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Comparative Study of Total Phenolic Content, Antioxidant Activities, and Polyphenol Oxidase Enzyme Inhibition of Quince Leaf, Peel, and Seed Extracts

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Abstract
Quince *(Cydonia oblonga* Miller) is a widely consumed, safe, and low-cost natural source of different classes of interesting metabolites. Phenolic compounds in natural foods (especially fruits and vegetables) have application as preventive or therapeutic agents in the treatment of diseases caused by free radicals. This study focused on total phenolic content, DPPH and ABTS activities as antioxidant properties, and polyphenol oxidase (PPO) inhibitory activity of the total phenolic compounds extracted from quince leaf, peel, and seed. Quince leaf and peel extracts showed higher ABTS and DPPH activity than quince seed extract. Additionally, all quince extracts inhibited PPO, with IC_{50} values of 36.47 ± 8.28 µg/ml (leaf extract), 173.25 ± 5.69 µg/ml (peel extract), and 386.75 ± 9.27 µg/ml (seed extract). Moreover, the effects of extracts on enzymatic browning in apple were examined by measuring the color parameters.

Keywords Enzymatic browning · ABTS assay · DPPH assay · Colour measurement · *Cydonia oblonga* Miller

Introduction

Quince is the fruit of a deciduous tree of the Rosaceae family, *Cydonia oblonga* Miller (Silva et al. 2002; Abbastabar et al. 2015). The homeland of quince, which is among the fruit varieties grown in a temperate climate, is northwestern Iran, the North Caucasus, the Caspian Sea, and North Anatolia (Ercisli et al. 1999). Quince is considered a functional fruit due to its medicinal and nutritional values and is mostly used in the production of jam, marmalades, jelly, liquor, and cakes (Iqbal et al. 2018).

Quince fruit is recognized as a good, cheap, and important dietary source of health-promoting compounds due to its biologically active constituents, which are characterized by their antioxidant, antimicrobial, and antiulcerative properties (Oliveira et al. 2008). Moreover, *Cydonia oblonga* Miller leaves have been used, after decoction or infusion, in folk medicine for their sedative, antipyretic, antidiarrheic, and antitussive properties and for the treatment of various skin diseases (Oliveira et al. 2007). Quince seeds have been used as a source of hydrocolloid, hydrogel, and biopolymer (Kirtil and Oztop 2016), whereas quince peel has not been widely used; however, it has been investigated for use as an anti-inflammatory and antimicrobial agent (Fattouch et al. 2007; Essafi-Benkhadir et al. 2012).

Quince peel contains 13 phenolics, including 3-O-caffeoylquinic, 4-O-caffeoylquinic, 5-O-caffeoylquinic, 3,5-di-O-caffeoylquinic acids, quercetin 3-galactoside, rutin, kaempferol 3-glucoside, kaempferol 3-rutinoside, and five unidentified compounds (Silva et al. 2004). Quince seeds and leaves also include a phenolic profile composed of 3-O-, 4-O-, 5-O-, and 3,5-O-di-caffeoylquinic acids; quercetin-3-O-galactoside; rutin; lucenin-2; vicenin-2; etc. (Silva et al. 2005; Costa et al. 2009) (Fig. 1).

Polyphenol oxidase (PPO; EC 1.14.18.1), known as tyrosinase, is an enzyme of the copper-containing oxidoreductase class that causes browning as a result of the oxidation products of phenol compounds on the cut surfaces of vegetables and fruits (Kiralp 2004). Polyphenol oxidase catalyzes monophenols to O-diphenols by hydroxylation in the presence of oxygen, and then to O-quinones by dehydrogenation in the presence of oxygen. Nonenzymatic polymerization of O-quinones forms melanin or melanin-like components by a series of oligomerization and polymerization reactions by the oxidative component (Bao 1999; Madand 2000).

