Foods and Raw Materials, 2022, vol. 10, no. 1
E-ISSN 2310-9599
ISSN 2308-4057

Research Article
Open Access

https://doi.org/10.21603/2308-4057-2022-1-51-66
Available online at https://jfrm.ru/en

Kumquat fruit and leaves extracted with different solvents: phenolic content and antioxidant activity

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Received August 08, 2021; Accepted in revised form September 14, 2021; Published online January 31, 2022

Abstract:
Introduction. Kumquat is a good source of vitamin C, as well as phenolic and flavonoid substances. In this study, we used various solvents to obtain extracts from fresh and lyophilized dried fruits and leaves of kumquat plant, as well as six mutants, to compare their total phenolic and flavonoid contents and antioxidant activities.
Study objects and methods. The total phenolic and flavonoid content was determined by the Folin-Ciocalteu method and the colorimetric method, respectively. The antioxidant capacities of the extracts were determined by commonly used antioxidant tests, such as the DPPH radical scavenging activity, reducing power, and metal chelating activity.
Results and discussion. The total phenolic content of the extracts was in the range of 3705–86 329 mg GAE/g extract. The total amount of flavonoid substance ranged from 5556 to 632 222 mg QUE/g extract. The highest free radical scavenging activity was observed in the kumquat leaves. We also found that the activity of dried fruit was lower than that of fresh fruit. According to our results, the differences in the phenolic contents of the studied plants affected their antioxidant properties. We determined that the extracts with a high phenolic content showed high antioxidant activity. No significant difference was detected between the rootstock kumquat type and its mutants. Finally, we found no chelating activity in the extracts obtained from fresh and lyophilized dried fruits.
Conclusion. Kumquat fruit and its leaves can be considered as functional foods due to phenolic compounds, which are able to neutralize free radicals.

Keywords: Antioxidant activity, flavonoid substance, kumquat, phenolic content, extract, solvent

Funding: The authors thank Inönü University, Turkey (BAPB – Grant No. TYL-2018-1108) for financial support.

Please cite this article in press as: Büyükkormaz Ç, Küçükbay FZ. Kumquat fruit and leaves extracted with different solvents: phenolic content and antioxidant activity. Foods and Raw Materials. 2022;10(1):51–66. https://doi.org/10.21603/2308-4057-2022-1-51-66.

INTRODUCTION

Constantly developing technology, environmental pollution, ultraviolet radiation, and many other factors cause us to be exposed to various toxic substances. This results in more diseases caused by external environmental effects, including more pronounced genetic diseases. Preventing these diseases should become our priority. Since most of them occur in people with a weak immune system, we must focus on strengthening it. For this, we should consume foods with high antioxidant capacity, especially fruits and green leafy vegetables that contain antioxidative phytochemicals [1, 2].

Phytochemicals, or "plant chemicals", are compounds of plant origin, mostly polyphenols, that are essential for human life. They work alongside macronutrients such as carbohydrates, fats, and proteins, as well as 13 essential vitamins and 17 minerals [3]. Antioxidant phytochemicals, especially in fruits and vegetables, combine with free radicals in the human body to protect cells from the attacks of harmful radicals [4]. Bioactive compounds in fruits contain ascorbic acid, organic and phenolic acids, flavonoids, anthocyanins, and carotenoid substances [5, 6].

Citrus fruits come in different types, varieties, and flavors and have positive effects on health and nutrition. Although they have been known as the best sources of vitamin C for a long time, studies on their use as an antioxidant substance have recently gained momentum, due to their richness in phenolic compounds [7]. These bioactive components are responsible for various health benefits of citrus fruits, such as prevention of various diseases or protective effects to lower the risk of various cancers [8–10].

Citrus is a fruit group belonging to the genus Citrus, which is a member of the *Aurantioideae* subfamily of the *Rutaceae* family. The most common citrus varieties are orange (*Citrus sinensis* L.), mandarin (*Citrus reticulata* L.), lemon (*Citrus limon* L.), golden ball (*Citrus paradisi* L.), bitter orange (*Citrus auranthium* L.), and bergamot (*Citrus bergami* L.) [11]. In addition to fresh table consumption, citrus fruits are used as jam, marmalade or fruit juice, as well as raw material in the cosmetics sector [11].

Citrus fruits grow in subtropical climate areas. While mainland China, Southeast Asia, and India are major producers of citrus fruits due to suitable ecological conditions, they are also cultivated in the Mediterranean and Aegean coastal regions and partly in the Eastern Black Sea region of Turkey [12, 13]. The distribution of species and varieties of citrus fruits has gained a regional identity. For example, Washington navel, as well as other navel oranges, and Jaffa are harvested in the Eastern Mediterranean region.

Orange is one of the most produced and consumed citrus fruits in Turkey due to its preference in the juice industry and its great potential in the oil industry [14]. Orange is followed by mandarin and lemon products, respectively. Apart from these species, kumquat, which is called the “little gem of the citrus family”, has recently grown in popularity, as well as such species as Altıntop and citrus, which are lower in production but can be considered important [15].

Kumquat is also called “citrus fortunella”, taking its name from the Scottish horticultural expert Robert Fortune (1812–1880). This species, referred to as “komquot” in some countries, is also called a “golden orange” [16]. It is like a tiny lemon in shape and orangish in color. However, while orange and lemon are consumed after they are peeled, kumquat is consumed with its peel. Its scent is reminiscent of bergamot. It tastes sweet and leaves a lasting scent when you hold it in your hand.

In addition to fresh consumption, kumquat can be used in products such as confectionery, marmalade, liquor, and wine [17, 18]. Essential oil and bioactive ingredients obtained from its peel are used in the perfumery, pharmaceutical, and food industries [19]. Kumquat is an excellent source of nutrients containing minerals, ascorbic acid, carotenoids, flavonoids, and essential oils [20]. It contains remarkable antioxidant properties due to its flavonoid content [18]. However, there are very few studies about kumquat grown in Turkey.

In this study, we aimed to determine the antioxidant capacity and the total phenolic and flavonoid contents of the extracts obtained from fresh and lyophilized dried fruits and leaves of kumquat and six mutants from the Mersin Alata Horticultural Research Institute Directorate.

STUDY OBJECTS AND METHODS

Plant materials. Kumquat leaf and fruit samples were obtained from the Mersin Alata Horticultural Research Institute in November 2017 and January 2018, respectively. We used EP (Old Parcel) with rootstock species; EP.4, EP.29, EP.31 and YP (New Parcel); YP.117, YP.141, YP.188 mutants. The leaf samples were dried in room conditions and in the shade, and stored in a dry and cool environment for analysis. The fruit samples were freeze-dried, or lyophilized.

Chemicals and equipment. We used chemicals and solvents of analytical purity produced by Sigma, Aldrich, and Riedel-de Haen.

The equipment used in the study included a lyophilizer (Christ Alpha 1-2 LC plus), a vortex (Fisons), a rotary evaporator (Laborota 4000-efficient Heidolph), a spectrophotometer (Shimadzu UV-1601), a shaking water bath (Clifton 100–400 rpm; with thermostat), an incubator (EnoLab MB-80), an analytical balance (Gec Avery), a centrifuge (Nüvefuge CN180), a pH-meter (WTW pH 330i), a heater and magnetic stirrer (Chiltern HS31), a disperser and micropipettes (Eppendorf).

Extraction process. Phenolic compounds were extracted from kumquat fruits and leaves with a Soxhlet extraction device, using 260 mL of 99, 80, 60, and 50% methanol and pure water as solvents. In addition, 1 and 0.5% acidified ethanol and hexane solvents were used for kumquat leaves.

For extraction, 20 g of the samples were weighed into the cartridge and then placed in the Soxhlet extractor. The solvent(s) was added to the glass flask and kept in the Soxhlet device for 8 h. The solvent used for extraction was concentrated from the obtained phenolic extracts using a laboratory scale rotary evaporator under vacuum. The remaining part was removed by standing in the open air. The extracts were weighed gravimetrically and stored in dark vials at +4°C in the refrigerator until analysis.

Determination of free radical capture capacity (DPPH method). We used 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical to determine the free radical capture capacity according to the Blois method [21]. This method is based on the ability of the extracts to donate a proton or electron and to decolorize the purple colored DPPH solution (from violet to yellow). A decrease in the absorbance of the reaction mixture is indicative of high free radical scavenging activity.

All the extracts, BHA and BHT standards, and α-tocopherol were dissolved in ethanol at 1 mg/mL. After taking the samples and standards into 5 different volumes of 50, 100, 150, 250, and 500 μL, ethanol was added to a total volume of 3 mL. 1000 μL of 0.1 mM DPPH was added to the tubes and vortexed. The absorbance of the mixture, which was incubated for 30 min in the dark at room temperature, was measured in the UV-visible spectrophotometer at 517 nm. Calculations were made using the following formula:

$$\% \text{ free-radical scavenging activity} = \frac{A_C - A_{S/S}}{A_C} \times 100 \quad (1)$$

where A_C is the absorbance of the control reaction; $A_{S/S}$ is the absorbance of the sample or standard.

Determination of reducing capacity. The Oyaizu method was used to determine the reduction capacity [22]. According to this method, the reducing agent in the medium reduces Fe^{3+} ions to Fe^{2+} ions and a complex is formed by adding $FeCl_3$. The absorbance of the resulting complex is measured in the UV-visible spectrophotometer at 700 nm. The increase in absorbance of the reaction mixture is directly proportional to the reducing power of the sample.

All the extracts, BHA and BHT standards, and α-tocopherol were dissolved in ethanol at 1 mg/mL. 100, 250, and 500 μL of the samples and standards were taken into test tubes in three different volumes, and 3400, 3250, and 3000 μL of pH 6.6 phosphate buffer was added to them, respectively, to a total volume of 3500 μL. Then, after adding 2500 μL of 1% K_3 [Fe $(CN)_6$] and vortexing, it was left to incubate for 20 min in a water bath at 50°C. After the incubation, 2500 μL of 10% trichloroacetic acid (TCA) was added to the test tubes and centrifuged at 3000 rpm for 10 min. 1250 μL of the resulting supernatant was taken into empty tubes and 1250 μL of distilled water and 500 μL of 0.1% $FeCl_3$ were added to them. The mixture was vortexed and its absorbance was measured at 700 nm in the UV-visible spectrophotometer.

Determination of iron (II) ions chelating activity. Antioxidants with metal chelating properties inactivate free iron by binding it and thus inhibit the formation of radicals such as hydroxyl and peroxide, which are formed as a result of Fenton reactions ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + HO^{-}$) [23]. The Dinis method was used to determine the activity of chelating iron (II) ions [24]. All the extracts and EDTA used as control were dissolved in ethanol to 1 mg/mL. The samples and standards were taken into 50, 100, 150, 250, and 500 μL test tubes, and 3700, 3650, 3600, 3500, and 3250 μL of ethanol was added to them, respectively, to a total volume of 3750 μL. Then, 50 μL of 2mM $FeCl_2$ was added and vortexed to incubate at room temperature for 10 min. Then, 200 μL of 5mM ferrosine was added. The resulting purple color was measured in the UV-visible spectrophotometer at 562 nm after the mixture was kept at room temperature for 25 min.

Determination of total phenolic content. The Folin-Ciocalteu method was used to determine the total phenolic content [25]. The Folin-Ciocalteu reagent (FCR) used in this method is molybophosphotungstic heteropolyacid ($3H_2O{\cdot}P_2O_5{\cdot}13WO_3{\cdot}5MoO_3{\cdot}10H_2O$). This method is based on the transfer of electrons from phenolic compounds and other reducing compounds to molybdenum. Phenolic compounds only react with the FCR in basic conditions (pH ~ 10) [26].

$$Mo(VI) + e^{-} (\text{antioxidant}) \rightarrow Mo(V)$$

Commercially available 2N Folin-Ciocalteu reagent was prepared daily by diluting it with purified water at a ratio of 1/1 (v/v). 500 μL of the extracts (1 mg/mL) was taken into test tubes and 500 μL of distilled water was added. After 250 μL of 1 N Folin reagent was added to the mixture, it was incubated for 5 min by vortexing. 1250 μL of 2% Na_2CO_3 solution was added to it, vortexed, and then kept at room temperature for 2 h. The absorbance of the resulting mixture was measured at 765 nm in the UV-visible spectrophotometer. The phenolic content of the extracts was given as mg gallic acid equivalent (GAE)/g extract.

