhibition.

chelating effect of P-Gum was higher than that of BHA (28.9), BHT (8.5), α-tocopherol (16.5) and trolox (7.5). In this assay, P-Gum disrupted the formation of the f Fe^{2+}–ferrozine complex. It suggests P-Gum have chelating activity and is able to capture Fe^{2+} before ferrozine.

Radical scavenging assays determine the antioxidant capacities of compounds by spectrophotometrically[55,56]. Antioxidants cause depolarization and reverse the $DPPH^{\bullet}$ formation $DMPD^{\bullet+}$ and $ABTS^{\bullet+}$cation. A freshly prepared $DPPH^{\bullet}$ solution exhibits a deep purple color with absorption maximum at 517 nm. This purple color of $DPPH^{\bullet}$ solution generally disappears when an antioxidant compound is present in the medium[24,43]. Thus, antioxidant molecules can scavenge free $DPPH^{\bullet}$ by providing hydrogen atoms or by electron donation, via a free-radical attack on the $DPPH^{\bullet}$ molecule and convert them to colorless or bleached product (DPPH-H). In this radical scavenging assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored diphenyl-picrylhydrazine (DPPH-H). In brief, this method is based on the DPPH reduction in alcoholic medium in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H in the reaction[16,50,55].

Table 2 indicates an important scavenging of DPPH radical due to the scavenging ability of P-Gum (23.9 μg/ml) and the reference compounds. BHA (10.2 μg/ml), BHT (27.7 μg/ml), α-tocopherol (11.8 μg/ml) and trolox (10.9 μg/ml) were used as references for radical scavenger activity. According to the IC_{50} value of P-Gum in DPPH radical scavenging assay results, DPPH radical scavenging decreased as follow: BHA ≈ Trolox > α-Tocopherol > P-Gum > BHT and were shown in Table 2.

Antioxidant compounds that can able to transfer a hydrogen atom to $DMPD^{\bullet+}$, turn off color of the solution and provide decolorization. Therefore, this study demonstrates capability of the radical hydrogen donor to scavenge a single electron from $DMPD^{\bullet+}$. Preliminary experiments have shown that the choice of oxidant solution and the ratio between the concentrations of $DMPD^{\bullet+}$ and the oxidative compound is very important for the effectiveness of the method. $DMPD^{\bullet+}$ shows a maximum absorbance at 505 nm[16]. As shown in Table 2, P-Gum exhibited a marked $DMPD^{\bullet+}$radical scavenging activity. IC_{50} value of $DMPD^{\bullet+}$radical scavenging activity of P-Gum was found to be 15.4 μg/ml. On the other hand, at the same concentration this value was found as 15.4 μg/ml for α-tocopherol and was shown the same effect with P-Gum. There was a significant decrease ($p < 0.05$) control value and $DMPD^{\bullet+}$ scavenging capacity of P-Gum.

In superoxide anion radical scavenging method, superoxide anion reduces NBT to the yellow dye (NBT^{2+}) to produce the blue formazan. The absorbance of formazan is spectrophotometrically measured at 560 nm[57]. The decrease in absorbance at 560 nm with antioxidants shows superoxide anion was dissipated. P-Gum had distinctive inhibition of superoxide radical generation. As seen in Table 2, IC_{50} values belonging to inhibition of superoxide anion radical generation of P-Gum was found to be 16.5 μg/ml. On the other hand, at the same concentration BHA, BHT, α-tocopherol and trolox exhibited 19.8, 20.4, 17.8 and 21.0 μg/ml IC_{50} values in superoxide anion radical scavenging activity, respectively. According to these results, P-Gum had higher superoxide anion radical scavenging activity than that all of tested reference compounds and these differences statically were found as significant ($p < 0.01$).

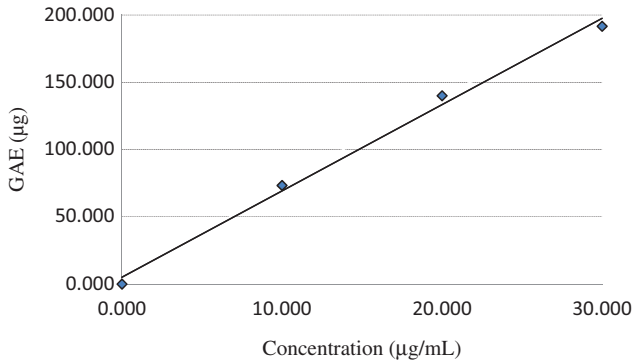

Figure 1. Total phenolic content as gallic acid equivalent (GAE/mg of extract) in P-Gum. P-Gum, pistachio (*Pistacia vera* L.) gum.