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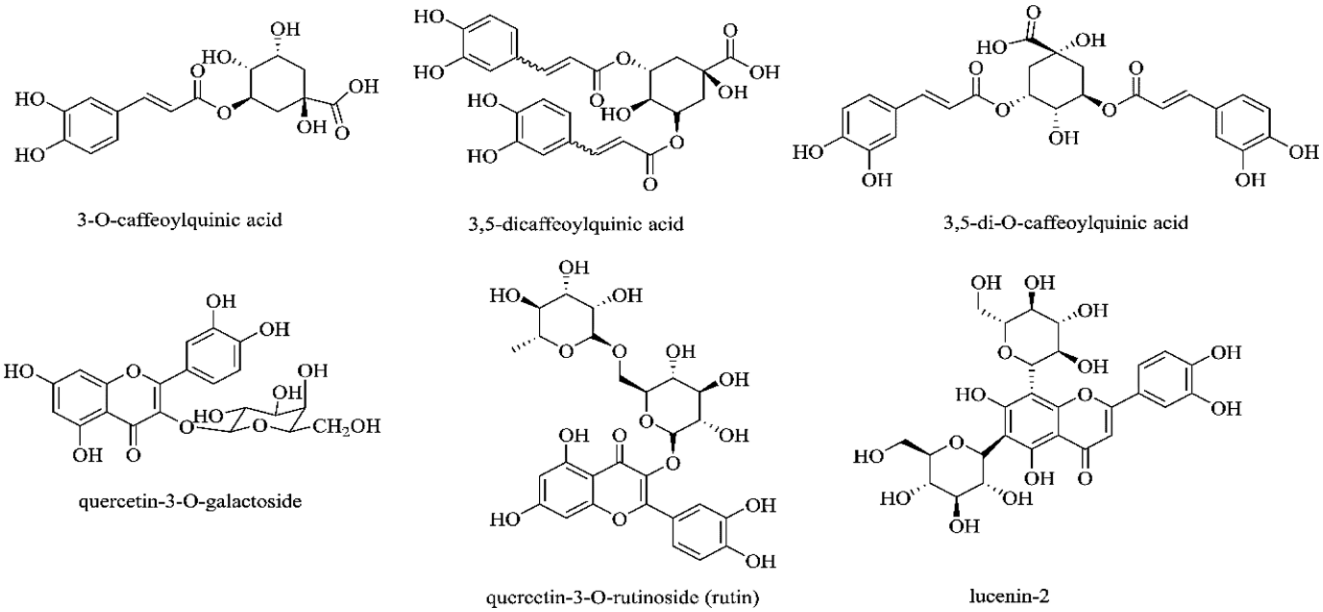

Fig. 1 Structure of some phenolic substances in quince leaf, peel, and seeds

It is important to recycle the wastes in the food and agriculture sectors and turn them into value-added products. This study aimed to reveal the potential usability of quince leaf, peel, and seed as value-added products instead of waste by examining their antioxidant and PPO inhibition properties, as quince leaf, peel, and seed are not used much in the processing of quince. In this study, total phenolic compounds were extracted from quince leaf, peel, and seed. Their ABTS and DPPH activities as future antioxidant and PPO inhibitory activities were investigated. In addition, the effects of extracts on enzymatic browning were investigated by measuring the color parameters.

Materials and Methods

Materials

Quince samples were collected from the Pamukova region in Sakarya, Turkey (latitude: 40°30′18.9648″N; longitude: 30°10′3.3204″E) in October 2017. Quince leaf, peel, and seed samples were analyzed in an appropriate stage of maturity. All samples were dried to avoid any deterioration from the maturity stage to the time of extraction. Dried samples were ground before extraction and kept at 4 °C.

All used chemicals were obtained from Sigma Aldrich, Alfa Aesar, and Merck. Tyrosinase from mushroom was purchased from Sigma Aldrich.

Extraction

Samples were extracted according to the method in the literature (Silva et al. 2004; Benzarti et al. 2015), with a few modifications. The details are given in Supplementary Information.

Total Phenolic Content

The Folin–Ciocalteu phenol reagent (consisting of lithium sulphate, hydrochloric acid, phosphoric acid, disodium wolframate dihydrate, and bromine) assay was used to determine the total phenolic content (Soong and Barlow 2004). Gallic acid (GAE) was used as a standard for the calibration curve (Fig. 2). The total amount of phenolic compounds was calculated as milligrams of GAE per gram of extract. The details are given in Supplementary Information.