Determination of total flavonoid content. The total flavonoid content was measured by an aluminum chloride colorimetric test according to Zhishen *et al.* [27]. All the extracts and a quercetin solution used as a standard were dissolved in 1 mg/mL ethanol. 500 μL was taken from the extracts prepared in the test tubes and pure water was added to a total volume of 5000 μL. To this mixture, 300 μL of 5% $NaNO_2$ solution was added and left to incubate at room temperature for 5 min, and then 300 μL of 10% $AlCl_3$ solution was added. After waiting for 6 min, 2 mL of 1.0 M NaOH solution was added and the volume was completed to 20 mL with distilled water. The absorbance of the solution was measured at 510 nm in the UV-visible spectrophotometer. The total flavonoid content of the extracts was given as mg quercetin equivalent (QUE)/g extract.

RESULTS AND DISCUSSION

The solubility and distribution of phenolic compounds in the solvent depend on the polarity of their structure, so the choice of solvent and method is one of the most important steps. In our study, for fresh and lyophilized dried fruits, we preferred methanol and its aqueous solutions, as well as pure water. For leaves, we preferred methanol and aqueous solutions, distilled water, and ethanol acidified with hexane.

Three different methods (DPPH radical scavenging activity, reducing capacity, and iron (II) ions chelating activity) were used to determine the antioxidant capacity. We thought that the extracts could show activity through different mechanisms depending on the diversity of phenolic substances. In addition, we determined the total phenolic content and flavonoid amounts in all the extracts in order to show that the antioxidant effect was proportional to the plant content.

Free radical scavenging activity. The DPPH method is commonly used to evaluate the antioxidant activity of natural products, as it is easy and highly sensitive. DPPH (2,2-diphenyl-1-picrylhydrazyl) is a commercially available stable organic nitrogen radical. The antioxidant effect is proportional to the removal of the DPPH radical. The DPPH radical ($DPPH^{\bullet}$) is purple in color due to the unpaired nitrogen atom. When the DPPH solution reacts with an oxygen atom of a substance (antioxidant chemical) that can give hydrogen atoms, the initial purple color disappears as the radical reduces, turning yellow [28]. The reaction takes place stoichiometrically according to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect was easily determined by following the decrease in UV absorbance at 517 nm until it stabilized.

We observed that the highest free radical scavenging activity was in kumquat leaves, and the activity of kumquat fruit decreased when dried (Table 1). There was no significant difference between the rootstock kumquat type and its mutants. The free radical scavenging activities of the extracts were slightly below the standards (BHA, BHT, and α-Tocopherol). The highest activity (81.66%) was seen in the YP.188 hybrid leaf extract using 80% methanol solvent. As for the fruits, the highest activity (61.37%) was in the EP.4 hybrid extract using a pure methanol solvent.

When we examined all the samples, we associated high phenolic content with high antioxidant activity. We found that the total phenolic content was higher in the samples with high antioxidant activity. As a matter of fact, the leaf extract with high antioxidant activity also had a high phenolic content (85.651 ± 0.030 mg GAE/g extract).

However, when we carefully examined the results, we saw that having a high amount of phenolic substances did not give high results in all antioxidant activity methods. For example, although the YP.188 Leaf 80% methanol and the YP.188 Leaf 50% methanol extracts contained almost the same amount of phenolic substances, the former had higher activity in the applied antioxidant activity methods. This could be explained by the differences between the phenolic substances they contained depending on the solvent used.

In fact, other studies have found that the antioxidant activity of methanol and ethanol extracts, which generally contained phenolic substances, was higher than in other solvent systems [29]. For example, Jayaprakasha *et al.* extracted powdered kumquat fruit in 5 different solvents and investigated the radical capture capacities of the extracts, their amounts in total phenolic matter, and their inhibitory properties for prostate cancer [30].

In this study, the extracts obtained from EtOAc and MeOH-water (4:1, v/v) solvents were found to have the highest and lowest total phenolics, respectively, according to the Folin-Ciocalteu method. It was also observed that the EtOAc and MeOH extracts exhibited the highest and lowest 1,1-diphenyl-2-picyrylhydrazyl (DPPH) radical scavenging activity, respectively [30].

Table 1 DPPH radical scavenging activity of kumquat fruit and leaf extracts, µg/mL (mean ± SD of triplicate)

Extracts and standards	12.5*	25.0*	37.5*	62.5*	125*
Rootstock fresh fruit pure methanol	7.22 ± 0.10	11.19 ± 0.2	12.64 ± 0.1	20.94 ± 0.1	30.32 ± 0.3
Rootstock fresh fruit 80% methanol	6.50 ± 0.10	7.94 ± 0.1	9.03 ± 0.2	12.64 ± 0.3	19.49 ± 0.1
Rootstock fresh fruit 60% methanol	4.69 ± 0.10	7.58 ± 0.1	8.66 ± 0.2	13.00 ± 0.3	21.30 ± 0.3
Rootstock fresh fruit 50% methanol	7.03 ± 0.10	9.03 ± 0.0	18.66 ± 0.1	22.02 ± 0.3	28.52 ± 0.1
Rootstock fresh fruit pure water	10.83 ± 0.0	14.08 ± 0.2	14.08 ± 0.2	22.02 ± 0.0	33.57 ± 0.3
Rootstock dry fruit pure methanol	3.09 ± 0.10	4.75 ± 0.2	7.56 ± 0.3	8.25 ± 0.3	9.97 ± 0.1
Rootstock dry fruit 80% methanol	5.15 ± 0.20	6.53 ± 0.0	8.25 ± 0.2	9.28 ± 0.3	12.37 ± 0.3
Rootstock dry fruit 60% methanol	4.81 ± 0.00	7.56 ± 0.1	8.25 ± 0.0	8.93 ± 0.0	9.62 ± 0.2
Rootstock dry fruit 50% methanol	3.78 ± 0.00	6.53 ± 0.1	7.22 ± 0.0	8.25 ± 0.0	10.31 ± 0.2
Rootstock dry fruit pure water	3.78 ± 0.20	4.47 ± 0.1	6.87 ± 0.0	7.22 ± 0.1	8.59 ± 0.2
Rootstock leaf pure methanol	12.46 ± 0.20	23.88 ± 0.3	32.87 ± 0.1	41.87 ± 0.1	57.09 ± 0.1
Rootstock leaf 80% methanol	21.45 ± 0.10	29.76 ± 0.2	37.72 ± 0.2	50.52 ± 0.1	65.74 ± 0.5
Rootstock leaf 60% methanol	13.49 ± 0.20	18.15 ± 0.1	33.91 ± 0.2	46.71 ± 0.0	65.40 ± 0.3
Rootstock leaf 50% methanol	20.76 ± 0.30	31.49 ± 0.0	39.10 ± 0.1	50.87 ± 0.2	66.44 ± 0.3
Rootstock leaf pure water	12.11 ± 0.10	21.11 ± 0.1	30.10 ± 0.3	36.33 ± 0.2	52.25 ± 0.2
Rootstock leaf 0.5% acidified ethanol	3.46 ± 0.10	8.30 ± 0.2	13.84 ± 0.3	20.42 ± 0.1	34.26 ± 0.4
Rootstock leaf 1% acidified ethanol	5.19 ± 0.10	13.15 ± 0.2	15.22 ± 0.1	25.61 ± 0.2	40.83 ± 0.1
Rootstock leaf hexane	n.d.	n.d.	2.42 ± 0.2	11.07 ± 0.1	12.04 ± 0.1
EP.4 fresh fruit pure methanol	16.97 ± 0.1	20.22 ± 0.3	35.02 ± 0.2	42.60 ± 0.1	61.37 ± 0.3
EP.4 fresh fruit 80% methanol	15.88 ± 0.1	16.61 ± 0.2	21.66 ± 0.2	28.52 ± 0.3	42.96 ± 0.5
EP.4 fresh fruit 60% methanol	13.36 ± 0.1	15.88 ± 0.1	15.88 ± 0.2	22.74 ± 0.3	31.05 ± 0.1
EP.4 fresh fruit 50% methanol	15.88 ± 0.2	18.05 ± 0.1	19.86 ± 0.1	23.10 ± 0.3	33.57 ± 0.2
EP.4 fresh fruit pure water	15.88 ± 0.2	22.38 ± 0.3	28.05 ± 0.1	34.55 ± 0.3	35.38 ± 0.2
EP.4 dry fruit pure methanol	2.06 ± 0.1	2.75 ± 0.3	10.31 ± 0.1	11.37 ± 0.1	14.43 ± 0.1
EP.4 dry fruit 80% methanol	6.25 ± 0.2	7.56 ± 0.0	8.25 ± 0.2	10.31 ± 0.1	14.43 ± 0.2
EP.4 dry fruit 60% methanol	6.53 ± 0.1	8.25 ± 0.1	9.97 ± 0.3	10.97 ± 0.4	13.06 ± 0.1
EP.4 dry fruit 50% methanol	7.56 ± 0.1	8.93 ± 0.1	9.08 ± 0.2	9.97 ± 0.4	13.06 ± 0.3
EP.4 dry fruit pure water	5.15 ± 0.2	8.93 ± 0.2	10.65 ± 0.3	11.68 ± 0.0	15.12 ± 0.3

