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## Evaluating the antioxidant activity in different citrus genotypes

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### Abstract

Citrus fruit consumption per capita has steadily increased over the world over the last 30 years. Citrus fruits are high in vitamin C as well as other active ingredients like phenols and flavonoids that are beneficial to human health. Therefore, the amounts of total phenol, total flavonoids, and antioxidant activity in 12 different genotypes of the citrus have been investigated in the present study. These genotypes have been selected from four citruses, including Lemon, Kiwi, Mandarin, and Orange. There are three genotypes for each of these citruses, and the numbering is done according to the place of harvesting the genotypes and also due to the ease of evaluation. Studies have been done for the leaves and fruits of the mentioned plant. Results showed that the average value of total phenol, flavonoid, and antioxidant activity in different citrus genotypes is 15.06 mg/g, 37.02 mg/g, and 75.54 µg/mL of dry extract, respectively. The extract of the fruit had the highest amount of phenol, and the leaf extract had the highest amount of flavonoids. The values of total phenol, total flavonoids, and antioxidant activity, from highest to lowest, belong to Kiwi, Orange, Lemon, and Mandarin groups.

**Keywords:** citrus genotypes; total phenol; total flavonoids; antioxidant activity.

**Practical Application:** It was tried to investigate the amounts of total phenol, total flavonoids, and antioxidant activity in different genotypes of the citrus.

### 1 Introduction

The use of plants for the treatment of diseases has a history dating back to human life. This knowledge, which is usually obtained by trial and error, has been passed down from generation to generation and has been passed on to modern man. At present, with the emergence of the side effects of chemical drugs, people are more interested in using medicinal plants. The lower cost of herbal medicines than chemical medicines and the greater compatibility with the body are also effective. One of the problems of the food and medicine industry is its expansion of microbial strains resistant to drugs and antibiotics (Zarei et al., 2019). Today, due to the toxic and carcinogenic properties of chemical and synthetic compounds, the use of medicinal plants to treat chronic diseases has attracted the attention of many researchers. The use of natural antimicrobial and antioxidant compounds such as organic acids, essential oils, and plant extracts can be a suitable and safe substitute in food (Lafarga et al., 2019; Ramos & Nascimento, 2020). Also, in oral diseases and arthritis, where collagen is prone to degeneration, these plants' phenolic compounds and antioxidants can prevent it (Ramírez et al., 2021).

Phenolic compounds are part of the human diet, and their greatest common benefit is their anticancer and antioxidant activity (Burzyńska, 2019). Phenolic compounds are a large group of natural plant materials containing flavonoids, tannins, and anthocyanins that are present in different parts of the plant. Flavonoids and other phenolic compounds in plants, such as phenolic acids, acetylene, tannins, are usually found in leaves and woody stems like branches (Chandra et al., 2020). These compounds play an important role in the plant's natural growth and have resistance to infection and impact. These substances also have significant benefits in the fields of food, chemistry, pharmacy, and medicine due to the wide range of favorable biological effects, including antioxidant properties (Cömert et al., 2020; Ferrari et al., 2021).

Free radicals and reactive oxygen species are essential parts of life that participate in important biological reactions. They are only useful when they are in the right place at the right time. Otherwise, they can be harmful and attack the best cell compounds such as fatty acids, proteins, nucleic acids, and pigments. Antioxidant compounds are needed to counteract the toxic effects of reactive oxygen species (Smetanska et al., 2021).

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Plant cells use both enzymatic and non-enzymatic antioxidant systems to solve this problem (Arifjanov et al., 2021).

Oxidative stress mostly affects chronic diseases such as cancers, arthritis, cardiovascular disease, and vascular disorders. As a holder of a wide range of antioxidant compounds, plants can be a rich source of active biological compounds. The natural antioxidants in citrus fruits and vegetables inhibit the growth of chronic diseases. Fruits and vegetables are diverse in terms of antioxidant compounds and antioxidant activities. In general, those with high antioxidant activity usually contain more antioxidants. Antioxidants, by eliminating free radicals or preventing their formation from damaging cells, protect against oxidants (Galhardo et al., 2021). The mechanism of phenolic compounds for antioxidant activity is to neutralize free radicals so that the concentration of these biomolecules increases rapidly after the plant is exposed to the problem and adjusts the response costs in the plant (Hemmati et al., 2020). Phenolic compounds in plants have different functions, including a) pigment in plants, b) anti-disease and antioxidant properties, c) antibacterial and natural pesticide, d) plant protection agent against UV waves, e) plant cell wall insulating agent against gases (Prakash et al., 2019).