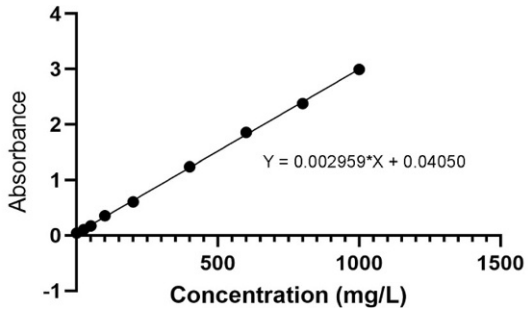

Fig. 2 Calibration curve of gallic acid

DPPH Assay

The DPPH radical scavenging activities of peel, seed, and leaf extract were measured according to the method in the literature (Cadi et al. 2020), with a few modifications. The details are given in Supplementary Information.

ABTS Assay

The ABTS scavenging activities of the extracts were measured according to the method in the literature (Sonmez et al. 2019). The details are given in Supplementary Information.

PPO Activity Assay

Enzyme activity was determined, using catechol, by measuring the increase in absorbance at 420 nm according to the method in the literature (Kamkaen et al. 2007), with a few modifications. The details are given in Supplementary Information.

Color Measurement

Lightness (L*), redness/greenness (a*), yellowness/blueness (b*), and fresh fruit color values (L_0*, a_0*, and b_0*) were determined using a colorimeter (CR-10; Konica Minolta, Japan). Total color difference (ΔE) was calculated according to the following formula (Onwude et al. 2019):

$$\Delta E = \sqrt{\left(L^* - L_0^*\right)^2 + \left(a^* - a_0^*\right)^2 + \left(b^* - b_0^*\right)^2} \quad (1)$$

Results and Discussion

The total phenolic contents (TPC), antioxidant values, and PPO activity values are presented in Table 1. Among leaf, seed, and peel extracts, leaf extract had the highest phenolic content (113.47 ± 9.35 mg GAE/g extract), followed by peel extract (18.91 ± 1.87 mg GAE/g extract) and seed extract (9.80 ± 1.41 mg GAE/g extract), respectively.

It was reported that the TPC of some fruit (avocado, walnut, mulberry, fig, carob, lemon, pomegranate, grape, loquat) tree leaves collected from the Mediterranean region of Turkey ranged from 22.24 mg GAE/g to 219.18 mg GAE/g in methanol extract. Among them, fig and mulberry leaves had the lowest TPC (22.24 mg GAE/g and 27.81 mg GAE/g, respectively), whereas pomegranate and carob leaves had the highest TPC (219.18 mg GAE/g and 161.95 mg GAE/g, respectively; Uysal et al. 2016). It was also reported that the TPC of thyme, mint, and rhubarb leaves was 49.67 mg GAE/g dry weight (dw), 37.87 mg GAE/g dw, and 78.52 mg GAE/g dw, respectively (Bulut et al. 2020). Quince leaf, used in this study, has moderate TPC (113.47 ± 9.35 mg

Table 1 Total phenolic content, extract concentration providing 50% inhibition (IC_{50}), and percentage of inhibition (I%) values of DPPH, ABTS, and PPO inhibitory activities of quince leaf, seed, and peel extracts

Sample	TPC (mg GAE/g extract)	DPPH (I %)	DPPH assay (IC_{50}, µg/ml)	ABTS (I%)	ABTS assay (IC_{50}, µg/ml)	PPO inhibitory activity (IC_{50}, µg/ml)
Quince leaf extract	113.47 ± 9.35	55.55 ± 2.38	49.51 ± 2.38	97.66 ± 0.44	11.05 ± 3.95	36.47 ± 8.28
Quince peel extract	18.91 ± 1.87	26.39 ± 2.19	259.27 ± 31.74	37.91 ± 6.45	110.84 ± 10.33	173.25 ± 5.69
Quince seed extract	9.80 ± 1.41	2.93 ± 0.43	Not calculated	18.37 ± 0.76	Not calculated	386.75 ± 9.27
Ascorbic acid	–	81.89 ± 3.31	2.36 ± 0.97	92.91 ± 5.09	29.23 ± 0.77	1.33 ± 0.5

GAE gallic acid equivalent, *PPO* polyphenol oxidase, *TPC* total phenolic content