Continuation of Table 1

Extracts and standards	12.5*	25.0*	37.5*	62.5*	125*
EP.4 leaf pure methanol	14.88 ± 0.1	23.53 ± 0.0	28.03 ± 0.1	36.33 ± 0.2	54.67 ± 0.7
EP.4 leaf 80% methanol	17.65 ± 0.1	32.53 ± 0.3	39.45 ± 0.2	47.06 ± 0.1	71.63 ± 0.5
EP.4 leaf 60% methanol	16.65 ± 0.1	25.61 ± 0.3	34.95 ± 0.2	44.64 ± 0.1	63.67 ± 0.3
EP.4 leaf 50% methanol	16.61 ± 0.2	26.99 ± 0.3	36.33 ± 0.1	46.02 ± 0.1	65.05 ± 0.1
EP.4 leaf pure water	18.34 ± 0.2	27.68 ± 0.2	34.26 ± 0.2	47.40 ± 0.3	55.36 ± 0.3
EP.4 leaf 0.5% acidified ethanol	1.73 ± 0.0	7.61 ± 0.1	9.69 ± 0.3	17.99	29.41 ± 0.5
EP.4 leaf 1% acidified ethanol	6.92 ± 0.1	12.80 ± 0.2	18.34 ± 0.3	24.57 ± 0.2	35.64 ± 0.3
EP.4 leaf hexane	n.d.	n.d.	n.d.	n.d.	7.96 ± 0.5
EP.29 fresh fruit pure methanol	9.42 ± 0.2	15.16 ± 0.2	22.38 ± 0.1	28.88 ± 0.1	37.91 ± 0.1
EP.29 fresh fruit 80% methanol	9.75 ± 0.0	14.08 ± 0.2	17.69 ± 0.1	22.02 ± 0.3	31.77 ± 0.5
EP.29 fresh fruit 60% methanol	12.64 ± 0.1	17.33 ± 0.2	21.30 ± 0.3	25.63 ± 0.3	36.10 ± 0.2
EP.29 fresh fruit 50% methanol	13.72 ± 0.1	17.69 ± 0.1	19.49 ± 0.0	26.71 ± 0.2	36.10 ± 0.4
EP.29 fresh fruit pure water	14.44 ± 0.1	15.16 ± 0.1	17.69 ± 0.1	20.94 ± 0.3	33.21 ± 0.4
EP.29 dry fruit pure methanol	7.90 ± 0.1	9.28 ± 0.1	10.31 ± 0.2	13.06 ± 0.1	15.81 ± 0.5
EP.29 dry fruit 80% methanol	7.56 ± 0.1	11.68 ± 0.1	14.09 ± 0.2	15.43 ± 0.1	19.59 ± 0.2
EP.29 dry fruit 60% methanol	7.90 ± 0.1	10.31 ± 0.1	12.65 ± 0.1	14.43 ± 0.2	19.93 ± 0.4
EP.29 dry fruit 50% methanol	6.80 ± 0.1	9.28 ± 0.1	10.31 ± 0.2	11.68 ± 0.3	14.09 ± 0.5
EP.29 dry fruit pure water	4.81 ± 0.1	5.84 ± 0.1	7.56 ± 0.1	9.28 ± 0.3	12.03 ± 0.1
EP.29 leaf pure methanol	15.57 ± 0.2	26.99 ± 0.1	29.76 ± 0.1	36.33 ± 0.0	52.25 ± 0.3
EP.29 leaf 80% methanol	9.00 ± 0.1	22.15 ± 0.1	31.49 ± 0.1	41.87 ± 0.4	59.86 ± 0.7
EP.29 leaf 60% methanol	12.46 ± 0.2	26.99 ± 0.2	32.53 ± 0.3	46.71 ± 0.1	63.32 ± 0.4
EP.29 leaf 50% methanol	16.96 ± 0.2	28.37 ± 0.1	33.22 ± 0.2	45.67 ± 0.2	60.55 ± 0.4
EP.29 leaf pure water	10.73 ± 0.1	20.7 ± 0.1	26.99 ± 0.1	35.99 ± 0.2	51.21 ± 0.2
EP.29 leaf 0.5% acidified ethanol	3.11 ± 0.1	8.65 ± 0.2	13.84 ± 0.1	20.42 ± 0.2	34.26 ± 0.5
EP.29 leaf 1% acidified ethanol	6.57 ± 0.2	11.07 ± 0.2	15.57 ± 0.1	24.91 ± 0.3	39.79 ± 0.2
EP.29 leaf hexane	n.d.	n.d.	n.d.	n.d.	5.54 ± 0.1
EP.31 fresh fruit pure methanol	2.89 ± 0.1	17.69 ± 0.0	22.74 ± 0.3	29.24 ± 0.2	40.7 ± 0.4
EP.31 fresh fruit 80% methanol	12.27 ± 0.2	16.97 ± 0.1	23.10 ± 0.1	33.94 ± 0.2	51.62 ± 0.5
EP.31 fresh fruit 60% methanol	11.91 ± 0.1	22.02 ± 0.1	25.63 ± 0.2	40.43 ± 0.3	54.51 ± 0.1
EP.31 fresh fruit 50% methanol	14.80 ± 0.1	15.52 ± 0.3	21.66 ± 0.1	28.16 ± 0.5	42.96 ± 0.1
EP.31 fresh fruit pure water	8.30 ± 0.2	13.00 ± 0.3	13.72 ± 0.0	18.41 ± 0.1	23.83 ± 0.1
EP.31 dry fruit pure methanol	7.38 ± 0.2	8.72 ± 0.1	30.20 ± 0.2	39.73 ± 0.1	43.42 ± 0.1
EP.31 dry fruit 80% methanol	7.05 ± 0.2	8.39 ± 0.2	8.39 ± 0.1	10.74 ± 0.3	14.43 ± 0.2
EP.31 dry fruit 60% methanol	3.69 ± 0.1	6.38 ± 0.1	8.72 ± 0.2	9.73 ± 0.1	11.07 ± 0.2
EP.31 dry fruit 50% methanol	3.36 ± 0.0	6.04 ± 0.2	6.38 ± 0.1	8.72 ± 0.0	11.41 ± 0.1
EP.31 dry fruit pure water	6.04 ± 0.1	8.39 ± 0.1	3.36 ± 0.1	3.02 ± 0.3	4.36 ± 0.1
EP.31 leaf pure methanol	13.49 ± 0.1	22.15 ± 0.2	28.37 ± 0.1	37.37 ± 0.2	53.98 ± 0.3
EP.31 leaf 80% methanol	20.42 ± 0.1	31.14 ± 0.2	39.45 ± 0.2	50.52 ± 0.3	68.17 ± 0.4
EP.31 leaf 60% methanol	17.99 ± 0.1	30.80 ± 0.1	39.10 ± 0.1	49.83 ± 0.5	65.05 ± 0.4
EP.31 leaf 50% methanol	19.72 ± 0.1	30.45 ± 0.1	33.22 ± 0.2	49.13 ± 0.1	63.67 ± 0.1
EP.31 leaf pure water	12.11 ± 0.1	21.11 ± 0.1	24.91 ± 0.2	36.33 ± 0.3	53.98 ± 0.5
EP.31 leaf 0.5% acidified ethanol	8.30 ± 0.1	17.30 ± 0.2	24.57 ± 0.2	33.56 ± 0.3	51.56 ± 0.5
EP.31 leaf 1% acidified ethanol	10.3 ± 0.2	16.96 ± 0.1	21.45 ± 0.0	32.18 ± 0.3	47.06 ± 0.3
EP.31 leaf hexane	n.d.	n.d.	n.d.	n.d.	8.65 ± 0.3
YP.117 fresh fruit pure methanol	10.83 ± 0.2	18.41 ± 0.2	21.66 ± 0.4	33.94 ± 0.1	46.93 ± 0.4
YP.117 fresh fruit 80% methanol	10.11 ± 0.2	15.75 ± 0.1	20.58 ± 0.2	27.08 ± 0.5	41.88 ± 0.4
YP.117 fresh fruit 60% methanol	12.27 ± 0.1	15.88 ± 0.1	18.41 ± 0.3	27.08 ± 0.1	40.7 ± 0.3
YP.117 fresh fruit 50% methanol	12.64 ± 0.1	16.61 ± 0.2	22.38 ± 0.1	30.69 ± 0.1	48.38 ± 0.4
YP.117 fresh fruit pure water	15.88 ± 0.1	13.36 ± 0.3	20.94 ± 0.2	28.16 ± 0.1	41.52 ± 0.4
YP.117 dry fruit pure methanol	2.68 ± 0.1	3.45 ± 0.1	4.68 ± 0.1	6.71 ± 0.0	10.40 ± 0.3
YP.117 dry fruit 80% methanol	3.69 ± 0.1	6.38 ± 0.2	7.05 ± 0.1	8.05 ± 0.1	8.39 ± 0.2
YP.117 dry fruit 60% methanol	5.03 ± 0.1	7.72 ± 0.1	8.71 ± 0.3	8.92 ± 0.1	11.74 ± 0.1
YP.117 dry fruit 50% methanol	4.70 ± 0.1	5.09 ± 0.2	6.38 ± 0.3	6.38 ± 0.1	6.38 ± 0.3
YP.117 dry fruit pure water	5.03 ± 0.3	5.18 ± 0.3	6.04 ± 0.3	7.05 ± 0.2	11.41 ± 0.2
YP.117 leaf pure methanol	13.84 ± 0.2	22.84 ± 0.1	30.45 ± 0.1	42.91 ± 0.5	60.55 ± 0.5
YP.117 leaf 80% methanol	20.70 ± 0.3	26.99 ± 0.2	33.56 ± 0.1	47.40 ± 0.2	67.47 ± 0.7
YP.117 leaf 60% methanol	14.53 ± 0.2	21.80 ± 0.3	39.45 ± 0.2	50.87 ± 0.2	65.40 ± 0.1

Continuation of Table 1

Extracts and standards	12.5*	25.0*	37.5*	62.5*	125*
YP.117 leaf 50% methanol	19.03 ± 0.3	33.56 ± 0.2	39.10 ± 0.2	52.25 ± 0.1	65.74 ± 0.1
YP.117 leaf pure water	14.53 ± 0.4	20.76 ± 0.2	32.18 ± 0.1	40.83 ± 0.3	57.44 ± 0.4
YP.117 leaf 0.5% acidified ethanol	7.22 ± 0.1	13.15 ± 0.2	19.72 ± 0.1	28.72 ± 0.1	45.67 ± 0.3
YP.117 leaf 1% acidified ethanol	7.96 ± 0.1	15.22 ± 0.4	17.99 ± 0.1	28.72 ± 0.1	46.37 ± 0.3
YP.117 leaf hexane	n.d.	n.d.	n.d.	2.69 ± 0.1	14.19 ± 0.2
YP.141 fresh fruit pure methanol	9.03 ± 0.1	11.91 ± 0.1	14.80 ± 0.2	23.47 ± 0.2	35.38 ± 0.2
YP.141 fresh fruit 80% methanol	9.39 ± 0.1	10.83 ± 0.2	16.61 ± 0.3	25.27 ± 0.1	35.74 ± 0.3
YP.141 fresh fruit 60% methanol	11.91 ± 0.1	16.08 ± 0.2	19.86 ± 0.2	26.35 ± 0.2	37.91 ± 0.5
YP.141 fresh fruit 50% methanol	5.05 ± 0.1	8.66 ± 0.1	14.08 ± 0.2	24.55 ± 0.3	41.88 ± 0.2
YP.141 fresh fruit pure water	9.39 ± 0.1	10.11 ± 0.1	15.16 ± 0.2	21.30 ± 0.0	31.05 ± 0.2
YP.141 dry fruit pure methanol	5.03 ± 0.1	5.70 ± 0.1	7.72 ± 0.1	10.40 ± 0.2	12.42 ± 0.2
YP.141 dry fruit 80% methanol	7.72 ± 0.1	9.40 ± 0.0	14.43 ± 0.2	10.40 ± 0.0	11.74 ± 0.4
YP.141 dry fruit 60% methanol	8.05 ± 0.1	8.72 ± 0.1	9.73 ± 0.2	30.87 ± 0.3	32.35 ± 0.1
YP.141 dry fruit 50% methanol	1.01 ± 0.1	6.71 ± 0.1	7.38 ± 0.3	10.40 ± 0.2	12.42 ± 0.5
YP.141 dry fruit pure water	7.05 ± 0.2	16.78 ± 0.1	15.7 ± 0.2	15.37 ± 0.1	16.7 ± 0.2
YP.141 leaf pure methanol	18.34 ± 0.2	28.03 ± 0.2	33.56 ± 0.1	48.79 ± 0.3	64.71 ± 0.1
YP.141 leaf 80% methanol	17.65 ± 0.0	33.56 ± 0.1	43.94 ± 0.2	57.09 ± 0.3	72.66 ± 0.4
YP.141 leaf 60% methanol	18.69 ± 0.2	32.53 ± 0.2	39.79 ± 0.1	53.63 ± 0.6	67.82 ± 0.4
YP.141 leaf 50% methanol	17.65 ± 0.3	31.49 ± 0.2	39.79 ± 0.1	51.90 ± 0.3	63.32 ± 0.5
YP.141 leaf pure water	16.61 ± 0.4	28.03 ± 0.2	32.87 ± 0.2	45.67 ± 0.7	61.59 ± 0.3
YP.141 leaf 0.5% acidified ethanol	7.61 ± 0.1	15.57 ± 0.1	21.45 ± 0.3	32.87 ± 0.2	50.52 ± 0.4
YP.141 leaf 1% acidified ethanol	8.30 ± 0.1	14.88 ± 0.1	19.03 ± 0.3	32.18 ± 0.2	48.79 ± 0.4
YP.141 leaf hexane	n.d.	n.d.	1.38 ± 0.1	5.88 ± 0.1	15.92 ± 0.1
YP.188 fresh fruit pure methanol	5.42 ± 0.2	10.83 ± 0.1	13.72 ± 0.2	21.66 ± 0.1	36.10 ± 0.6
YP.188 fresh fruit 80% methanol	5.39 ± 0.2	9.42 ± 0.1	11.91 ± 0.3	22.74 ± 0.1	33.57 ± 0.2
YP.188 fresh fruit 60% methanol	9.39 ± 0.2	12.27 ± 0.2	14.08 ± 0.2	23.10 ± 0.1	33.94 ± 0.2
YP.188 fresh fruit 50% methanol	11.05 ± 0.2	11.19 ± 0.2	15.88 ± 0.5	22.74 ± 0.3	32.85 ± 0.4
YP.188 fresh fruit pure water	13.00 ± 0.2	13.72 ± 0.1	22.38 ± 0.3	33.57 ± 0.3	46.93 ± 0.3
YP.188 dry fruit pure methanol	3.09 ± 0.2	4.75 ± 0.2	7.56 ± 0.2	8.25 ± 0.1	9.97 ± 0.1
YP.188 dry fruit 80% methanol	5.15 ± 0.0	6.53 ± 0.2	8.25 ± 0.2	9.28 ± 0.1	12.37 ± 0.1
YP.188 dry fruit 60% methanol	4.81 ± 0.2	7.56 ± 0.2	8.25 ± 0.1	8.93 ± 0.1	9.62 ± 0.2
YP.188 dry fruit 50% methanol	3.78 ± 0.2	6.53 ± 0.1	7.22 ± 0.3	8.25 ± 0.2	10.31 ± 0.5
YP.188 dry fruit pure water	3.78 ± 0.2	4.47 ± 0.2	6.87 ± 0.3	7.22 ± 0.1	8.59 ± 0.1
YP.188 leaf pure methanol	21.11 ± 0.1	31.14 ± 0.4	35.99 ± 0.2	53.98 ± 0.2	73.70 ± 0.1
YP.188 leaf 80% methanol	25.26 ± 0.2	41.18 ± 0.1	47.06 ± 0.3	66.09 ± 0.3	81.66 ± 0.4
YP.188 leaf 60% methanol	29.07 ± 0.0	46.02 ± 0.5	52.25 ± 0.2	66.78 ± 0.4	80.97 ± 0.4
YP.188 leaf 50% methanol	20.42 ± 0.2	33.56 ± 0.1	45.67 ± 0.1	57.09 ± 0.0	73.70 ± 0.1
YP.188 leaf pure water	13.15 ± 0.2	18.69 ± 0.2	21.45 ± 0.1	48.79 ± 0.4	67.82 ± 0.7
YP.188 leaf 0.5% acidified ethanol	7.27 ± 0.1	15.22 ± 0.3	22.49 ± 0.2	37.02 ± 0.2	57.44 ± 0.2
YP.188 leaf 1% acidified ethanol	10.38 ± 0.1	16.96 ± 0.0	21.11 ± 0.2	32.18 ± 0.3	50.17 ± 0.2
YP.188 leaf hexane	n.d.	n.d.	n.d.	4.50 ± 0.2	17.30 ± 0.2
BHA	73.36 ± 0.2	79.58 ± 0.2	80.62 ± 0.1	83.39 ± 0.3	84.43 ± 0.2
BHT	65.74 ± 0.0	72.32 ± 0.1	73.01 ± 0.2	73.36 ± 0.1	72.32 ± 0.0
α-tocopherol	76.12 ± 0.2	76.12 ± 0.1	81.66 ± 0.2	84.78 ± 0.2	84.43 ± 0.0