Phenolic compounds are one of the best sources of natural antioxidants in plants. Flavonoids are the largest group of plant phenols and comprise more than half of the 8,000 natural phenolic compounds present (Orsavová et al., 2019). The increase in the number of hydroxyl groups is directly related to the antioxidant power of flavonoid compounds (Lou et al., 2020). Flavonoids directly inhibit the active molecules of superoxide and hydroxyl.

Anthocyanins are the most important group of natural pigments after chlorophyll, which are non-toxic and water-soluble and widely used in plant cell fluid is present (Dziadek et al., 2019). These flavonoid pigments are responsible for the red, blue, and purple colors in many fruits, vegetables, and flowers (Nardini & Garaguso, 2020). Anthocyanins have certain physical and chemical properties that give them a unique color and stability. They are highly reactive molecules and are therefore sensitive to destructive reactions. Oxygen, temperature, light, enzymes, and pH are some of the factors that may affect the chemistry of anthocyanins and thus their stability and color (Santos et al., 2019). Anthocyanins are used for a variety of therapeutic purposes. Anthocyanins can protect against blood pressure and atherosclerosis problems. The benefits of anthocyanins for diabetes and pancreatic disorders have also been discovered in recent years. This factor is attributed to several simultaneous biological effects of these pigments in the body, including preventing the production of free radicals, reducing lipid peroxidation, and reducing pancreatic swelling, which reduces the concentration of blood sugar in the urine. Anthocyanin bioflavonoids prevent DNA damage and lipid peroxidation. In addition, they have anti-inflammatory effects and help boost the production of cytokines that regulate immune responses.

Anthocyanins support hormonal balance by reducing estrogen activity and help regulate the production of enzymes that absorb nutrients as well as reduce cell membrane permeability and fragility. Diets high in antioxidants such as anthocyanins lead to changes in some age-related deficiencies that affect neurological and behavioral parameters, including memory

(Tkacz et al., 2021). Juices that contain anthocyanins, such as cherry and blueberry juice, have antioxidant and anti-inflammatory effects and positively affect muscle damage following exercise and improve recovery (Cruz et al., 2019). Anthocyanins help enhance night vision and better vision by protecting the eyes from free radical damage. Enhancing rhodopsin regeneration and protection against inflammation are at least two mechanisms that use anthocyanins to improve vision and protect the eyes (Wang et al., 2020). Anthocyanins have a high ability to absorb free radicals present in the body. Nitric oxide radical is one of the most important secondary metabolites in plant and animal cells that accumulate in the cell wall of plants under stress (Leite et al., 2019). Superoxide anion is a reduced form of molecular oxygen, a free radical composed of mitochondrial electron transport systems. Some of the electrons that pass through the mitochondrial chains react directly with oxygen to form the superoxide anion (Vuolo et al., 2019; Rios-Corripio & Guerrero-Beltrán, 2019).

The source of phenols and flavonoids in different parts of the world depends on the diet of the people in the region. For example, in countries such as Japan and China, the consumption of green tea provides these compounds needed by the body. While in western countries, these substances are supplied by consuming apples and onions, and in eastern countries by consuming fermented vegetables and foods (Yahia et al., 2019).

The objectives of the present study were to evaluate the levels of total phenol, total flavonoids and antioxidant activities of 12 different genotypes. The genotypes are related to four different fruits including lemon, kiwi, mandarin and orange. Also, the desired parameters have been investigated in 3 parts including leaf, fruit and the combination of these two parts.