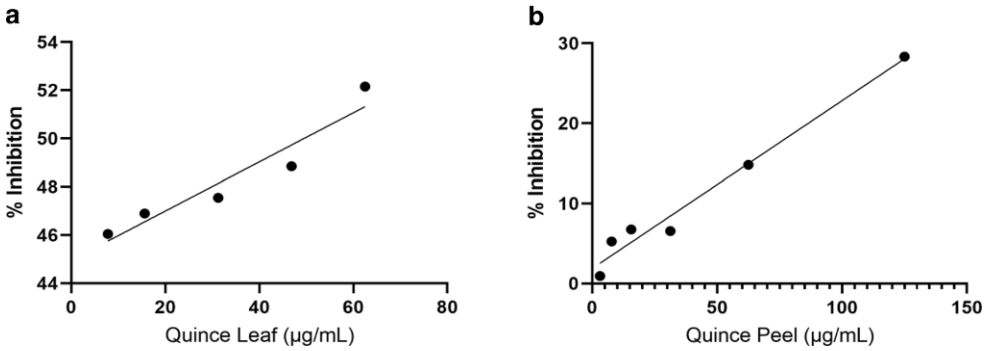

Fig. 3 Scavenging activity on DPPH radicals (IC_{50}) of the quince leaf (**a**) and peel (**b**)

Table 2 Results of color measurement of extracts applied to apple

Sample[a]	L*	a*	b*	ΔE
Fresh apple	68.36 ± 1.46	–0.13 ± 0.69	13.68 ± 1.65	–
1	66.62 ± 1.82	1.53 ± 0.61	16.22 ± 3.11	3.49 ± 2.09
2	62.42 ± 2.91	2.22 ± 0.54	19.01 ± 2.39	8.31 ± 3.20
3	64.42 ± 1.66	1.80 ± 0.41	18.41 ± 2.77	6.45 ± 2.31

[a]*1* Quince leaf extract applied to apple; *2* quince peel extract applied to apple; *3* quince seed extract applied to apple
*L** lightness; *a** redness/greenness; *b** yellowness/blueness

GAE/g extract); however, it has higher TPC than the leaves of seven reported fruits (avocado, walnut, mulberry, fig, lemon, grape, loquat).

The TPC in seed extracts of some plants (*Juniperus oxycedrus* ssp., *Rhus coriaceae, Humulus lupulus, Umbelliferuae foeniculum, Nigella sativa* L., *Coriandrum sativum, Linum usitatissimum*) collected from Turkey was determined to range from 1.27 mg GAE/g to 37.20 mg GAE/g. Among them, *Linum usitatissimum* and *Coriandrum sativum* seeds had the lowest TPC (1.27 mg GAE/g, and 1.51 mg GAE/g, respectively), while *Juniperus oxycedrus* ssp. and *Rhus coriaceae* seeds had the highest TPC (37.20 mg GAE/g and 24.60 mg GAE/g, respectively; Kirca and Arslan 2008). Quince seed, used in this study, has moderate TPC (9.80 ± 1.41 mg GAE/g extract); however, it has higher TPC than the seeds of four reported plants (*Umbelliferuae foeniculum, Nigella sativa* L., *Coriandrum sativum, Linum usitatissimum*).

It was reported that the TPC of pomegranate and grape peels collected from Turkey were 3.55 mg GAE/g fruit weight (FW) and 149 mg GAE/100 g FW, respectively (Gozlekci et al. 2011; Kupe et al. 2021). Quince peel, used in this study, has lower TPC (18.91 ± 1.87 mg GAE/g extract) than grape peel, whereas it has higher TPC than pomegranate peel.

The extract of quince leaf showed the highest DPPH activity in this report, with an IC_{50} value of 49.51 ± 2.38 µg/ml. The IC_{50} graphs of the extracts are summarized in Fig. 3. It was reported that total anthocyanins in various sweet cherry cultivars such as 'Stella,' '0–900 Ziraat' (one of the cultivars of sweet cherry), 'Regina,' 'Noble,' 'Berryessa,' and 'Mazzard' showed DPPH activity. Their inhibition percentages for DPPH activity were 7.859%, 50.674%, 19.611%, 28.368%, 19.611%, and 35.778%, respectively (Sonmez et al. 2022). The obtained extract from quince leaf (55.55 ± 2.38%) in this study had higher DPPH activity than that of reported sweet cherry cultivars.