*It represents the concentrations of the solutions prepared by taking 50, 100, 150, 250, and 500 µL of standard and extract stock solutions prepared as 1 mg/mL and completing the total volume of 3 mL

n.d.: not detected

The chelating activity of iron (II) ions. Antioxidants with metal chelating properties inactivate it by binding free iron and thus inhibit the formation of radicals such as hydroxyl and peroxide, which are formed as a result of Fenton reactions. Therefore, metal chelating plays an important role in determining antioxidant activity [31].

We evaluated the metal ion chelating activity according to the competition between plant extracts with ferrosine in order to bind Fe^{2+} ions in the solution. We observed no chelating activity in the extracts obtained from moist and lyophilized dried fruits (Table 2). The pure methanol extracts showed weak activity in kumquat leaves, while the extracts obtained from aqueous solvents showed no activity at all.

Table 2 Metal chelating capacities of kumquat fruit and leaf extract, µg/mL (mean ± SD of triplicate)

Extracts and standards	12.5*	25.0*	37.5*	62.5*	125*
Rootstock leaf pure methanol	10.70 ± 0.20	14.95 ± 0.1	20.44 ± 0.1	20.71 ± 0.3	5.76 ± 0.1
Rootstock leaf 0.5% acidified ethanol	4.39 ± 0.10	5.12 ± 0.0	4.39 ± 0.1	5.95 ± 0.1	6.73 ± 0.1
Rootstock leaf 1% acidified ethanol	3.51 ± 0.10	10.10 ± 0.1	11.86 ± 0.2	13.47 ± 0.1	18.59 ± 0.3
Rootstock leaf hexane	2.99 ± 0.0	8.52 ± 0.1	15.10 ± 0.2	18.30 ± 0.1	17.19 ± 0.4
EP.4 leaf pure methanol	10.56 ± 0.10	21.26 ± 0.1	23.32 ± 0.2	28.94 ± 0.1	16.74 ± 0.2
EP.4 leaf 0.5% acidified ethanol	3.51 ± 0.1	10.10 ± 0.2	11.86 ± 0.1	13.47 ± 0.1	18.59 ± 0.3
EP.4 leaf 1% acidified ethanol	4.10 ± 0.1	3.07 ± 0.2	5.42 ± 0.1	6.59 ± 0.3	6.83 ± 0.2
EP.4 leaf hexane	9.87 ± 0.2	14.20 ± 0.1	24.22 ± 0.2	34.08 ± 0.3	25.41 ± 0.3
EP.29 leaf pure methanol	13.03 ± 0.1	25.24 ± 0.1	32.24 ± 0.2	36.90 ± 0.3	19.48 ± 0.1
EP.29 leaf 0.5% acidified ethanol	4.93 ± 0.0	16.29 ± 0.1	23.47 ± 0.1	37.07 ± 0.1	24.96 ± 0.3
EP.29 leaf 1% acidified ethanol	5.38 ± 0.0	10.91 ± 0.2	17.32 ± 0.1	30.19 ± 0.2	31.24 ± 0.1
EP.29 leaf hexane	1.35 ± 0.1	2.54 ± 0.1	6.13 ± 0.1	12.26 ± 0.2	8.97 ± 0.2
EP.31 leaf pure methanol	27.36 ± 0.2	43.84 ± 0.1	44.64 ± 0.1	42.06 ± 0.3	31.20 ± 0.4
EP.31 leaf 0.5% acidified ethanol	2.54 ± 0.2	5.38 ± 0.2	10.46 ± 0.1	15.40 ± 0.3	15.99 ± 0.1
EP.31 leaf 1% acidified ethanol	2.69 ± 0.0	6.43 ± 0.1	9.72 ± 0.2	10.27 ± 0.1	7.92 ± 0.1
EP.31 leaf hexane	8.37 ± 0.2	9.57 ± 0.1	18.22 ± 0.1	20.33 ± 0.2	20.78 ± 0.3
YP.117 leaf pure methanol	11.17 ± 0.2	16.48 ± 0.3	22.35 ± 0.3	24.21 ± 0.2	24.58 ± 0.1
YP.117 leaf 0.5% acidified ethanol	3.44 ± 0.1	9.87 ± 0.0	11.36 ± 0.2	24.66 ± 0.0	19.28 ± 0.3
YP.117 leaf 1% acidified ethanol	5.23 ± 0.2	5.48 ± 0.1	14.20 ± 0.2	14.35 ± 0.2	14.05 ± 0.2
YP.117 leaf hexane	8.67 ± 0.1	20.63 ± 0.4	30.64 ± 0.3	36.32 ± 0.2	38.57 ± 0.3
YP.141 leaf pure methanol	14.79 ± 0.2	30.1 ± 0.2	35.43 ± 0.2	38.53 ± 0.3	35.56 ± 0.1
YP.141 leaf 0.5% acidified ethanol	2.09 ± 0.1	2.64 ± 0.1	6.43 ± 0.2	8.37 ± 0.2	8.74 ± 0.1
YP.141 leaf 1% acidified ethanol	1.20 ± 0.1	5.53 ± 0.1	10.31 ± 0.1	11.96 ± 0.0	14.80 ± 0.2
YP.141 leaf hexane	6.13 ± 0.1	11.36 ± 0.2	13.49 ± 0.1	17.04 ± 0.3	19.73 ± 0.1
YP.188 leaf pure methanol	16.84 ± 0.1	27.38 ± 0.2	31.63 ± 0.3	37.67 ± 0.3	44.39 ± 0.2
YP.188 leaf 0.5% acidified ethanol	2.54 ± 0.0	6.28 ± 0.1	8.07 ± 0.2	11.96 ± 0.0	17.32 ± 0.3
YP.188 leaf 1% acidified ethanol	2.69 ± 0.1	6.88 ± 0.1	10.91 ± 0.1	16.89 ± 0.1	16.35 ± 0.3
YP.188 leaf hexane	13.15 ± 0.1	28.10 ± 0.2	42.75 ± 0.2	50.37 ± 0.1	42.75 ± 0.3
EDTA	3.30 ± 0.0	25.93 ± 0.1	64.18 ± 0.2	91.40 ± 0.1	92.26 ± 0.1

*It represents the concentrations of the solutions prepared by taking 50, 100, 150, 250, and 500 µL of standard and extract stock solutions prepared as 1 mg/mL and completing the total volume of 3 mL

In addition, weak chelating activity was detected in the 0.5 and 1% acidified ethanol extracts of kumquat leaves and the hexane solvent extracts. The highest activity (50.37%) was found in 62.5 µg/mL concentration of the extract obtained from kumquat leaves with a hexane solvent. We determined no correlation between the chelating activity of the extracts and their concentration. No significant difference was found between the rootstock kumquat type and its hybrids.

When we evaluated all the activities, we concluded that the extracts obtained from kumquat fruits and leaves were not good at chelating iron (II) ions. The most important feature that affects the metal chelating activity depends on the functional groups in the structure of phenolic compounds and the position and amount of these functional groups. For this reason, the difference in the chelating activity of the samples can be explained by different amounts of phenolic substances, as well as phenolic substance groups in different structures and positions [32].

The reducing capacity of the extracts. The reducing agent in the environment reduces Fe^{3+} ions to Fe^{2+} ions depending on its antioxidant capacity. The absorbance of the Prussian blue complex ($Fe_4[Fe(CN)_6]$) formed by adding $FeCl_3$ to the reduced product is measured at 700 nm [22]. The increase in absorbance of the reaction mixture is directly proportional to the reducing power of the sample.

We found that the capacity of kumquat leaves to reduce Fe^{3+} ions was higher than that of lyophilized and wet kumquat fruits (Table 3). We observed that lyophilizing and drying of kumquat fruits did not cause a significant change in their reducing capacity. The reducing capacity of the fruit and leaf extracts was lower than the standards (BHA, BHT and α-tocopherol).