The necessity and importance of this study in order to achieve the amounts of phenol, flavonoids and antioxidant properties in different parts of fruits with vitamin C. Studies by other researchers have shown differences in the properties of vitamin C-rich fruits in different harvest areas. According to studies, the fruits in question differ in the parameters of phenol, flavonoids and antioxidant properties. In fact, areas with fruit with good properties and areas with fruit with poor properties. Therefore, in this study, the mentioned fruits from different regions have been studied to determine the difference between the desired parameters. Therefore, for each of the 4 mentioned fruits, 3 different genotypes have been defined, each of which is related to a specific region. Also, the values of the desired parameters in the three areas of shell, mantle and their combination, have improved the results of the present study.

It is worth noting that firstly, fruits with high vitamin C and antioxidant properties were considered. Secondly, the location of the fruit harvest, which is from 12 different regions, affects the properties of each.

## 2 Material and methods

In this study, 12 different citrus genotypes were evaluated, and according to the place of collection, they are divided into four groups, including Lemon, Kiwi, Mandarin, and Orange.

Also, for ease of evaluation, the genotypes are numbered from 1 to 12, respectively.

### 2.1 Method of preparation of hydro-alcoholic extract

10 g of dried leaves were soaked in the shade and in the vicinity of air, mill, and then in 100 cc of solution (containing 30% distilled water and 70% alcohol) and kept for 48 h at room temperature on a shaker. After the desired time, the extracts were refined, then the solvent was evaporated at a temperature of less than 38 °C by the rotary apparatus, and the remaining after drying was kept in the refrigerator at 38 °C for experiments. Then, to measure the amount of total phenol and flavonoids, 100 mg of the extract powder was dissolved in 1 cc of methanol.

### 2.2 Calculation method of total phenol

To measure the total phenol content, 100 µL of plant extract, 2 mL of sodium carbonate, 3 mL of distilled water, and 100 µL of cocaltin folate reagent were added. After 30 min, their absorption was recorded at 720 nm. Gallic acid was used as the standard to draw the standard curve. The phenol content of total extracts based on mg equivalent of gallic acid per gram of plant dry weight was reported.

### 2.3 Calculation method of total flavonoids

To measure total flavonoids, add 2 mL of methanol, 100 µL of aluminum chloride solution, 100 µL of 1 M potassium acetate solution, and 3 mL of distilled water to 500 µL of each extract. The adsorption of the mixture was measured after 40 min at a wavelength of 415 nm relative to the blank. Blank contained all of the above ingredients, but the same volume of 80% methanol was added instead of the extract. Quercetin was used to draw the standard curve. The flavonoid content of all extracts was reported based on mg equivalent of quercetin per gram of plant dry weight.

Aluminum chloride colorimetric method was used to determine the amount of total flavonoids. Each plant extract was combined separately with methanol, aluminum chloride, potassium acetate and distilled water. Experiments were performed 3 times and their average was reported.

### 2.4 Assessment of antioxidant properties

Stable radical diphenylpicrylhydrazyl was used to determine free radical scavenging activity. For this purpose, 40 mg of the extract was dissolved in 25 cc of methanol. Then, three concentrations of 40, 70, and 100 µL were taken from this

solution, increased to 4 cc with DPPH (with a concentration of 0.1 mmol), and then placed at room temperature for 2 h and absorbed light with a wavelength of 500 nm done.

## 3 Results and discussion

The results for total phenol, total flavonoids, and antioxidant activity values for different citrus genotypes are presented in this section, respectively. The mentioned results have also been discussed, and the groups and genotypes with the highest and lowest values are identified.

### 3.1 Total phenol values

The results showed that the amount of phenol in leaf extract of different genotypes ranged from 1.47 to 14.29, and the average was 8.38 mg/g of dry extract and was significant at the level of 1%. The maximum phenol content of leaf (14.29 mg/g) extract was obtained for genotype 4 and the minimum (1.47 mg/g) for genotype 8. Also, the amount of phenol in fruit extract of different genotypes ranged from 15.64 to 31.52, and the average was 23.35 mg/g of dry extract and was significant at the level of 1%. The maximum phenol content of fruit (31.52 mg/g) extract was obtained for genotype 11 and the minimum (15.64 mg/g) for genotype 7. Fruit extract (23.35 mg/g) generally had more phenol than leaf extract (8.38 mg/g). Finally, the amount of phenol in leaf and fruit extracts of different genotypes ranged from 10.09 to 19.68, and the average was 15.06 mg/g of dry extract. Also, genotypes of group Kiwi with 17.59 and group Mandarin with 11.32 mg/g have maximum and minimum phenol values, respectively. Total phenol values in different genotypes are shown in Table 1. Also, the phenol values of all genotypes in order from the largest to the smallest are shown in Figure 1.