The ABTS method is based on the ability of hydrogen-donating or electron-donating antioxidants to decolorize the performed radical monocation of 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) generated due to oxidation of ABTS with potassium persulfate (Re et al. 1999). Leaf extract exhibited the strongest ABTS activity, with an IC_{50} value of 11.05 ± 3.95 µg/ml. The IC_{50} graphs of the extracts are given in Fig. 4. It was reported that the total phenolic contents extracted from various plants such as '*Arum maculatum,*' '*Rumex acetosella,*' '*Capsella bursa-pastoris,*' '*Hypericum orientale,*' '*Achillea millefolium,*' '*Silene vulgaris,*' and '*Rumex crispus*' showed ABTS activity. Their IC_{50} values for ABTS activity were 106.01 µg/ml, 47.45 µg/ml, 66.14 µg/ml, 42.39 µg/ml, 83.31 µg/ml, 91.59 µg/ml, and 40.22 µg/ml, respectively (Gazioglu et al. 2018). The extract obtained from quince leaf (IC_{50} = 11.05 ± 3.95 µg/mL) in this study exhibited stronger ABTS activity than that reported for these plants.

All extracts obtained from quince leaf, peel, and seed inhibited PPO enzyme. Additionally, leaf extract (IC_{50} = 36.47 ± 8.28 µg/ml) had higher inhibitory activity than seed (IC_{50} = 386.75 ± 9.27 µg/ml) or peel (IC_{50} = 173.25 ± 5.69 µg/ml) extracts. The IC_{50} graphs of these are summarized in Fig. 5. It was reported that some cherry cultivars such as 'Hasan Kazak,' 'Napoleon,'

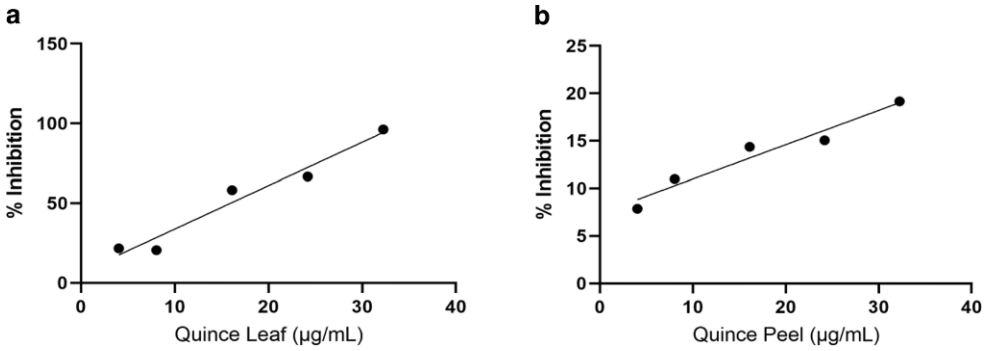

Fig. 4 Scavenging activity on ABTS radicals (IC_{50}) of the quince leaf (**a**) and peel (**b**)

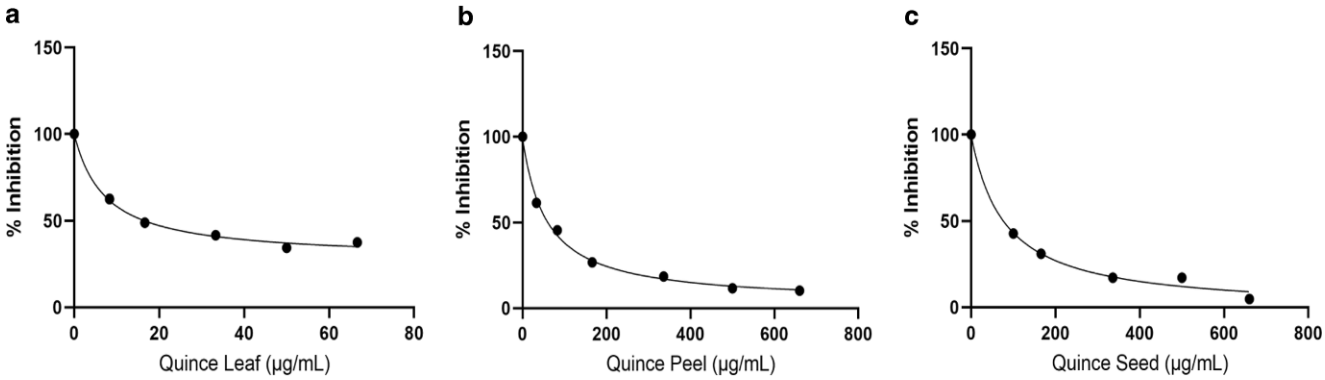

Fig. 5 IC_{50} graphs of quince leaf (**a**), peel (**b**), and seed (**c**) for polyphenol oxidase activity