Table 3 The reducing power of extracts and standards, µg/mL (mean ± SD of triplicate)

Extracts and standards	5.88*	14.7*	29.41*
Rootstock fresh fruit pure methanol	0.104 ± 0.001	0.115 ± 0.003	0.138 ± 0.002
Rootstock fresh fruit 80% methanol	0.105 ± 0.002	0.106 ± 0.001	0.124 ± 0.001
Rootstock fresh fruit 60% methanol	0.120 ± 0.001	0.133 ± 0.001	0.140 ± 0.003
Rootstock fresh fruit 50% methanol	0.096 ± 0.001	0.100 ± 0.002	0.104 ± 0.001
Rootstock fresh fruit pure water	0.082 ± 0.002	0.098 ± 0.003	0.115 ± 0.001
Rootstock dry fruit pure methanol	0.075 ± 0.001	0.082 ± 0.003	0.094 ± 0.001
Rootstock dry fruit 80% methanol	0.074 ± 0.002	0.087 ± 0.006	0.097 ± 0.005
Rootstock dry fruit 60% methanol	0.076 ± 0.001	0.081 ± 0.001	0.089 ± 0.001
Rootstock dry fruit 50% methanol	0.076 ± 0.002	0.082 ± 0.001	0.089 ± 0.003
Rootstock dry fruit pure water	0.078 ± 0.003	0.081 ± 0.001	0.089 ± 0.001
Rootstock leaf pure methanol	0.103 ± 0.002	0.145 ± 0.001	0.241 ± 0.004
Rootstock leaf 80% methanol	0.098 ± 0.001	0.149 ± 0.001	0.227 ± 0.003
Rootstock leaf 60% methanol	0.093 ± 0.001	0.136 ± 0.005	0.218 ± 0.003
Rootstock leaf 50% methanol	0.097 ± 0.002	0.148 ± 0.001	0.240 ± 0.003
Rootstock leaf pure water	0.089 ± 0.001	0.143 ± 0.003	0.209 ± 0.005
Rootstock leaf 0.5% acidified ethanol	0.074 ± 0.001	0.096 ± 0.002	0.128 ± 0.001
Rootstock leaf 1% acidified ethanol	0.076 ± 0.001	0.098 ± 0.003	0.129 ± 0.001
Rootstock leaf hexane	0.091 ± 0.002	0.125 ± 0.003	0.179 ± 0.002
EP.4 fresh fruit pure methanol	0.111 ± 0.002	0.144 ± 0.001	0.199 ± 0.001
EP.4 fresh fruit 80% methanol	0.108 ± 0.001	0.110 ± 0.003	0.100 ± 0.001
EP.4 fresh fruit 60% methanol	0.104 ± 0.002	0.095 ± 0.003	0.112 ± 0.001
EP.4 fresh fruit 50% methanol	0.099 ± 0.003	0.092 ± 0.001	0.143 ± 0.001
EP.4 fresh fruit pure water	0.086 ± 0.001	0.093 ± 0.001	0.115 ± 0.002
EP.4 dry fruit pure methanol	0.070 ± 0.001	0.077 ± 0.001	0.091 ± 0.001
EP.4 dry fruit 80% methanol	0.071 ± 0.002	0.078 ± 0.001	0.089 ± 0.003
EP.4 dry fruit 60% methanol	0.074 ± 0.001	0.076 ± 0.001	0.087 ± 0.003
EP.4 dry fruit 50% methanol	0.071 ± 0.001	0.075 ± 0.003	0.085 ± 0.001
EP.4 dry fruit pure water	0.070 ± 0.002	0.072 ± 0.001	0.081 ± 0.001
EP.4 leaf pure methanol	0.087 ± 0.002	0.134 ± 0.004	0.201 ± 0.001
EP.4 leaf 80% methanol	0.097 ± 0.001	0.145 ± 0.003	0.245 ± 0.004
EP.4 leaf 60% methanol	0.093 ± 0.003	0.139 ± 0.001	0.211 ± 0.003
EP.4 leaf 50% methanol	0.091 ± 0.002	0.149 ± 0.003	0.227 ± 0.005
EP.4 leaf pure water	0.116 ± 0.001	0.193 ± 0.003	0.307 ± 0.001
EP.4 leaf 0.5% acidified ethanol	0.075 ± 0.002	0.093 ± 0.001	0.125 ± 0.001
EP.4 leaf 1% acidified ethanol	0.079 ± 0.001	0.102 ± 0.002	0.133 ± 0.006
EP.4 leaf hexane	0.091 ± 0.003	0.125 ± 0.001	0.179 ± 0.001
EP.29 fresh fruit pure methanol	0.107 ± 0.004	0.118 ± 0.004	0.135 ± 0.006
EP.29 fresh fruit 80% methanol	0.107 ± 0.001	0.114 ± 0.002	0.108 ± 0.002
EP.29 fresh fruit 60% methanol	0.109 ± 0.000	0.109 ± 0.000	0.138 ± 0.000
EP.29 fresh fruit 50% methanol	0.113 ± 0.000	0.117 ± 0.001	0.133 ± 0.000
EP.29 fresh fruit pure water	0.086 ± 0.001	0.092 ± 0.000	0.100 ± 0.001
EP.29 dry fruit pure methanol	0.072 ± 0.000	0.081 ± 0.001	0.098 ± 0.000
EP.29 dry fruit 80% methanol	0.073 ± 0.000	0.080 ± 0.001	0.093 ± 0.000
EP.29 dry fruit 60% methanol	0.072 ± 0.001	0.077 ± 0.001	0.090 ± 0.001
EP.29 dry fruit 50% methanol	0.071 ± 0.001	0.078 ± 0.000	0.088 ± 0.000
EP.29 dry fruit pure water	0.073 ± 0.000	0.076 ± 0.001	0.090 ± 0.000
EP.29 leaf pure methanol	0.090 ± 0.000	0.125 ± 0.001	0.206 ± 0.002
EP.29 leaf 80% methanol	0.093 ± 0.000	0.145 ± 0.001	0.236 ± 0.000
EP.29 leaf 60% methanol	0.106 ± 0.001	0.158 ± 0.000	0.260 ± 0.000
EP.29 leaf 50% methanol	0.103 ± 0.000	0.163 ± 0.000	0.281 ± 0.000
EP.29 leaf pure water	0.101 ± 0.000	0.158 ± 0.001	0.244 ± 0.000
EP.29 leaf 0.5% acidified ethanol	0.086 ± 0.000	0.103 ± 0.001	0.135 ± 0.000
EP.29 leaf 1% acidified ethanol	0.077 ± 0.001	0.094 ± 0.001	0.119 ± 0.001
EP.29 leaf hexane	0.088 ± 0.000	0.136 ± 0.000	0.193 ± 0.000
EP.31 fresh fruit pure methanol	0.091 ± 0.001	0.098 ± 0.001	0.109 ± 0.001
EP.31 fresh fruit 80% methanol	0.087 ± 0.000	0.095 ± 0.000	0.117 ± 0.000
EP.31 fresh fruit 60% methanol	0.081 ± 0.000	0.103 ± 0.001	0.129 ± 0.001

Continuation of Table 3

Extracts and standards	5.88*	14.7*	29.41*
EP.31 fresh fruit 50% methanol	0.089 ± 0.000	0.115 ± 0.001	0.104 ± 0.000
EP.31 fresh fruit pure water	0.088 ± 0.001	0.094 ± 0.000	0.105 ± 0.001
EP.31 dry fruit pure methanol	0.093 ± 0.000	0.099 ± 0.000	0.125 ± 0.000
EP.31 dry fruit 80% methanol	0.095 ± 0.000	0.102 ± 0.000	0.099 ± 0.000
EP.31 dry fruit 60% methanol	0.099 ± 0.000	0.085 ± 0.001	0.096 ± 0.001
EP.31 dry fruit 50% methanol	0.099 ± 0.000	0.092 ± 0.001	0.097 ± 0.000
EP.31 dry fruit pure water	0.107 ± 0.000	0.100 ± 0.001	0.111 ± 0.001
EP.31 leaf pure methanol	0.089 ± 0.001	0.119 ± 0.001	0.176 ± 0.001
EP.31 leaf 80% methanol	0.093 ± 0.000	0.133 ± 0.001	0.200 ± 0.000
EP.31 leaf 60% methanol	0.101 ± 0.001	0.148 ± 0.001	0.214 ± 0.000
EP.31 leaf 50% methanol	0.100 ± 0.001	0.142 ± 0.000	0.212 ± 0.001
EP.31 leaf pure water	0.094 ± 0.001	0.133 ± 0.000	0.206 ± 0.001
EP.31 leaf 0.5% acidified ethanol	0.089 ± 0.000	0.127 ± 0.000	0.184 ± 0.000
EP.31 leaf 1% acidified ethanol	0.088 ± 0.000	0.113 ± 0.001	0.155 ± 0.000
EP.31 leaf hexane	0.098 ± 0.001	0.119 ± 0.000	0.202 ± 0.001
YP.117 fresh fruit pure methanol	0.099 ± 0.001	0.117 ± 0.000	0.153 ± 0.000
YP.117 fresh fruit 80% methanol	0.096 ± 0.000	0.099 ± 0.000	0.117 ± 0.000
YP.117 fresh fruit 60% methanol	0.100 ± 0.000	0.100 ± 0.001	0.114 ± 0.000
YP.117 fresh fruit 50% methanol	0.107 ± 0.000	0.116 ± 0.001	0.142 ± 0.000
YP.117 fresh fruit pure water	0.088 ± 0.000	0.094 ± 0.000	0.114 ± 0.000
YP.117 dry fruit pure methanol	0.077 ± 0.000	0.082 ± 0.000	0.108 ± 0.001
YP.117 dry fruit 80% methanol	0.074 ± 0.000	0.079 ± 0.001	0.085 ± 0.000
YP.117 dry fruit 60% methanol	0.081 ± 0.000	0.088 ± 0.001	0.093 ± 0.000
YP.117 dry fruit 50% methanol	0.085 ± 0.001	0.080 ± 0.000	0.087 ± 0.000
YP.117 dry fruit pure water	0.079 ± 0.000	0.083 ± 0.000	0.089 ± 0.000
YP.117 leaf pure methanol	0.092 ± 0.001	0.141 ± 0.001	0.206 ± 0.000
YP.117 leaf 80% methanol	0.093 ± 0.000	0.133 ± 0.001	0.201 ± 0.000
YP.117 leaf 60% methanol	0.101 ± 0.001	0.157 ± 0.000	0.235 ± 0.001
YP.117 leaf 50% methanol	0.109 ± 0.001	0.159 ± 0.001	0.262 ± 0.001
YP.117 leaf pure water	0.105 ± 0.000	0.152 ± 0.000	0.242 ± 0.000
YP.117 leaf 0.5% acidified ethanol	0.091 ± 0.000	0.116 ± 0.001	0.165 ± 0.000
YP.117 leaf 1% acidified ethanol	0.087 ± 0.001	0.113 ± 0.001	0.163 ± 0.001
YP.117 leaf hexane	0.072 ± 0.000	0.091 ± 0.000	0.154 ± 0.000
YP.141 fresh fruit pure methanol	0.096 ± 0.001	0.104 ± 0.000	0.124 ± 0.000
YP.141 fresh fruit 80% methanol	0.091 ± 0.000	0.091 ± 0.001	0.105 ± 0.000
YP.141 fresh fruit 60% methanol	0.146 ± 0.000	0.138 ± 0.001	0.139 ± 0.000
YP.141 fresh fruit 50% methanol	0.092 ± 0.000	0.103 ± 0.001	0.142 ± 0.000
YP.141 fresh fruit pure water	0.091 ± 0.000	0.099 ± 0.000	0.117 ± 0.001
YP.141 dry fruit pure methanol	0.092 ± 0.001	0.091 ± 0.001	0.102 ± 0.000
YP.141 dry fruit 80% methanol	0.102 ± 0.000	0.105 ± 0.001	0.120 ± 0.000
YP.141 dry fruit 60% methanol	0.093 ± 0.000	0.090 ± 0.001	0.097 ± 0.000
YP.141 dry fruit 50% methanol	0.097 ± 0.001	0.088 ± 0.001	0.095 ± 0.000
YP.141 dry fruit pure water	0.094 ± 0.001	0.087 ± 0.000	0.098 ± 0.000
YP.141 leaf pure methanol	0.105 ± 0.000	0.155 ± 0.000	0.241 ± 0.001
YP.141 leaf 80% methanol	0.108 ± 0.000	0.165 ± 0.001	0.254 ± 0.000
YP.141 leaf 60% methanol	0.100 ± 0.000	0.154 ± 0.001	0.250 ± 0.000
YP.141 leaf 50% methanol	0.106 ± 0.001	0.162 ± 0.000	0.252 ± 0.002
YP.141 leaf pure water	0.101 ± 0.000	0.141 ± 0.000	0.247 ± 0.001
YP.141 leaf 0.5% acidified ethanol	0.088 ± 0.000	0.123 ± 0.001	0.186 ± 0.001
YP.141 leaf 1% acidified ethanol	0.082 ± 0.000	0.108 ± 0.000	0.148 ± 0.000
YP.141 leaf hexane	0.070 ± 0.001	0.102 ± 0.000	0.162 ± 0.000
YP.188 fresh fruit pure methanol	0.092 ± 0.001	0.111 ± 0.000	0.146 ± 0.000
YP.188 fresh fruit 80% methanol	0.094 ± 0.000	0.107 ± 0.001	0.136 ± 0.001
YP.188 fresh fruit 60% methanol	0.090 ± 0.000	0.104 ± 0.001	0.123 ± 0.000
YP.188 fresh fruit 50% methanol	0.095 ± 0.000	0.096 ± 0.001	0.112 ± 0.000
YP.188 fresh fruit pure water	0.099 ± 0.000	0.103 ± 0.000	0.126 ± 0.000
YP.188 dry fruit pure methanol	0.090 ± 0.001	0.086 ± 0.000	0.110 ± 0.000