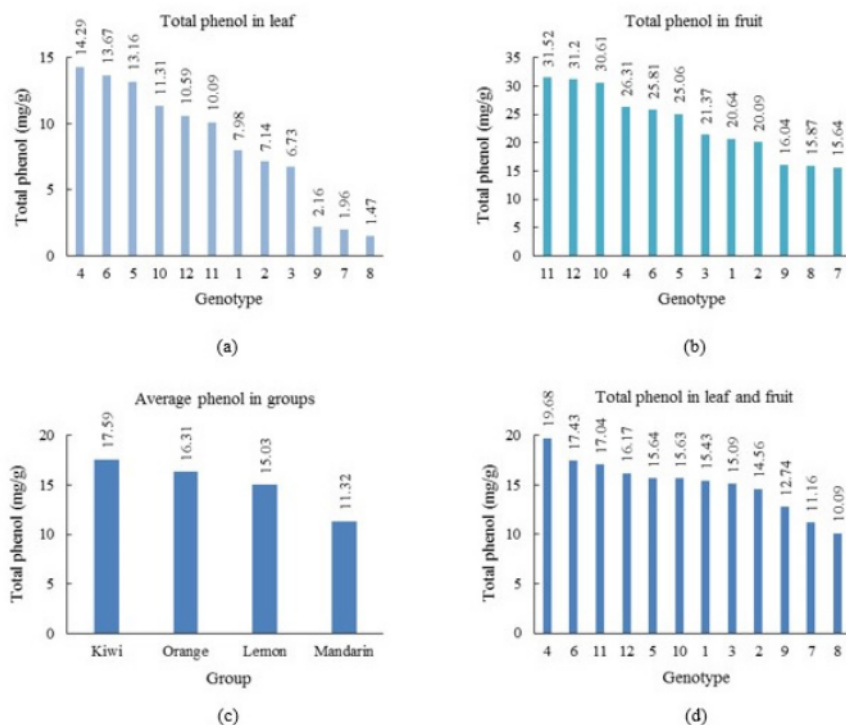
### 3.2 Total flavonoids values

The results showed that the amount of flavonoid in leaf extract of different genotypes ranged from 34.41 to 101.56, and the average was 71.70 mg/g of dry extract and was significant at the level of 1%. The maximum flavonoid content of leaf (101.56 mg/g) extract was obtained for genotype 4 and the minimum (34.41 mg/g) for genotype 7. Also, the amount of flavonoid in fruit extract of different genotypes ranged from 0.27 to 6.83, and the average was 3.55 mg/g of dry extract and was significant at the level of 1%. The maximum flavonoid content of fruit (6.83 mg/g) extract was obtained for genotype 12 and the minimum (0.27 mg/g) for genotype 8. In general, leaf extract (71.70 mg/g) had more flavonoid than fruit extract (3.55 mg/g). Finally, the amount of flavonoid in leaf and fruit

**Table 1.** Total phenol values in different genotypes (mg/g).

Genotypes Study state	Lemon			Kiwi			Mandarin			Orange		
	1	2	3	4	5	6	7	8	9	10	11	12
Leaf	7.98	7.14	6.73	14.29	13.16	13.67	1.96	1.47	2.16	11.31	10.09	10.59
Fruit	20.64	20.09	21.37	26.31	25.06	25.81	15.64	15.87	16.04	30.61	31.52	31.2
Leaf and fruit	15.43	14.56	15.09	19.68	15.64	17.43	11.16	10.09	12.74	15.63	17.04	16.17
Average		15.03			17.59			11.32			16.31	

The evaluation of the antioxidant activity



**Figure 1.** Evaluation of different genotypes based on total phenol: (a) total phenol in leaf; (b) total phenol in fruit; (c) average phenol in groups; (d) total phenol in leaf and fruit.