For determining total phenolic contents, standard graphic were obtained using known quantities of standard gallic acid. As can be seen in Figure 1, the phenolic compound of 30 μg/ml of P-Gum exhibited 191.7 μg GAE. Flavonoids are very effective antioxidants and it has been proposed that they protect against cardiovascular disease by reducing oxidation of low-density proteins[16,23]. At the same time, total amount of flavonoid in 30 μg/ml P-Gum was determined spectrophotometrically and found to be 0.841 μg quercetin equivalents (Figure 2).

Lipid peroxidation consists of a series of free radical mediated chain reaction and is associated with several types of biological damages. It is the process in which free radicals get electrons from the lipids in cell membranes, resulting in cell damage. In this assay, we measured the amount of peroxide produced from linoleic acid emulsion by auto-oxidation during the experiment period, indirectly[58–61]. P-Gum exhibited effective antioxidant activity in the linoleic acid emulsion system and is showed in graphic (Figure 3). The effect of 10 μg/ml concentration of P-Gum on lipid peroxidation of linoleic acid emulsion is shown in Table 2 and was found to be 54.2%. On the other hand BHA, BHT, α-tocopherol and trolox exhibited 80.3%, 73.5%, 36.2% and 72.0% on peroxidation of linoleic acid emulsion at the same concentration, respectively. The autoxidation of linoleic acid emulsion without P-Gum or standard compounds was accompanied by a rapid increase of lipid peroxides.

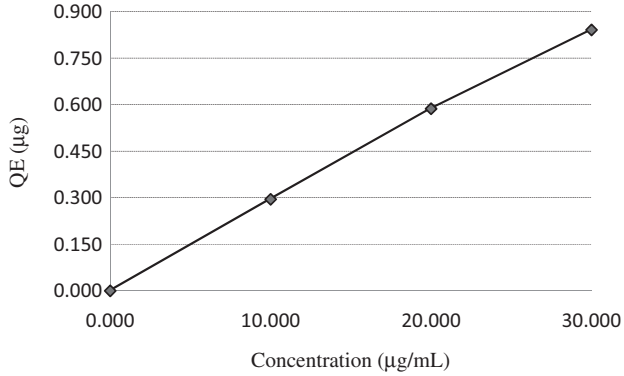

Figure 2. Total flavonoids content as quercetin equivalent (QE/mg of extract) in P-Gum. P-Gum, pistachio (*Pistacia vera* L.) gum.

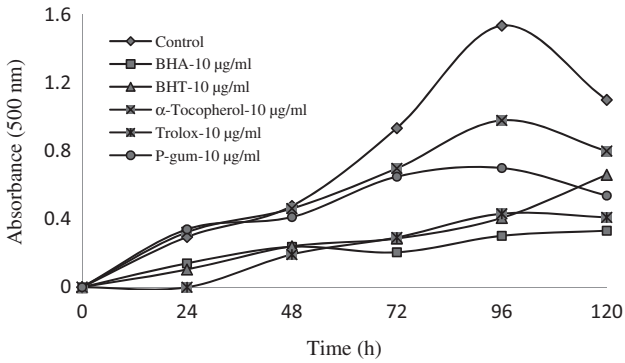

Figure 3. The total antioxidant activity of P-Gum [pistachio (*Pistacia vera* L.) gum] and standard antioxidants such as BHA, BHT, α-tocopherol and trolox at the same concentration(10 µg/ml).

In conclusion, P-Gum was found to be an effective antioxidant in different bioanalytical assays including reducing power, $DPPH^{\bullet}$, $DMPD^{\bullet+}$ and $O_2^{\bullet-}$ radical scavenging, and metal-chelating activities when it is compared to standard antioxidant compounds such as popular synthetic antioxidants BHA and BHT, α-tocopherol, trolox. Based on the discussion above, P-Gum can be used for minimizing or preventing lipid oxidation in pharmaceutical products, retarding the formation of toxic oxidation products, maintaining nutritional quality and prolonging the shelf life of pharmaceuticals.

Declaration of interest

The authors declare no conflict of interest.

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