Continuation of Table 3

Extracts and standards	5.88*	14.7*	29.41*
YP.188 dry fruit 80% methanol	0.091 ± 0.000	0.088 ± 0.000	0.094 ± 0.001
YP.188 dry fruit 60% methanol	0.089 ± 0.001	0.087 ± 0.000	0.098 ± 0.000
YP.188 dry fruit 50% methanol	0.092 ± 0.000	0.094 ± 0.001	0.099 ± 0.000
YP.188 dry fruit pure water	0.093 ± 0.000	0.087 ± 0.001	0.100 ± 0.000
YP.188 leaf pure methanol	0.102 ± 0.000	0.182 ± 0.001	0.252 ± 0.001
YP.188 leaf 80% methanol	0.115 ± 0.001	0.164 ± 0.000	0.263 ± 0.000
YP.188 leaf 60% methanol	0.116 ± 0.000	0.176 ± 0.000	0.279 ± 0.000
YP.188 leaf 50% methanol	0.109 ± 0.001	0.159 ± 0.000	0.253 ± 0.001
YP.188 leaf pure water	0.111 ± 0.000	0.169 ± 0.000	0.271 ± 0.000
YP.188 leaf 0.5% acidified ethanol	0.098 ± 0.000	0.157 ± 0.001	0.218 ± 0.0001
YP.188 leaf 1% acidified ethanol	0.088 ± 0.000	0.110 ± 0.001	0.147 ± 0.000
YP.188 leaf hexane	0.076 ± 0.001	0.102 ± 0.001	0.167 ± 0.001
BHA	0.690 ± 0.001	1.346 ± 0.000	1.984 ± 0.000
BHT	0.504 ± 0.000	0.939 ± 0.000	1.290 ± 0.002
α-tokeferol	0.234 ± 0.000	0.477 ± 0.001	0.872 ± 0.000

*It represents the concentrations of the solutions prepared by taking 100, 250, and 500 µL of standard and extract stock solutions prepared as 1 mg/mL and completing the total volume of 3.750 µmL

The highest reducing capacity (0.307 ± 0.001) was observed at a concentration of 29.41 µg/mL of the EP.4 mutant leaf extract obtained with pure water. Among the fruits, the highest reducing capacity (0.199 ± 0.001) was found at a concentration of 29.41 µg/mL of the EP.4 hybrid wet fruit extract obtained with pure methanol. The reducing capacities of the standards were 1.984 ± 0.001, 1.290 ± 0.002, 0.872 ± 0.001 for BHA, BHT, and α-toceferol, respectively, at the highest concentration of 29.41 µg/mL.

No significant difference was observed between the rootstock kumquat plant and its mutants. Although the reducing power is an important factor of antioxidant activity, in our study, the reducing power was lower in the extracts with high antioxidant activity. Other studies also show that extracts with high antioxidant activity may have low reducing power [33, 34]. This is because in the systems where free iron ions are present in trace amounts, the net oxidation rate increases with the Fenton reaction. Substances with high reducing power may cause further acceleration of oxidation by reducing Fe(III) to Fe(II). The presence of trace levels of iron ions in kumquat materials may have caused its low reducing power and ncreased antioxidant activity [35].

Phenolic and flavonoid content. Since phenolic and flavonoid compounds contain hydroxyl groups in their structures and can easily give a hydrogen radical in hydroxyl groups, they have free radical quenching properties. Therefore, it is important to know the total phenolic and flavonoid contents of the samples to determine their contribution to the antioxidant activity, including radical scavenging activity tests. For this, we used the Folin-Ciocalteu method, a standard method in antioxidant studies. The basis of the method is that phenolic compounds dissolved in water and other organic solvents form a colored complex with a Folin reagent in an alkaline medium. The total phenolic content of the extracts obtained by Soxhlet extraction with different solvents was calculated using the regression equation (y = 0.0292x + 0.0749 and

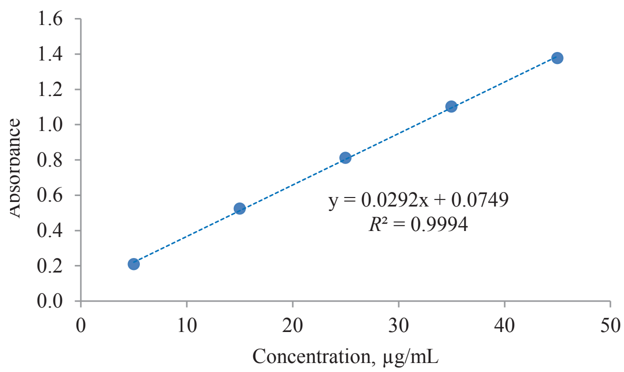

Figure 1 Standard calibration curve of gallic acid to determine total phenolic content

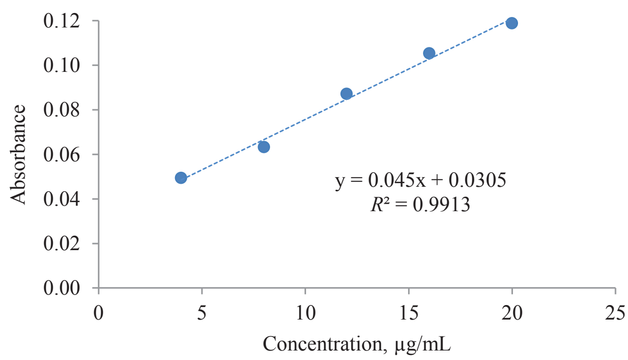

Figure 2 Calibration curve of standard quercetin to determine total flavonoid content

Table 4 Total phenolic and total flavonoid contents in kumquat fruit and leaf extracts

Extracts	Total Phenolic Substance, mg GAE/g extract	Total Flavonoid Substance, mg QUE/g extract
Rootstock fresh fruit pure methanol	16.096 ± 0.045	42.222 ± 0.018
Rootstock fresh fruit 80% methanol	8.432 ± 0.024	24.444 ± 0.014
Rootstock fresh fruit 60% methanol	5.808 ± 0.012	22.222 ± 0.012
Rootstock fresh fruit 50% methanol	7.089 ± 0.018	26.667 ± 0.018
Rootstock fresh fruit pure water	13.747 ± 0.011	41.111 ± 0.020
Rootstock dry fruit pure methanol	8.959 ± 0.038	46.667 ± 0.016
Rootstock dry fruit 80% methanol	9.856 ± 0.033	10.022 ± 0.010
Rootstock dry fruit 60% methanol	5.829 ± 0.011	10.100 ± 0.012
Rootstock dry fruit 50% methanol	5.425 ± 0.010	5.556 ± 0.011
Rootstock dry fruit pure water	3.705 ± 0.011	14.444 ± 0.016
Rootstock leaf pure methanol	66.356 ± 0.034	454.444 ± 0.046
Rootstock leaf 80% methanol	72.548 ± 0.021	258.889 ± 0.024
Rootstock leaf 60% methanol	68.979 ± 0.023	213.333 ± 0.034
Rootstock leaf 50% methanol	67.096 ± 0.018	248.889 ± 0.032
Rootstock leaf pure water	54.062 ± 0.023	174.444 ± 0.024
Rootstock leaf 0.5% acidified ethanol	31.925 ± 0.030	314.444 ± 0.042
Rootstock leaf 1% acidified ethanol	31.062 ± 0.018	308.889 ± 0.014
Rootstock leaf hexane	n.d.	n.d.
EP.4 fresh fruit pure methanol	20.281 ± 0.013	67.778 ± 0.026
EP.4 fresh fruit 80% methanol	8.678 ± 0.025	32.222 ± 0.024
EP.4 fresh fruit 60% methanol	5.479 ± 0.012	26.667 ± 0.018
EP.4 fresh fruit 50% methanol	7.760 ± 0.021	35.556 ± 0.012
EP.4 fresh fruit pure water	7.534 ± 0.011	25.556 ± 0.010
EP.4 dry fruit pure methanol	11.247 ± 0.013	25.556 ± 0.014
EP.4 dry fruit 80% methanol	11.315 ± 0.022	16.667 ± 0.016
EP.4 dry fruit 60% methanol	14.288 ± 0.023	27.778 ± 0.022
EP.4 dry fruit 50% methanol	9.137 ± 0.014	30.000 ± 0.023
EP.4 dry fruit pure water	7.521 ± 0.021	23.333 ± 0.024
EP.4 leaf pure methanol	63.438 ± 0.015	410.000 ± 0.032
EP.4 leaf 80% methanol	64.797 ± 0.017	271.111 ± 0.023
EP.4 leaf 60% methanol	64.685 ± 0.010	231.111 ± 0.023
EP.4 leaf 50% methanol	65.568 ± 0.022	248.889 ± 0.023
EP.4 leaf pure water	73.034 ± 0.015	255.556 ± 0.023
EP.4 leaf 0.5% acidified ethanol	33.068 ± 0.032	315.556 ± 0.023
EP.4 leaf 1% acidified ethanol	33.952 ± 0.014	355.556 ± 0.023
EP.4 leaf hexane	n.d.	n.d.
EP.29 fresh fruit pure methanol	14.596 ± 0.011	42.222 ± 0.023
EP.29 fresh fruit 80% methanol	8.884 ± 0.021	31.111 ± 0.023
EP.29 fresh fruit 60% methanol	8.842 ± 0.021	20.000 ± 0.023
EP.29 fresh fruit 50% methanol	11.534 ± 0.018	30.000 ± 0.023
EP.29 fresh fruit pure water	13.404 ± 0.016	21.111 ± 0.023
EP.29 dry fruit pure methanol	12.404 ± 0.012	65.556 ± 0.023
EP.29 dry fruit 80% methanol	12.918 ± 0.012	16.667 ± 0.023
EP.29 dry fruit 60% methanol	9.623 ± 0.018	26.667 ± 0.023
EP.29 dry fruit 50% methanol	9.747 ± 0.017	21.111 ± 0.023
EP.29 dry fruit pure water	7.205 ± 0.013	13.333 ± 0.023
EP.29 leaf pure methanol	60.836 ± 0.022	438.889 ± 0.023
EP.29 leaf 80% methanol	67.589 ± 0.032	223.333 ± 0.023
EP.29 leaf 60% methanol	70.226 ± 0.043	256.667 ± 0.023
EP.29 leaf 50% methanol	64.822 ± 0.023	268.889 ± 0.023
EP.29 leaf pure water	50.390 ± 0.013	184.444 ± 0.023
EP.29 leaf 0.5% acidified ethanol	41.158 ± 0.011	486.667 ± 0.023
EP.29 leaf 1% acidified ethanol	25.856 ± 0.033	242.222 ± 0.023
EP.29 leaf hexane	n.d.	n.d.
EP.31 fresh fruit pure methanol	6.384 ± 0.014	38.889 ± 0.023
EP.31 fresh fruit 80% methanol	9.952 ± 0.012	20.000 ± 0.023