**Table 2.** Total flavonoid values in different genotypes (mg/g).

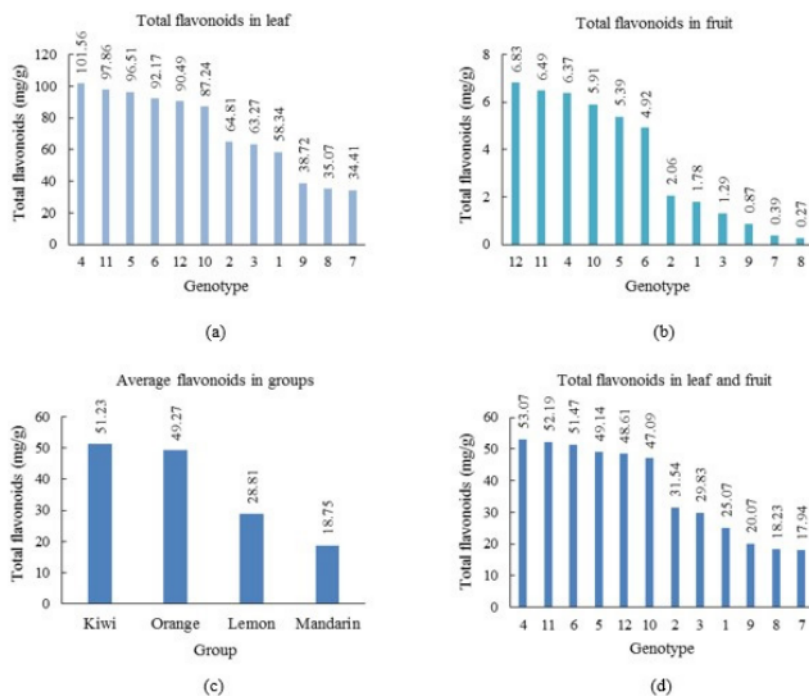
Genotypes	Lemon			Kiwi			Mandarin			Orange		
	1	2	3	4	5	6	7	8	9	10	11	12
Leaf	58.34	64.81	63.27	101.56	96.51	92.17	34.41	35.07	38.72	87.24	97.86	90.49
Fruit	1.78	2.06	1.29	6.37	5.39	4.92	0.39	0.27	0.87	5.91	6.49	6.83
Leaf and fruit	25.07	31.54	29.83	53.07	49.14	51.47	17.94	18.23	20.07	47.09	52.19	48.61
Average		28.81			51.23			18.75			49.27	

extracts of different genotypes ranged from 17.94 to 53.07, and the average was 37.02 mg/g of dry extract. Also, genotypes of group Kiwi with 51.23 and group Mandarin with 18.75 mg/g have maximum and minimum flavonoid values, respectively. Total flavonoid values in different genotypes are shown in Table 2. Also, the flavonoid values of all genotypes in order from the largest to the smallest are shown in Figure 2.