'0–900 Ziraat,' 'Lambert,' 'Churcill,' and 'Karakiraz' inhibited the polyphenol oxidase enzyme, with IC_{50} values of 495.40 µg/ml, 386.80 µg/ml, 441.14 µg/ml, 195.06 µg/ml, 1239.44 µg/ml, and 324.19 µg/ml, respectively (Demir et al. 2013). The extracts obtained from quince leaf ($IC_{50} = 36.47 \pm 8.28$ µg/ml) and peel ($IC_{50} = 173.25 \pm 5.69$ µg/ml) in this study inhibited PPO stronger than reported for cherry cultivars. Moreover, quince seed extract ($IC_{50} = 386.75 \pm 9.27$ µg/ml) has higher inhibitory activity against PPO than 'Hasan Kazak,' '0–900 Ziraat,' and 'Churcill' cultivars.

Color measurement is an important assay for the food industry during the harvesting, processing, and preservation of foods (Wu and Sun 2013). The XYZ, RGB, and L*, a*, b* color spaces, which are conventional colorimeters, are determined with this assay (Mendoza and Aguilera 2004). L*, which is the luminance or lightness component, ranges from 0 to 100, while parameters a* (from green to red) and b* (from blue to yellow), which are the two chromatic components, range from –120 to 120 (Yam and Papadakis 2004). The aqua solutions of the extracts were applied to fresh apple slices using the immersion method. The effects of extracts on enzymatic browning in apple were examined by measuring these color parameters. The color measurement results are presented in Table 2. The L_o*, a_o*, and b_o* values of fresh apple were determined as 68.36 ± 1.46, –0.13 ± 0.69, and 13.68 ± 1.65, respectively. The L* values of quince leaf, peel, and seed extracts were 66.62 ± 1.82, 62.42 ± 2.91, 64.42 ± 1.66, respectively. Among them, quince leaf extract was found to have the closest L* value to that of fresh apple. The decreasing of the L* value and increasing of the a* value are indicators of browning. The results also showed that quince leaf extract had a higher ΔE value (3.49 ± 2.09) than quince peel (8.31 ± 3.20) and seed (6.45 ± 2.31) extracts applied to apple. This result was associated with the inhibition activity to control the enzymatic browning reaction. It is considered that these extracts, especially that of quince leaf, can prevent enzymatic browning by reacting with O-quinone to produce stable molecules instead of the brown pigments.

Conclusions

In conclusion, it was determined that the leaf of quince had a significantly higher phenolic content than that of seed or peel. Moreover, the leaf extract showed higher DPPH and ABTS activity than the seed and peel extracts, as well as stronger PPO inhibitory activity than the seed and peel extracts. The leaf extract had high total phenolic content (113.47 ± 9.35 mg GAE/g extract) and strong ABTS ($IC_{50} = 11.05 \pm 3.95$ µg/ml and I% = 97.66 ± 0.44) and DPPH ($IC_{50} = 49.51 \pm 2.38$ µg/ml and I% = 55.55 ± 2.38) activities regarding antioxidant properties and inhibitory activities against polyphenol oxidase ($IC_{50} = 36.47 \pm 8.28$ µg/ml). Therefore, quince leaf extract may be used as a PPO enzyme inhibitor and for protective additives in fruits after some further testing, such as cytotoxicity studies, in vivo studies, testing of various application methods to foods, etc. This presented study of the antioxidant properties of quince leaf, peel, and seed shows that quince waste can be a natural source of antioxidants.

Supplementary Information The online version of this article (https://doi.org/10.1007/s10341-022-00696-5) contains supplementary material, which is available to authorized users.

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Conflict of interest F. Sonmez and Z. Sahin declare that they have no competing interests.

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