Continuation of Table 4

Extracts	Total Phenolic Substance, mg GAE/g extract	Total Flavonoid Substance, mg QUE/g extract
EP.31 fresh fruit 60% methanol	17.500 ± 0.023	42.222 ± 0.023
EP.31 fresh fruit 50% methanol	5.822 ± 0.023	14.444 ± 0.023
EP.31 fresh fruit pure water	8.164 ± 0.013	27.778 ± 0.023
EP.31 dry fruit pure methanol	12.212 ± 0.015	105.556 ± 0.023
EP.31 dry fruit 80% methanol	7.452 ± 0.028	25.556 ± 0.023
EP.31 dry fruit 60% methanol	7.767 ± 0.026	23.333 ± 0.023
EP.31 dry fruit 50% methanol	7.486 ± 0.024	26.667 ± 0.023
EP.31 dry fruit pure water	6.568 ± 0.022	13.333 ± 0.023
EP.31 leaf pure methanol	61.973 ± 0.022	450.000 ± 0.023
EP.31 leaf 80% methanol	64.739 ± 0.018	284.444 ± 0.023
EP.31 leaf 60% methanol	74.082 ± 0.020	260.000 ± 0.023
EP.31 leaf 50% methanol	72.363 ± 0.014	281.111 ± 0.023
EP.31 leaf pure water	50.274 ± 0.024	180.000 ± 0.023
EP.31 leaf 0.5% acidified ethanol	47.699 ± 0.010	454.444 ± 0.023
EP.31 leaf 1% acidified ethanol	43.603 ± 0.018	632.222 ± 0.033
EP.31 leaf hexane	n.d.	n.d.
YP.117 fresh fruit pure methanol	13.322 ± 0.022	36.667 ± 0.023
YP.117 fresh fruit 80% methanol	8.527 ± 0.012	16.667 ± 0.023
YP.117 fresh fruit 60% methanol	8.486 ± 0.014	17.778 ± 0.023
YP.117 fresh fruit 50% methanol	7.349 ± 0.022	158.889 ± 0.023
YP.117 fresh fruit pure water	8.308 ± 0.018	112.222 ± 0.023
YP.117 dry fruit pure methanol	9.445 ± 0.012	36.667 ± 0.023
YP.117 dry fruit 80% methanol	8.822 ± 0.010	16.667 ± 0.023
YP.117 dry fruit 60% methanol	7.705 ± 0.016	17.778 ± 0.023
YP.117 dry fruit 50% methanol	6.986 ± 0.020	158.889 ± 0.023
YP.117 dry fruit pure water	5.740 ± 0.018	112.222 ± 0.023
YP.117 leaf pure methanol	65.356 ± 0.016	458.889 ± 0.023
YP.117 leaf 80% methanol	70.205 ± 0.014	194.444 ± 0.023
YP.117 leaf 60% methanol	68.514 ± 0.023	298.889 ± 0.023
YP.117 leaf 50% methanol	65.616 ± 0.022	285.556 ± 0.023
YP.117 leaf pure water	55.425 ± 0.020	248.889 ± 0.023
YP.117 leaf 0.5% acidified ethanol	43.603 ± 0.016	312.222 ± 0.023
YP.117 leaf 1% acidified ethanol	41.205 ± 0.022	381.111 ± 0.023
YP.117 leaf hexane	n.d.	n.d.
YP.141 fresh fruit pure methanol	9.342 ± 0.022	313.333 ± 0.023
YP.141 fresh fruit 80% methanol	7.630 ± 0.020	40.000 ± 0.023
YP.141 fresh fruit 60% methanol	10.740 ± 0.014	40.000 ± 0.023
YP.141 fresh fruit 50% methanol	9.164 ± 0.018	31.111 ± 0.023
YP.141 fresh fruit pure water	8.432 ± 0.012	27.778 ± 0.023
YP.141 dry fruit pure methanol	15.637 ± 0.020	97.778 ± 0.023
YP.141 dry fruit 80% methanol	9.089 ± 0.022	26.667 ± 0.023
YP.141 dry fruit 60% methanol	10.918 ± 0.018	50.000 ± 0.023
YP.141 dry fruit 50% methanol	8.295 ± 0.014	55.556 ± 0.023
YP.141 dry fruit pure water	6.144 ± 0.022	26.667 ± 0.023
YP.141 leaf pure methanol	72.342 ± 0.023	564.444 ± 0.023
YP.141 leaf 80% methanol	76.658 ± 0.010	387.778 ± 0.023
YP.141 leaf 60% methanol	64.322 ± 0.022	354.444 ± 0.023
YP.141 leaf 50% methanol	63.767 ± 0.016	357.778 ± 0.023
YP.141 leaf pure water	60.082 ± 0.014	305.556 ± 0.023
YP.141 leaf 0.5% acidified ethanol	51.048 ± 0.012	470.000 ± 0.023
YP.141 leaf 1% acidified ethanol	32.329 ± 0.012	300.000 ± 0.023
YP.141 leaf hexane	n.d.	n.d.
YP.188 fresh fruit pure methanol	11.336 ± 0.010	111.111 ± 0.023
YP.188 fresh fruit 80% methanol	8.993 ± 0.012	87.778 ± 0.023
YP.188 fresh fruit 60% methanol	9.986 ± 0.008	86.667 ± 0.023
YP.188 fresh fruit 50% methanol	8.979 ± 0.016	104.444 ± 0.023
YP.188 fresh fruit pure water	20.144 ± 0.022	102.222 ± 0.023

Continuation of Table 4

Extracts	Total Phenolic Substance, mg GAE/g extract	Total Flavonoid Substance, mg QUE/g extract
YP.188 dry fruit pure methanol	9.151 ± 0.014	15.556 ± 0.023
YP.188 dry fruit 80% methanol	8.212 ± 0.028	16.667 ± 0.023
YP.188 dry fruit 60% methanol	7.048 ± 0.014	21.111 ± 0.023
YP.188 dry fruit 50% methanol	7.021 ± 0.012	38.889 ± 0.023
YP.188 dry fruit pure water	5.418 ± 0.008	26.667 ± 0.023
YP.188 leaf pure methanol	72.637 ± 0.010	446.667 ± 0.023
YP.188 leaf 80% methanol	85.651 ± 0.030	330.000 ± 0.023
YP.188 leaf 60% methanol	86.329 ± 0.022	345.556 ± 0.023
YP.188 leaf 50% methanol	75.418 ± 0.022	300.000 ± 0.023
YP.188 leaf pure water	70.849 ± 0.018	313.333 ± 0.023
YP.188 leaf 0.5% acidified ethanol	62.890 ± 0.020	582.222 ± 0.023
YP.188 leaf 1% acidified ethanol	33.226 ± 0.018	275.556 ± 0.023
YP.188 leaf hexane	n.d.	n.d.

n.d.: not detected

R^2 = 0.9994) of the calibration line of the standard gallic acid solution prepared in the concentration range of 5–50 μg/mL and expressed as gallic acid equivalent (mg GAE/g extract). The gallic acid standard curve is shown in Fig. 1. We found that the kumquat leaf extracts had the highest total phenolic content (Table 4). In particular, the highest total phenolic content (86.329 ± 0.022 mg GAE/g extract) was in the YP.188 mutant extract obtained with 60% methanol. In the fruit samples, the highest total phenolic content (20.281 mg GAE/g extract) was found in the EP.4 mutant extract obtained with pure methanol. There was no significant difference in total phenolic contents between the fresh and dried fruit samples.

Lou *et al.* compared total phenolic contents in fresh and dried kumquat fruits [36]. The scientists investigated changes in total phenolic matter by changing the drying degree and time. They found that the total amount of phenolic substances increased with drying, amounting to 15–17 mg GAE/g extract and 48–50 mg GAE/g extract in fresh and dried fruit (130°C), respectively [36].

In another study, Özcan *et al.* dried kumquat fruit in hot air, under vacuum, and in a microwave oven [27]. The authors found that the total phenolic content of hot air-dried fruit was approximately 5 mg GAE/g extract, but with other drying methods, it varied in the range of 25–30 mg GAE/g extract [37].

Yıldız Turgut *et al.* studied the functional quality parameters of the powder obtained from Fortunella margarita kumquat varieties grown in Turkey. They reported the total phenolic content of kumquat between 2.62 ± 0.051 – 6.97 ± 0.053 mg GAE/g depending on the type of drying method [38].

Having determined the total phenolic content, we measured the total flavonoid content of the samples. Total flavonoid concentration was determined colorimetrically using a UV spectrophotometer according to the method applied by Zhishen *et al.* [27].

In our study, quercetin was used as a standard and the results were calculated as quercetin equivalent (mg QUE/g extract) from the quercetin standard calibration chart ($y = 0.0185x - 0.0019$ and $R^2 = 0.9666$) (Fig. 2). The highest amount of total flavonoid substance was seen in kumquat leaves (Table 4). In particular, the highest flavonoid content was found in the EP.31 mutant extract (632.222 ± 0.033 mg QUE/g extract) obtained with 1% acidified ethanol.

Among the fruit samples, the highest amount (313.333 ± 0.023 mg QUE/g extract) was found in the YP.141 mutant extract obtained with pure methanol. There were no significant differences between the total flavonoid amounts in the fresh and dried fruits.

Lou *et al.* reported that the total amount of flavonoid substance in kumquat varied between 58.23–91.42 mg/g depending on the drying temperature [36]. In another study, Lou *et al.* found that the total phenolic and flavonoid contents were higher in the extracts from kumquat and calamondin peel compared to fruit pulp, and that they were higher in the extracts from unripe kumquat compared to those from ripe kumquat [39, 40].

CONCLUSION

In antioxidant activity studies, it is common to use a different polarity solvent system in order to determine which compound types have the highest activity. There may be a relationship between phenolic or flavonoid amounts and antioxidant capacity determination methods. In particular, a relationship between methods such as the DPPH, which is based on radical capture, and total phenolic and flavonoid amounts may be important in some plant structures. Phenolic acids and flavonoids are soluble in polar solvents and show strong activity in polar systems.

In this study, we investigated the effect of different solvents and their concentrations on the bioactivity of kumquat fruit and leaf extracts. We found that the solvent type was extremely important for the extracts' bioactivity. In particular, the extraction performed with pure methanol in the fruits and 60 or 80% methanol in the leaves showed the highest total phenolic and flavonoid contents, the highest extraction efficiency (50.18–59.95%), and the highest antioxidant capacity.

We found no statistically significant difference between the total amount of phenolic/flavonoid substances and % inhibition value in the extraction performed with 60 and 80% methanol solutions. This shows that the amount of phenolic substances was affected by the polarity of the solvent, depending on the difference in phenolic compounds found in kumquat fruit and leaves. We concluded that phenolic components in the structure of a kumquat fruit could be extracted with a single solvent type, whereas those in the structure of a kumquat leaf could be extracted better with an aqueous solution of the relevant solvent, rather than a single solvent type.

We also observed that the aqueous solutions gave better results than the pure solutions in the production of phenolics from kumquat leaves, maximizing at certain water ratios and showing different distributions according to the solvents. These results can be explained by the fact that water increases diffusion by causing swelling in the leaf structure. In this context, methanol was the most effective solvent for bioactive component extraction from the kumquat fruits, whereas methanol + water was most effective for the leaves.

Having examined the effect of a solvent amount, we concluded that the extraction with 260 mL solvent ensured the highest total phenolic content, extraction efficiency, and antioxidant capacity. In addition, since methanol is a toxic solvent, it must be removed so that the obtained extract can be used in foods or consumed as a food supplement.