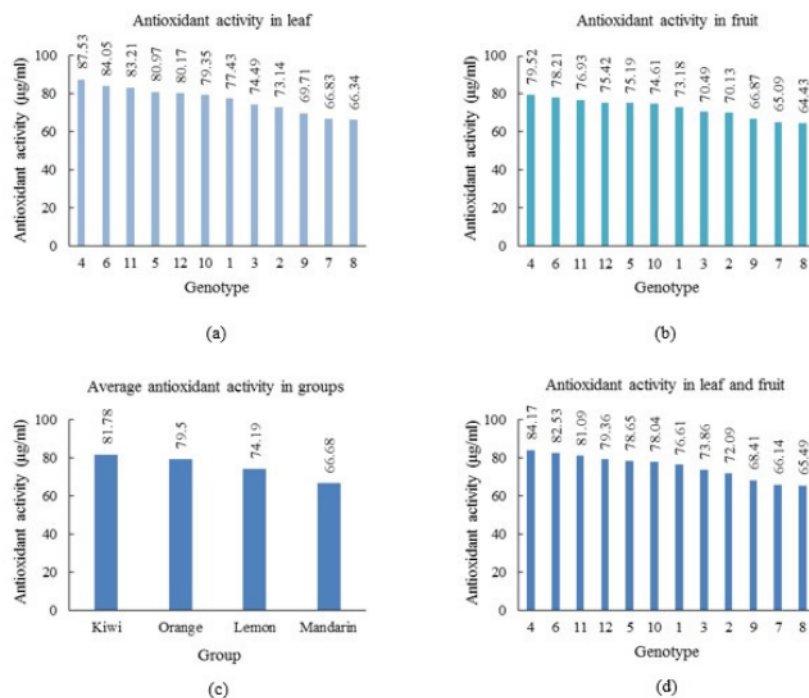
### 3.3 Antioxidant activity values

The results showed that the amount of antioxidant activity in leaf extract of different genotypes ranged from 66.34 to 87.53, and the average was 76.94 µg/mL of dry extract and was significant at the level of 1%. The maximum antioxidant activity content of leaf (87.53 µg/mL) extract was obtained for genotype 4 and the minimum (66.34 µg/mL) for genotype 8.

Also, the amount of antioxidant activity in fruit extract of different genotypes ranged from 64.43 to 79.52, and the average was 72.51 µg/mL of dry extract and was significant at the level of 1%. The maximum antioxidant activity content of fruit (79.52 µg/mL) extract was obtained for genotype 4 and the minimum (64.43 µg/mL) for genotype 8. In general, leaf extract (76.94 µg/mL) had more antioxidant activity than fruit extract (72.51 µg/mL). Finally, the amount of antioxidant activity in leaf and fruit extracts of different genotypes ranged from 65.49 to 84.17, and the average was 75.54 µg/mL of dry extract. Also, genotypes of group Kiwi with 81.78 and group Mandarin with 66.68 µg/mL have maximum and minimum antioxidant activity values, respectively. Antioxidant activity values in different genotypes are shown in Table 3. Also, the antioxidant activity values of all genotypes in order from the largest to the smallest are shown in Figure 3.



**Figure 2.** Evaluation of different genotypes based on total flavonoid: (a) total flavonoid in leaf; (b) total flavonoid in fruit; (c) average flavonoid in groups; (d) total flavonoid in leaf and fruit.



**Figure 3.** Evaluation of different genotypes based on antioxidant activity: (a) antioxidant activity in leaf; (b) antioxidant activity in fruit; (c) average antioxidant activity in groups; (d) antioxidant activity in leaf and fruit.

**Table 3.** Antioxidant activity values in different genotypes ( $\mu\text{g/mL}$ ).

Genotypes Study state	Lemon			Kiwi			Mandarin			Orange		
	1	2	3	4	5	6	7	8	9	10	11	12
Leaf	77.43	73.14	74.49	87.53	80.97	84.05	66.83	66.34	69.71	79.35	83.21	80.17
Fruit	73.18	70.13	70.49	79.52	75.19	78.21	65.09	64.43	66.87	74.61	76.93	75.42
Leaf and fruit	76.61	72.09	73.86	84.17	78.65	82.53	66.14	65.49	68.41	78.04	81.09	79.36
Average		74.19			81.78			66.68			79.5	

#### 4 Conclusion

The results clearly show the effect of harvest location of lemon, kiwi, Mandarin and orange fruits on the results. Each genotype is location-specific with different amounts of phenol, flavonoids, and antioxidants. In general, the results showed that the extract of the fruit had the highest amount of phenol and the leaf extract had the highest amount of flavonoids. Genotypes 4 and 6 with 84.17 and 82.53  $\mu\text{g/mL}$ , respectively, have the highest values of antioxidant activity. The values of total phenol, total flavonoids, and antioxidant activity, from highest to lowest, belong to groups Kiwi, Orange, Lemon, and Mandarin, respectively. Therefore, it is better to use group Kiwi genotypes as one of the paternal or maternal bases or gene donors for citrus modification.

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## ORIGINALITY REPORT

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