Plants are complex systems by nature and have multiple reaction characteristics and dissolution properties in different phases. Thus, it is not possible for a single method to reveal all of their radical sources or antioxidants [41–43]. For these reasons, we used a combination of methods, namely the DPPH, metal chelation, and iron reduction. In addition, we used the Folin-Ciocalteu method and the aluminum chloride method to determine the total phenol and flavonoid contents, respectively. The results clearly showed that the differences in the phenolic contents affected the plants' antioxidant properties.

We found that having a high phenolic content or high radical scavenging activity did not yield high results in all antioxidant activity studies. Thus, we concluded that determining the antioxidant activity with a single method was not the right approach and that it would be more accurate to simulate biochemical events in living systems by using a variety of methods. In summary, antioxidant structures can demonstrate their antioxidant activities by different mechanisms such as binding transition metal ions, breaking down peroxides, preventing hydrogen absorption, and removing radicals.

Our study revealed that the kumquat leaf extracts had a higher DPPH radical scavenging power than the fruit extracts. However, both the fruit and leaf extracts showed high levels of free radical scavenging activity with high antioxidant activity at a 125 μg/mL concentration. Due to high antioxidant activity, kumquat leaves can be recommended to be used as food, just as kumquat fruit, against many diseases – from gastrointestinal to infertility, from cardiovascular to respiratory and excretory disorders, especially to prevent cell damage caused by free radicals in human and animal bodies.

CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this manuscript.

ACKNOWLEDGMENTS

The authors are thankful to Mr. M. Murat HOCAGİL for his helpful contribution with the plant material collection.

REFERENCES

1. Patel S, Chaubey MK, Das I, Pandey VN. Review on bioactive and antioxidant potential of coloured fruits and vegetables. Journal of Drug Delivery and Therapeutics. 2019;9(2):433–441. https://doi.org/10.22270/jddt.v9i2.2371.
2. Nayak B, Liu RH, Tang J. Effect of processing on phenolic antioxidants of fruits, vegetables, and grains – A review. Critical Reviews in Food Science and Nutrition. 2015;55(7):887–918. https://doi.org/10.1080/10408398.2011.654142.
3. Suffredini IB, Sader HS, Gonçalves AG, Reis AO, Gales AC, Varella AD, et al. Screening of antibacterial extracts from plants native to the Brazilian Amazon Rain Forest and Atlantic Forest. Brazilian Journal of Medical and Biological Research. 2004;37(3):379–384. https://doi.org/10.1590/s0100-879x2004000300015.
4. Rahman K. Studies on free radicals, antioxidants, and co-factors. Clinical Interventions in Aging. 2007;2(2):219–226.
5. Jugde H, Nguy D, Moller I, Cooney JM, Atkinson RG. Isolation and characterization of a novel glycosyltransferase

that converts phloretin to phlorizin, a potent antioxidant in apple. FEBS Journal. 2008;275(15):3804–3814. https://doi.org/10.1111/j.1742-4658.2008.06526.x.

6. Monagas M, Gómez-Cordovés C, Bartolomeä B, Laureano O, Ricardo da Silva JM. Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. Cv. Graciano, tempranillo, and cabernet sauvignon. Journal of Agricultural and Food Chemistry. 2003;51(22):6475–6481. https://doi.org/10.1021/jf030325+.
7. Knekt P, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A, et al. Flavonoid intake and risk of chronic diseases. American Journal of Clinical Nutrition. 2002;76(3):560–568. https://doi.org/10.1093/ajcn/76.3.560.
8. Rossa SA, Ziskac DS, Zhaod K, ElSohlya MA. Variance of common flavonoids by brand of grapefruit juice. Fitoterapia. 2000;71(2):154–161. https://doi.org/10.1016/S0367-326X(99)00131-8.
9. Knekt P, Ritz J, Pereira MA, O'Reilly EJ, Augustsson K, Fraser GE, et al. Antioxidant vitamins and coronary heart disease risk: A pooled analysis of 9 cohorts. American Journal of Clinical Nutrition. 2004;80(6):1508–1520. https://doi.org/10.1093/ajcn/80.6.1508.
10. Craig WJ. Phytochemicals: guardians of our health. Journal of the American Dietetic Association. 1997;97(10):S199–S204. https://doi.org/10.1016/S0002-8223(97)00765-7.
11. Shofinita D, Feng S, Langrish TAG. Comparing yields from the extraction of different citrus peels and spray drying of the extracts. Advanced Powder Technology. 2015;26(6):1633–1638. https://doi.org/10.1016/J.APT.2015.09.007.
12. Habermann G, de Souza MC. History, ecology and challenges of citrus production in tropical and subtropical areas. In: Hayat K, editor. Citrus molecular phylogeny, antioxidant properties and medicinal uses. New York: Nova Publishers; 2014. pp. 1–12.
13. Faber B, Yesiloglu T, Eskalen A. Citrus production in Turkey. Citrograph. 2010:34–36.
14. Tercan E, Dereli MA. Development of a land suitability model for citrus cultivation using GIS and multi-criteria assessment techniques in Antalya province of Turkey. Ecological Indicators. 2020;117. https://doi.org/10.1016/j.ecolind.2020.106549.
15. Ozkan B, Akcaoz H, Karadeniz F. Energy requirement and economic analysis of citrus production in Turkey. Energy Conversion and Management. 2004;45(11–12):1821–1830. https://doi.org/10.1016/j.enconman.2003.10.002.
16. Yıldız Turgut D, Topuz A. Bioactive compounds and biological activities of kumquat (*Fortunella* spp.). Turkish Journal of Agriculture – Food Science and Technology. 2019;7(10):1581–1588. https://doi.org/10.24925/TURJAF.V7I10.1581-1588.2628.
17. Koyasako A, Bernhard RA. Volatile constituents of the essential oil of kumquat. Journal of Food Science. 1983;48(6):1807–1812. https://doi.org/10.1111/j.1365-2621.1983.tb05090.x.
18. Barreca D, Bellocco E, Caristi C, Leuzzi U, Gattuso G. Kumquat (*Fortunella japonica* Swingle) juice: Flavonoid distribution and antioxidant properties. Food Research International. 2011;44(7):2190–2197. https://doi.org/10.1016/j.foodres.2010.11.031.
19. Nouri A, Shafaghatlonbar A. Chemical constituents and antioxidant activity of essential oil and organic extract from the peel and kernel parts of *Citrus japonica* Thunb. (kumquat) from Iran. Natural Product Research. 2016;30(9):1093–1097. https://doi.org/10.1080/14786419.2015.1101692.
20. Schirra M, Palma A, D'Aquino S, Angioni A, Minello EV, Melis M, et al. Influence of postharvest hot water treatment on nutritional and functional properties of kumquat (*Fortunella japonica* Lour. Swingle Cv. Ovale) fruit. Journal of Agricultural and Food Chemistry. 2008;56(2):455–460. https://doi.org/10.1021/jf0714160.
21. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199–1200. https://doi.org/10.1038/1811199a0.
22. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. The Japanese Society of Nutrition and Dietetics. 1986;44(6):307–315. https://doi.org/10.5264/eiyogakuzashi.44.307.
23. Salgado P, Melin V, Contreras D, Moreno Y, Mansilla HD. Fenton reaction driven by iron ligands. Journal of the Chilean Chemical Society. 2013;58(4):2096–2101. https://doi.org/10.4067/S0717-97072013000400043.
24. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of Biochemistry and Biophysics. 1994;315(1):161–169. https://doi.org/10.1006/abbi.1994.1485.
25. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology. 1999;299:152–178. https://doi.org/10.1016/S0076-6879(99)99017-1.
26. Karadag A, Ozcelik B, Saner S. Review of methods to determine antioxidant capacities. Food Analytical Methods. 2009;2(1):41–60. https://doi.org/10.1007/s12161-008-9067-7.
27. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999;64(4):555–559. https://doi.org/10.1016/S0308-8146(98)

00102-2.

28. Fukumoto LR, Mazza G. Assessing antioxidant and prooxidant activities of phenolic compounds. Journal of Agricultural and Food Chemistry. 2000;48(8):3597–3604. https://doi.org/10.1021/jf000220w.
29. Oreopoulou A, Tsimogiannis D, Oreopoulou V. Extraction of polyphenols from aromatic and medicinal plants: an overview of the methods and the effect of extraction parameters. In: Watson RR, editor. Polyphenols in plants: Isolation, purification and extract preparation. Academic Press; 2019. pp. 243–259. https://doi.org/10.1016/B978-0-12-813768-0.00025-6.
30. Jayaprakasha G, Chidambara Murthy KN, Etlinger M, Mantur SM, Patil BS. Radical scavenging capacities and inhibition of human prostate (LNCaP) cell proliferation by *Fortunella margarita*. Food Chemistry. 2012;131(1):184–191. https://doi.org/10.1016/j.foodchem.2011.08.058.
31. Arora A, Nair MG, Strasburg GM. Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. Free Radical Biology and Medicine. 1988;24(9):1355–1363. https://doi.org/10.1016/s0891-5849(97)00458-9.
32. Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15(10):7313–7352. https://doi.org/10.3390/molecules15107313.
33. Yıldırım A, Oktay M, Bilaloğlu V. The antioxidant activity of the leaves of *Cydonia vulgaris*. Turkish Journal of Medical Sciences. 2001;31:23–27.
34. Yıldırım A, Mavi A, Oktay M, Kara AA, Algur ÖF, Bilaloğlu V. Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argentea Desf Ex Dc*), Sage (*Salvia triloba L.*), Black Tea (*Camellia sinensis*). Journal of Agricultural and Food Chemistry. 2000;48(10):5030–5034. https://doi.org/10.1021/jf000590k.
35. Mei L, Decker EA, McClements DJ. Evidence of iron association with emulsion droplets and its impact on lipid oxidation. Journal of Agricultural and Food Chemistry. 1998;46(12):5072–5077. https://doi.org/10.1021/jf9806661.
36. Lou S-N, Lai Y-C, Huang J-D, Ho C-T, Ferng L-HA, Chang Y-C. Drying effect on flavonoid composition and antioxidant activity of immature kumquat. Food Chemistry. 2015;171:356–363. https://doi.org/10.1016/j.foodchem.2014.08.119.
37. Ozcan-Sinir G, Ozkan-Karabacak A, Tamer CE, Copur OU. The effect of hot air, vacuum and microwave drying on drying characteristics, rehydration capacity, color, total phenolic content and antioxidant capacity of Kumquat (*Citrus japonica*). Food Science and Technology. 2018;39(2):475–484. https://doi.org/10.1590/fst.34417.
38. Turgut DY, Çınar O, Seçmen T. Determination of functional properties of kumquat (*Fortunella Margarita* Swing.) powders obtained by different methods. Gıda. 2019;44(4):605–617 (In Turkish). https://doi.org/10.15237/gida.GD18118.
39. Lou S-N, Lai Y-C, Hsu Y-S, Ho C-T. Phenolic content, antioxidant activity and effective compounds of kumquat extracted by different solvents. Food Chemistry. 2016;197:1–6. https://doi.org/10.1016/j.foodchem.2015.10.096.
40. Lou S-N, Ho C-T. Phenolic compounds and biological activities of small-size citrus: Kumquat and calamondin. Journal of Food and Drug Analysis. 2017;25(1):162–175. https://doi.org/10.1016/j.jfda.2016.10.024.
41. Wong SP, Leong LP, William Koh JH. Antioxidant activities of aqueous extracts of selected plants. Food Chemistry. 2006;99(4):775–783. https://doi.org/10.1016/j.foodchem.2005.07.058.
42. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants. 2017;6(4). https://doi.org/10.3390/plants6040042.
43. Abd Aziz NA, Hasham R, Sarmidi MR, Suhaimi SH, Idris MKH. A review on extraction techniques and therapeutic value of polar bioactives from Asian medicinal herbs: Case study on *Orthosiphon aristatus*, *Eurycoma longifolia* and *Andrographis paniculata*. Saudi Pharmaceutical Journal. 2021;29(2):143–165. https://doi.org/10.1016/j.jsps.2020.12.016.

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